Life History and Chemical Ecology of *Xenos peckii* (Strepsiptera, Xenidae)

by

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Abstract

I investigated life history traits and pheromonal communication of *Xenos peckii*, a parasitoid of the paper wasp *Polistes fuscatus*. To emerge, males cut the puparium with their mandibles, and then push aside the pupal cap with their head. The endoparasitic females engage in active calling (pheromone release) behaviour assuming a particular body posture. Seasonal and diel emergence periods of males coincide with seasonal and diel calling periods of females. Mate-seeking males land on the anterior portion of the host wasp's abdomen, and then step backward until their mesothoracic legs contact the female's cephalothorax, upon which the male initiates copulation. Analyzing pheromone gland extracts by gas chromatographic-electroantennographic detection revealed a candidate pheromone component (CPC) that consistently elicited responses from male antennae. CPC was identified as (7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal based on mass and NMR spectra, and the synthesis of an authentic standard. In field experiments, traps baited with synthetic CPC captured *X. peckii* males.

Keywords: Strepsiptera; Twisted wing parasite; *Xenos peckii*; Stylopization; Mate signalling; Sex pheromone

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Glossary

apolysis	separation of the cuticle from the epidermis during moulting in many invertebrates
apomorphy	character that is unique to a related group of species ("derived trait"), but is not present in the ancestral forms
autapomorphy	an apomorphy which is distinct to a single terminal group; thus, an apomorphy that occurs within a single taxon
campodeiform larva	dorsoventrally flattened, bilaterally symmetric larva, which has well developed sense organs and legs, is motile and active, and possesses cerci
cephalotheca	sclerotized anterior region of the puparium of male strepsipterans
cephalothorax	fused head and first two thoracic segments of female strepsipterans; the bodypart of the female that extrudes between the host's tergites
ecdysial suture line	a line of thin cuticle that encircles the puparium of male strepsipterans, delineating the boundary of the cephalotheca
ecdysis	process of shedding the old cuticle (exuvia) during moulting
extrusion	process by which a developmentally mature strepsipteran 4th instar larva pokes its anterior region through the abdominal intersegmental membrane of its host
heteromorphosis	development characterized by a major change between successive larval instars that proceed from a campodeiform 1st instar to carabeiform (grub-like, immobile) 2nd instar
neoteny	retention of juvenile characteristics by an adult insect
phoresy	a mode of transport or dispersal whereby small insects attach themselves to the bodies of larger ones
planidium	first instar larva of the Strepsiptera commonly (but erroneously) termed "triungulin"
stylopized	host which is stylopized (= visibly parasitized) by one or more strepsipterans extruding between sclerites of the host

Uniting to declare the same truths, and, with one voice, . . . May that day ever more and more approach; to hasten its dawn is the peculiar office and duty of the naturalist, who is the Hierophant in the great temple of nature; and this can only be effected by opening our eyes to the light which nature herself affords to those who seek truth; by recording, not our own private hypotheses, but our discoveries; by improving, instead of destroying, what others have done; by retaining what is already discovered of the natural system, and endeavouring to add to it; remembering always that we are not the heralds of our own fame, but of the glory of our God.

Rev. William Kirby, 1802

Chapter 1.

Life History and Chemical Ecology of *Xenos peckii* (Strepsiptera, Xenidae)

1.1. Introduction

The Strepsiptera, commonly referred to as "twisted winged parasites", are a small insect order comprising only 618 currently described species (Catalogue of Life, May 18, 2015). All species are obligate entomophagous endoparasitoids, parasitizing a broad range of hosts encompassing 7 orders and 34 families of Insecta, in various habitats worldwide (Kathirithamby 2009). Host insects comprise endo-, exo-, and apterygotes that include Thysanura, Orthoptera, Blattodea, Mantodea, Hemiptera, Diptera, and Hymenoptera (Kathirithamby 1989, 2009). Strepsiptera are most prevalent in tropic regions, but are found on all continents, and in essentially any environment that sustains insects.

Defining features of Strepsiptera are their extreme sexual dimorphism, obligate endoparasitism in the larval stages, and entomophagy (McMahon *et al*. 2011). The primitive Strepsiptera (Mengenillidia; Fig. 1.1) are represented by few extant species, including those that parasitize Thysanura. The Mengenillidia exhibit some degree of neoteny in that 4th instar larvae exit the host to pupate and become free living, yet not very mobile, adult females with shortened limbs and wing stubs that are non-functional (Kinzelbach 1971a,b; Kathirithamby 2009). Derived species comprise the suborder Stylopidia and are characterized by extreme neoteny. Females of Stylopidia are permanently endoparasitic and lack a pupal instar, whereas the free-living males pupate

and become winged adults. Nonetheless, Stylopidia males also exhibit a reduced body plan, including fewer legs and tarsi in the pupal stage, and as adults fewer spiracles, and a vestigial mouth and digestive tract. The Corioxenidae represent a transitional stage, and are a sister-group to all other Stylopidia (McMahon *et al.* 2011; Fig. 1.1). Stylopidia comprise the vast majority of extant strepsipteran species.

Despite their broad host range and cosmopolitan distribution very little is known about the Strepsiptera. The paucity of research is likely due to their cryptic, endoparasitic lifestyle, the extremely short lifespan of the free-living adult males, and a general understanding among entomologists that strepsipterans lack any economic impact either as pests or as potential biocontrol agents.

1.1.1. Discovery and naming of Strepsiptera

Xenos vesparum (Rossi) constitutes the earliest record of a strepsipteran species, described in 1793 by Pietro Rossi, a lepidopterist at the University of Pisa. He discovered it parasitizing the paper wasp *Polistes gallica* Latrielle. Rossi recognized that his specimen did not conform to any previously described insect order and defined it as a “new kind of insect, neighbouring ichneumons”. Owing to the specimen’s confusing mix of characters that defied placement into any one family, Rossi disregarded physical characters and based his tentative placement solely on the parasitic habit of the tiny insect, which parallels that of the Ichneumonoidea (Rossi 1793, cited in Pierce 1909).

Nine years later, an English clergyman and naturalist, Reverend William Kirby, in a treatise on *Apis*, described a peculiar creature parasitizing the solitary bee *Andrena melittae*. Kirby accounted for his specimen under "miscellaneous", as he noted that it did not fit into any insect kind, as prescribed by the Linnean system (Kirby 1802).

Ordinal status was not proposed for another nine years, until William Kirby received a correspondence from William Peck, professor at Harvard, Cambridge. Peck,

inspired by Kirby's description of *Stylops melittae*, sent a sample of a creature he had witnessed parasitizing *Polistes fuscatus,* in North America. Upon receiving the New World specimen, Kirby immediately connected it with Rossi's description of *Xenos vesparum*, noting that these two geographically disparate species appeared to be closely related to each other. Kirby proposed that Rossi's specimen was not merely an anomalous-looking ichneumon wasp, but rather that these specimens exemplified a hitherto undescribed Order of insect (Kirby 1811).

Contrary to popular belief, Kirby (1811) chose the name Strepsiptera (*Strepsi* = "twisted" + *pteryx* = "wing") not on account of the hind wings of adult males, which appear to be wrinkly and misshapen (particularly in dried museum specimens), but on account of their haltere-like forewing structures which he likened to "distorted elytra of the Coleoptera". He put forth a strong argument for ordinal status, going as far as to propose a set of rules to outline the preconditions necessary to delineate an insect order. Kirby's effort to define the ordinal status of Strepsiptera resulted in the manifestation of a framework, which subsequently served to refine the ordinal ranking of all Insecta.

1.1.2. Phylogenetic placement of Strepsiptera

Strepsiptera represent an ancient lineage. Fossil specimens are typically preserved in amber deposits and are occasionally discovered. The earliest fossil specimens, of *Cretostylops engeli*, were found in Burmese amber, dating back approximately 100 million years (Grimaldi and Engel 2005). These fossils establish the presence of Strepsiptera in the mid-Cretaceous; however, they were already highly specialized, with many autapomorphies characteristic of modern Strepsiptera, suggesting a much earlier divergence from the holometabola (Pohl and Beutel 2008). Phylogenetic analysis revealed the monophyly of the derived Strepsiptera (McMahon *et al*. 2011). This clade, comprising the suborder Stylopidia, is composed of the Corioxenidae, and the highly derived Stylopiformia.

The placement of Strepsiptera within the Insecta has been a source of controversy (Whiting and Wheeler 1994; Carmean and Crespi 1995; Whiting *et al*. 1997; Huang *et al.* 1998; Huelsenbeck 2001; Beutel and Pohl 2006) ever since Kirby's 1811 seminal work; however, recent studies confirm that Strepsiptera are a sister group to the Coleoptera (McMahon and Kathirithamby 2008; Longhorn *et al.* 2010; McKenna and Farrel 2010; McMahon *et al.* 2011; Niehuis *et al*. 2012; Boussau *et al*. 2014). Estimates of divergence times using nuclear genes, fossil calibrations, and Bayesian methods suggest an early divergence of Strepsiptera from the holometabolous orders, approximately 274 mya, and thus a co-evolution with the holometabola (Hedges and Kumar 2009, and references cited therein). More recent phylogenomic analyses place Strepsiptera-Coleoptera divergence *ca*. 284 mya (Misof *et al*. 2014). Extant basal species still persist but are represented by only a few species in the suborder Mengenillidia (Kinzelbach 1972; Kathirithamby *et al.* in press; Fig. 1.1). In addition, recent identifications of female sex pheromones in three Strepsiptera species (Cvačka *et al*. 2012; Tolasch *et al*. 2012; Chapter 3), coupled with more advanced genetic analysis, support the hypothesis that Strepsiptera are, indeed, closely allied with the Coleoptera (Niehuis *et al*. 2012; Pohl and Beutel 2013; Boussau *et al*. 2014); however, much remains to be discovered.

1.1.3. Life history traits of Strepsiptera

Strepsiptera are unique in many respects. They possess the most extreme sexual dimorphism of any insect group. The males are holometabolous and free-living, whereas the females are neotenic and remain bound to their host for life (Grimaldi and Engel 2005; Erezyilmaz *et al*. 2014). Obligate female endoparasitism is an apomorphy of the Stylopidia (excluding the basal Corioxenidae, which exhibit transitional features). The extreme neoteny that characterizes strepsipteran females represents a recent development; ancestral strepsipteran females were free living, a characteristic still observed in the two remaining basal families Mengenillidae and Corioxenidae (Kathirithamby 2009; McMahon *et al*. 2011; Kathirithamby *et al*. in press).

Strepsiptera exhibit a trend towards specialization by reduction (Bohart 1941). Despite their free-living habit, females of the primitive Strepsiptera (suborder Mengenillidia; Fig. 1.1) are wingless with greatly reduced limbs, and exhibit no apparent activity. An evolutionary shift towards complete endoparasitism of females, in the more derived suborder Stylopidia, is presumably a consequence of a developmental gene mutation, which resulted in extreme neoteny and a total loss of all limbs, characteristic of the Stylopidia (Kathirithamby *et al*. in press). As a result, the adult females remain permanently embedded in their host, and are thought to be totally immobile and inactive from the point that they extrude from their host, in effect serving solely as a receptacle for sperm and a repository for eggs. The extreme modification of females as amorphous repositories for eggs has inspired the label "viviparous reproductive machine" (Kathirithamby 2009), noting that a single female can release *ca.* 1000 to over 750 000 planidia larvae, depending on the species.

In contrast to the neotenic adult females, adult males possess many features characteristic of typical holometabolous insects, although they exhibit extreme specializations that have likely arisen from a co-evolution with their hosts. Soon after extruding between the host's abdominal tergites, male 4th instars of both the Mengenillidia and Stylopidia undergo a molt and pupate into winged adults. Male strepsipterans live for only a few hours, during which time they must find a female and mate. Males exhibit extreme sensory adaptations for mate finding and recognition, including large branched antennae, stalked eyes that have no equal in the extant Animalia, and a specific sensory patch on the inner margin of certain tarsomeres. Sensory perception remains largely unstudied, but the vision of Strepsiptera is an exception. The eyes of adult males are composed of a cluster of eyelets, each containing a retina. The cluster of images produced by these eyelets is stitched into a single, composite image (Bushbeck *et al*. 1999; 2003) that affords the high level of visual acuity, which may be needed for locating females (Pix *et al*. 2000; Maksimovic *et al*. 2007).

Stage-dependent, obligate endoparasitism is a defining characteristic of the Strepsiptera; however, they defy easy categorization. Strepsiptera are referred to as

parasites in the early literature, although it has been argued that they are better characterized as parasitoids (which typically kill their hosts) because they castrate their hosts, effectively preventing their reproduction (Hughes *et al*. 2003; Kathirithamby 2009). More recent observations that males of paper wasp *Polistes dominula* that were parasitized with *Xenos vesparum* occasionally inseminate conspecific gynes have invoked doubt that the term parasitoid really applies to Strepsiptera (Cappa *et al*. 2014). Exhibiting features of both koinobiont and idobiont strategists (Kathirithamby 2009), Strepsiptera certainly do not fit the typical parasitoid classifications.

The development of both males and females is characterized by heteromorphosis (Reynolds 2013), whereby the campodeiform (motile, elongated and flattened) larva molts into a carabaeiform (grub-like) form in subsequent instars. In many ways, strepsipteran larval development resembles that of hymenopteran, dipteran, and coleopteran koinobiont endoparasitoids (Manfredini *et al*. 2010a, and references cited therein), which also possess planidial larvae and undergo heteromorphosis. First instars are tiny (*ca*. ≤ 0.2 mm long) but are highly active fast runners with long caudal filaments that enable them to jump onto potential host vectors (Kirkpatrick 1937; O'Connor, 1959).

First instars are commonly, but erroneously, termed “triungulins”. This misnomer has a historical basis, stemming from an early misidentification of strepsipteran first instars, which were mistaken for meloid beetle larvae (ex. Newport 1847; discussed in Pierce 1964) due to their morphological similarity. The misclassification escaped correction in some entomological circles, and even appeared in certain taxonomic keys (e.g., LeConte *et al*. 1883; Vaurie 1983), primary research articles (e. g., Kritsky *et al*. 1977), and periodicals (e.g., Crowson 2013) despite an otherwise overwhelming acceptance of the ordinal status of Stepsiptera. The term triungulin denotes first instars of meloid and rhipiphorid beetles which have planidial, motile larvae with typically three claws (*tri* = “three”; *ungue* = “claw”; hence “three-clawed”). In contrast, first instars of Strepsiptera either possess disk-like pulvilli on their prothoracic and mesothoracic legs with spine-shaped tarsi on the metathoracic (hind) legs, or spine-like tarsi without claws on all legs (Kathirithamby 1989). The misnomer, triungulin, used to denote strepsipteran

larvae is unfortunate, as it is linguistically inelegant, confuses literature searches, and fails to reflect the nature of strepsipteran larvae. In recognition of this error, some scientists refer to strepsipteran first instars as being “triungulinid” ("triungulin-like"); however, the proper nomenclature for strepsipteran first instars is planidia (singular: planidium) (Kathirithamby *et al*. in press).

Host foraging is a daunting task, requiring both long- and short-range travel. Upon emergence, the tiny planidia are tasked with finding their way from their current host insect to a new, healthy, larval host, which is often in a distant nest or burrow. First instars must quickly locate, travel to, and infect a host. Therefore, one would expect that they possess specific sensory and locomotory abilties. Once they arrive at the nest, they must identify a suitable new host, and burrow inside. The specific cues planidia utilize in host finding remain largely undiscovered. They could include visual, semiochemical, or other cues. The sensory modalities employed may differ between times of long-range and short-range foraging.

Planidia rely on their host insect to assist them during their long-range search for a new host. Planidia have been observed to take advantage of phoretic transport, and even possibly behavioural manipulation of their host insect to aid in carrying them to a suitable host insect. Saunders (1853) first described his observations of phoretic transmission of *Stylops*, whereby planidia emerged from, and then jumped off, their host bee onto a flower while the bee was foraging. The planidia waited for, and then 'hitched a ride' on a healthy bee which, in turn, carried them to the nest it was provisioning. A peculiar variant to phoretic transmission is suggested by an observation of *Stylops pacifica*, a parasitoid of *Andrena complexa*, whereby numerous planidia were found in the honey crop of their host bee (Young 1987). Young suggests that the planidia may be ingested by a foraging adult bee, and then deposited in a nest together with larval food provisions. Since the larvae are encased in the food provision, Young suspects that they may enter the host larvae via ingestion. Whether this is, in fact, the case remains to be studied.

An alternate mode of long-range transportation may occur in species parasitizing social wasps. Beani and Massolo (2007), noting field observations by Pardi (1946) of foreign nest visitations by parasitized gynes, suggest that, at least in parasitoids of social wasps, the parasitized host wasp may directly vector planidia. Beani and Massolo (2007) propose that behavioural manipulation may be employed in causing the *Polistes dominula* wasp host to visit these non-natal nests. A bimodal distribution of parasitization rates within the wasp population suggest that both phoresis and direct transport are involved in transporting planidia to host nests. Hughes *et al.* (2004) observed a very high parasitization rate of certain nests but a much lower rate in the population in general. The high parasitization rate of certain nests suggests that a parasitized wasp had visited, dispensing numerous planidia during the visit. Phoretic transport would account for a lower baseline parasitization rate over the entire wasp population.

Once transferred to a nest, the planidium locates and enters its larval host. The mode of entry into the host is unknown and may differ between species, although it is speculated that the planidium enzymatically dissolves the host cuticle with a salivary excretion. It is further assumed that entry occurs during host moulting when the cuticle is not yet fully sclerotized (Riek's [unpubl.] records cited in Kathirithamby 1989). Riek observed planidia of *Pseudoxenos sp.* penetrating first instars of their sphecid host wasp *Sceliphron laetum*. Entry of a planidium took about 5–10 min during which time it exuded from its mouth a "considerable quantity" of fluid, enough to effectively immerse it in its own exudate. The exudate hardened after 3 to 4 min, while at the same time it presumably softened the host's cuticle, possibly by some sort of enzymatic action (Linsley and MacSwain 1957). During this time, planidia were observed to expand and contract, as if they were exerting an effort to rupture the cuticle of their host. Eventually, the planidia sunk into the host tissue. The above observations suggest that planidia may utilize both physical and chemical means to gain entry into their host. The transition of a planidium through the host epidermis appears to be accomplished through a phagocytotic process whereby the planidium presumably wraps itself with a host-derived "epidermal bag" as it enters the host (Kathirithamby and Johnston 2004). Wrapping itself in host-

derived tissue may be a means of immune system avoidance. If so, an alternate method of immune avoidance is necessary once the planidium moults, because its first instar cuticle is shed, along with the epidermal bag (Manfredini *et al*. 2007).

Soon after planidia penetrate their host, both males and females molt into a grub-like 2nd instar, shedding their body appendages. While the first instar casts its exuvia, the two successive instars undergo "apolysis without ecdysis" (Kathirithamby 1989), developing within, rather than casting, their old exuviae – like a nested doll. In a process termed extrusion, the 4th instar then penetrates the host's intersegmental membrane, and protrudes its head and first two thoracic segments outside of the host. The head and thorax of a female planidium quickly sclerotize into a structure termed 'cephalothorax' (Nassonow 1892); thereafter, the female is considered a neotenic adult and will not pupate. In contrast, after the 4th instar of a male planidium has extruded from its host, the nested cuticles (see above) of the 2nd, 3rd, and 4th instars sclerotize to form a puparium. The male then moults into a winged adult.

1.1.4. Chemoreception

Specific chemoreception strategies may be utilized during the free-living stages of the strepsipteran life cycle: the host-seeking larvae (both male and female), and the mate-searching adult male. Few studies have investigated the sensory modalities utilized by Strepsiptera during specific life stages.

First instars have been shown to utilize visual (Kirkpatrick 1937) and chemical (Manfredini *et al.* 2010b) cues to locate and evaluate an appropriate host. Observations of phoretic transmission have been periodically discussed in the literature (Saunders 1853; Smith 1859; Janet 1897, cited in Wheeler 1919; Kirkpatrick 1937; Linsley & MacSwain 1957; Batra 1963; Young 1987; Kathirithamby *et al*. 2012); however, thus far only two studies (Kirkpatrick 1937; Manfredini *et al.* 2010b) have attempted to discern the specific cues involved in host finding.

Kirkpatrick (1937) investigated whether vision may play a role during long-range host foraging. Kirkpatrick revealed evidence for visual host recognition by *Corioxenos antestia* (Xenidae), with first instars more likely to jump (by springing their caudal filaments) when an object corresponding to the colour of their host bee was passed overhead. Kirkpatrick's findings comply with earlier field observations of phoretic transportation, whereby emerging first instars disembark from their host at a foraging site, and then later jump onto an unparasitized host that forages the same flower that they were deposited on. The healthy insect acts as a phoretic agent, transporting the planidia to the nest of a potential new host (Saunders 1853).

Investigating short-range foraging of *Xenos vesparum,* Manfredini *et al.* (2010b) tested, but could not show conclusively, whether host-derived (cuticular) semiochemical cues are involved in directing planidia to their host. Despite Kirkpatrick's finding, Manfredini *et al*. (2010b) argue that, in *Xenos*, light simply mediates the emergence of planidia from their mother, while carbon dioxide directs them to their new host.

Male adults have a particular challenge in locating a receptive female. Owing to their tiny size, endoparasitic habit, and mobile insect hosts, chemical communication had long ago been a suspected mate signalling modality by Strepsiptera. Hofeneder (1910) first suggested that females might signal males by releasing a semiochemical. This is remarkable considering that he proposed it nearly 50 years before the term "pheromone" was coined (Karlson and Lüscher 1959, cited in Tolasch *et al*. 2012). Recent studies have corroborated Hofeneder's hypothesis, with the identification of the sex pheromone of the bee parasitoids *Stylops melittae* (Tolasch *et al*. 2012) and *Stylops muelleri* (Cvačka *et al.* 2012), and that of the wasp parasitoid *Xenos peckii* (chapter 3).

1.2. Conclusion

Strepsiptera have puzzled biologists ever since the first male was discovered about 200 years ago. Still today, little is known about this enigmatic group of insects.

They break from the norm, and much of their biology and life history challenges common sense. They continue to generate controversy and debate within the entomological field. They are tiny, ephemeral, and economically irrelevant. Strepsiptera are therefore largely overlooked, with just one or two pages consigned to them in entomological textbooks.

With their unique biology, Strepsiptera are ideal model systems for studying a broad array of biological questions, ranging from insect evolution and ecology, the role of parasites in food webs, behavioural manipulation of host insects (Hughes *et al*. 2003; Hughes *et al*. 2004; Hughes 2005; Beani 2006; Dapporto *et al*. 2006; Beani *et al*. 2007; Manfredini *et al*. 2007), modes of endoparasitoid immune system avoidance (Kathirithamby *et al.* 2003; Hughes and Kathirithamby 2005), sexual selection in insects (Kathirithamby *et al.* in press), and even the evolution of arthropod vision (Bushbeck *et al*. 1999; Pix *et al.* 2000; Bushbeck *et al.* 2003).

Although Strepsiptera occupy the peripheral backwater of entomology, there is much we can learn from these little creatures. Controversy surrounding various aspects of their biology has served as fodder to catalyze heated scientific inquiry and debate. Lessons learned have served more than to satisfy idle curiosity, but have furthered our knowledge, such as taxonomic classification, evolution, and communication, in other insect groups.

1.3. Research objectives

Xenos peckii Kirby (Strepsiptera: Xenidae) is a parasite of the common North American paper wasp *Polistes fuscatus* (Fabricius) (Hymenoptera: Vespidae). Many life history traits of *X. peckii* are unknown, controversial, or hardly described. In Chapter 2, my research objective was to investigate the reproductive biology of *X. peckii,* studying in particular (1) the timing of sexual maturity in females and males, (2) the role of females during sexual communication and potential diel periodicity of communication,

(3) the emergence process of males and potential diel periodicity of emergence, and (4) the mating sequence.

There is also little known about the sexual communication systems of Strepsiptera in general, and of *X. peckii* in particular. In 2010, Tolasch and colleagues reported the identification, synthesis, and field-testing of the *Xenos vesparum* (Xenidae) pheromone at the annual meeting of the International Society of Chemical Ecology in Tours (France) but the authors neither revealed the pheromone structure nor published their results later. More recently, Tolasch *et al*. (2012) studying the pheromone of the bee parasitoid *Stylops mellittae* (Strepsiptera: Stylopidae) near Bayreuth (Germany), and Cvačka *et al*. (2012) studying the pheromone of *Styplops muelleri* near Prague (Czech Republic), reported that (*R,R,R*)-3,5,9-trimethyldodecanal is the single-component sex pheromone of both species. My research objective in Chapter 3 was to identify and field-test the sex pheromone of *X. peckii.*

Suborder / **Infraorder** / **Family**	**Genera**	
Protoxenidae	*Protoxenos*	Extinct
Cretostylopidae	*Cretostylops*	
Mengenillidia		
Mengeidae	*Mengea*	
Mengenilloidea		Extant
Bahiaxenidae	*Bahiaxenos*	
Mengenillidae	*Congoxenos, Eoxenos, Mengenilla*	
Stylopidia		
Corioxenidae	*Australoxenos, Blissoxenos, Corioxenos, Dundoxenos, Floridoxenos, Loania, Mufagaa, Proceroxenos, Triozocera, Uniclavis, Viridipromontoxius*	
Stylopiformia		
Bohartilidae	*Bohartilla*	
Elenchidae	*Colacina, Deinelenchus, Elencholax, Elenchus, Protelencholax*	
Halictophagidae	*Blattodeaphagus, Callipharixenos, Coriophagus, Dipterophagus, Halictophagus, Stenocranophilus, Tridactylophagus*	
Lychnocolacidae	*Lychnocolax*	
Myrmecolacidae	*Caenocholax, Myrmecolax, Stichotrema*	
Protelencholacidae	*Protelencholax*	
Stylopidae	*Crawfordia, Eurostylops, Erystylops, Halictoxenos, Hylecthrus, Melittostylops, Paragioxenos, Stylops, Ulrichia*	
* Xenidae	*Paraxenos, Pseudoxenos, Xenos*	

Figure 1-1. Phylogeny of Strepsiptera
Adapted from Kathirithamby *et al*. in press. Note: Placements of taxa are still in flux.
* *Xenos peckii* is placed within Xenidae.

1.4. References

Batra S.W.T. 1963. Organisms associated with *Lasioglossum zephyrun* (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society* 38: 267–389.

Beani, L. 2006. Crazy wasps: when parasites manipulate the *Polistes* phenotype. *Annales Zooligici Fennici* 43: 564–574.

Beani, L. and Massolo, A. 2007. *Polistes dominulus* wasps (Hymenoptera Vespidae), if parasitized by *Xenos vesparum* (Strepsiptera Stylopidae), wander among nests during the pre-emergence phase. *Redia* 90: 161–164.

Beutel, R.G. and Pohl, H. 2006. Endopterygote systematics – where do we stand and what is the goal (Hexapoda, Arthropoda)? *Systematic Entomology* 31: 202–219.

Bohart, R.M. 1941. A revision of the Strepsiptera with special reference to the species of North America. *University of California Publications in Entomology* 7: 91–160.

Boussau, B., Zaak, W., Delgado, J.A., Collantes, F., Beani, L., Steward, I.J., Cameron, S.A., Whitfield, J.B., Johnston, J.S., Holland, P.W.H., Bachtrog, D., Kathirithamby, J., and Huelsenbeck, J.P. 2014. Strepsiptera, phylogenomics and the long branch attraction problem. *PLOS ONE* 9(10): e107709.

Brodeur, J. and Boivin, G. 2004. Functional ecology of immature parasitoids. *Annual Review of Entomology* 49: 27–49.

Bushbeck, E., Ehmer, B., and Hoy, R. 1999. Chunk versus point sampling: visual imaging in a small insect. *Science* 286: 1178–1180.

Bushbeck, E., Ehmer, B., and Hoy, R. 2003. The unusual visual system of the Strepsiptera: external eye and neuropils. *Journal of Comparative Physiology* 189: 617–630.

Cappa, F., Manfredini, F., Dallai, R., Gottardo, M., and Beani, L., 2014. Parasitic castration by *Xenos vesparum* depends on host gender. *Parasitology* 141: 1080–1087.

Carmean, D. and Crespi, B.J. 1995. Do long branches attract flies? *Nature* 373: 666–666.

Catalogue of Life. May 18, 2015. http://www.catalogueoflife.org/col/browse/tree/id/22372804.

Cook, J.L. 2014. Review of the biology of parasitic insects in the order Strepsiptera. *Comparative Parasitology* 81: 134–151.

Cvačka, J., Jiroš, P., Kalinová, B., Straka, J., Černá, K., Šebesta, P., Tomčala, A., Vašíčková, K., Jahn, U., and Šobotník J. 2012. Stylopsal: the first identified female-produced sex pheromone of Strepsiptera. *Journal of Chemical Ecology* 38: 1483–1491.

Dapporto, L., Cini, A., Palagi, E., Morelli, M., Simonti, A., and Turillazzi, S. 2006. Behaviour and chemical signature of pre-hibernating females of *Polistes dominulus* infected by the strepsipteran *Xenos vesparum*. *Parasitology* 134: 545–552.

Grimaldi, D. and Engel, M.S. 2005. *Evolution of the Insects.* New York, N.Y. Cambridge.

Erezyilmaz, D.F., Hayward A., Huang Y., Paps J., Acs Z., Delgado J., Collantes F., Kathirithamby J. 2014. Expression of the pupal determinant *broad* during metamorphic and neotenic development of the strepsipteran *Xenos vesparum* Rossi. *PLOS ONE* 9: E93614.

Hedges, S.B. and Kumar, S. (eds). 2009. The Timetree of Life *Oxford U.P.*, Oxford N.Y.

Hofeneder K. 1910. Untersuchungen zur Naturgeschichte der Strepsipteren. Von Prof. Dr. Nikolai Viktrovich Nassonov, Mitglied des Kaiserlichen *Naturwissenchaftlich-Medizinischen Vereins, Innsbruck* 33: 1-205

Hrabar, M., Danci, A., McCann, S., Schaefer, P., Gries, G. 2014. New findings on life history traits of *Xenos peckii* (Strepsiptera, Xenidae). *The Canadian Entomologist* 146: 512–527.

Huelsenbeck, J.P. 2001. Systematic bias in phylogenetic analysis: Is the Strepsiptera problem solved? *Systematic Biology* 47: 519–37.

Hwang, U.W., Kim, W., Tautz, D., and Frierich, M. 1998. Molecular phylogenetics at the Felsenstein zone: approaching the Strepsiptera problem using 5.8S and 28S rDNA sequences. *Molecular Phylogenetics and Evolution* 9: 470–480.

Hughes, D.P. 2005. Parasitic manipulation: a social context. *Behavioural Processes* 68: 263–266.

Hughes, D.P., Beani, S., Turillazzi, S., and Kathirithamby, J. 2003. Prevalence of the parasite Strepsiptera in *Polistes* as detected by dissection of immatures. *Insectes Sociaux* 50: 62–68.

Hughes, D.P. and Kathirithamby, J. 2005. Cost of strepsipteran macroparasitism for immature wasps: Does sociality modulate virulence? *Oikos* 110: 428–434.

Hughes, D.P., Pamillo, P., and Kathirithamby, J. 2004. Horizontal transmission of *Wolbachia* by strepsipteran endoparasites? A response to Noda *et al*. 2001. *Molecular Ecology* 13: 507–509.

Janet, C. 1897. Etudes sur les fourmis, les guêpes et les abeilles. Note 14, rapports des animaux Myrmécophiles avec les fourmis, les guepes, et abeilles, Limoges. *Société entomologique de France*. 14: 21.

Karlson P., and Lüscher M. 1959. "Pheromones": a new term of a class of biologically active substances. *Nature* 183: 55–56.

Kathirithamby, J. 1989. Review of the order Strepsiptera. *Systematic Entomology* 14: 41–92.

Kathirithamby, J. 2009. Host-parasitoid associations in Strepsiptera. *Annual Review of Entomology* 54: 227–249.

Kathirithamby, J., Hrabar, M., Delgado, J.A., Collantes, F., Dötterl, S., Windsor, D., and Gries, G. 2015. We do not select nor are we choosy: a case study of reproductive synchrony in Strepsiptera. *Biological Journal of the Linnean Society,* in press.

Kathirithamby, J. and Johnston, S. 2004. The discovery after 94 years of the elusive female of a myrmecolacid (Strepsiptera), and the cryptic species of *Caenocholax fenyesi* Pierce *sensu lato*. *Proceeding of The Royal Society London, B.* 271: S5–S8.

Kathirithamby, J., Lechner, G.K., McMahon, D.P., Bryson A.L., and Johnston, S. 2012. A free ride and lunch: Stylopization in the solitary hunting wasp, *Ammophila fernaldi* Murray and *A. pictipennis* (Walsh) (Hymenoptera: Sphecidae) by *Paraxenos lugubris* Pierce (Strepsiptera). *Proceedings of the Entomological Society of Washington* 114: 464–475.

Kathirithamby, J., Ross, L.D., and Johnston, J.S. 2003. Masquerading as self? Endoparasitic Strepsiptera (Insecta) enclose themselves in host-derived epidermal bag. *Proceedings of the National Academy of Science* 100: 7655–7659.

Kinzelbach, R.K. 1971a. Morphologische Befunde an Fächerflüglern und ihre phylogenetische Bedeutung (Insecta: Strepsiptera). *Zoologica Stuttgart* 41(119-1): I–xiii,1–128.

Kinzelbach, R.K. 1971b. Morphologische Befunde an Fächerflüglern und ihre phylogenetische Bedeutung (Insecta: Strepsiptera). *Zoologica Stuttgart* 41(119-2): 129–256.

Kinzelbach, R.K. 1972. The fan-wings from the Senckenberg museum: part 2, Mengenillidae (Inseta, Strepsiptera). *Senckenbergiana Biologica* 53: 403–409.

Kinzelbach, R. and Pohl, H. 1994. The fossil Strepsiptera (Insecta: Strepsiptera). *Annals of the Entomological Society of America* 87: 59–70.

Kirby, W. 1802. Monographia Apum Angliae, vol 1. *Ipswich.* London, England.

Kirby, W. 1811. Strepisptera, a new order of insects proposed; and the characters of the order, with those of its genera, laid down. *Transactions of the Linnean Society of London* 11: 86–124.

Kirkpatrick, T.W. 1937. Colour vision in the triungulin larva of a strepsipteran (*Corioxenos antestiae* Blair). *Proceedings of the Royal Entomological Society* 12: 40–44.

Kritsky, G., Wells, D.W., and Mutt, J.A.M. 1977. Some observations on the fine-structure morphology of *Xenos peckii* (Coleoptera: Stylopidae). *The Coleopterist's Bulletin* 31: 93–96.

LeConte, J.L., Horn, G.H., and Henwhaw, S. 1883. Classification of the Coleoptera of North America. *Smithsonian Institution.* Washington, USA.

Linsley, E.G. and MacSwain. J.W. 1957. Observations on the habits of *Stylops pacifica* Bohart. *University of California Publications in Entomology* 11: 395–430.

Longhorn, S.J., Pohl, H.W., and Vogler, A.P. 2010. Ribosomal protein genes of holometabolan insects reject the Halteria, instead revealing a close affinity of Strepsiptera with Coleoptera. *Molecular Phylogenetics and Evolution* 55: 846–859.

Manfredini, F., Giusti, F., Beani, L., and Dallai, R. 2007. Developmental strategy of the endoparasite *Xenos vesparum* (Strepsiptera, Insecta): host invasion and elusion of its defense reactions. *Journal of Morphology* 268: 588–601.

Manfredini, F., Benati, D., and Beani, L. 2010a. The strepsipteran endoparasite *Xenos vesparum* alters the immunocompetence of its host, the paper wasp *Polistes dominulus*. *Journal of Insect Physiology* 56: 253–259.

Manfredini, F., Massolo, A., and Beani, L. 2010b. A difficult choice for tiny pests: host-seeking behaviour in *Xenos vesparum* triungulins. *Ethology Ecology & Evolution* 22: 247–256.

Maksimovic S., Layne J.E., and Bushbeck E.K. 2007. Behavioral evidence for within-eyelet resolution in twisted-winged insects (Strepsiptera). *Journal of Experimental Biology* 210: 2819–2828.

McKenna, D.D. and Farrell, B.D. 2010. 9-Genes reinforce the phylogeny of holometabola and yield alternate views on the phylogenetic placement of Strepsiptera. *PLOS ONE* 5(7):ARTN e11887.

McMahon, D.P. and Kathirithamby, J. 2008. A molecular phylogeny of Strepsiptera (Insecta). In *23rd International Congress of Entomology. 6-12th July, Durban.* Abstr. p.1906.

McMahon D.P., Hayward A., and Kathirithamby J. 2011. The first molecular phylogeny of Strepsiptera (Insecta) reveals an early burst of molecular evolution correlated with the transisiton to endoparasitism. *PLOS ONE* 6:e21206.

Misof B., Liu S., Meusemann K., Peters R.S., Donath A., Mayer C., Frandsen P.B., Ware J., Flouri T., Beutel R.G., Niehuis O., Petersen M., Izquierdo-Carrasco F., Wappler T., Rust J., Aberer A.J., Aspöck U., Aspöck, H., Barte., D., Blanke A., Berger S., Böhm A., Buckley T.R., Calcott B., Chen J., Friedrich F., Fukui M., Fujita M., Breve C., Grobe P., Gu S., Huang Y., Jermiin L.S., Kawahara A.Y. Krogmann L., Kubiak M., Lanfear R., Letsch H., Li Y., Li Z., Li J., lu H., Machida R., Mashimo Y., Kapli P., McKenna D.D., Meng G., Nakagaki Y., Navarrete-Heredia J.L., Ott M., Ou Y., Pass G., Podsiadlowski L, Pohl H., von Reumont B.M., Schütte K., Sekiya K., Shimizu S., Slipinski A., Stamatakis A., Song W., Su X., Szucsich N.U., Tan M., Tan X., Tang M., Tang J Timelthaler G., Tomizuka S., Trautwein M., Tong X., Uchifune T., Walzl M.G., Wiegmann B.M., Wilbrandt J., Wipfler B., Wong T.K.F., Wu Q., Wu G., Xie Y., Yang S., Yang Q., Yeates DK., Yoshizawa K., Zhang Q., Zhang R., Zhang W., Zhang Y., Zhao J., Zhou C., Zhou L., Ziesmann T., Zou S., Li Y., Xu X., Zhang Y., Yang H., Wang J., Wang J., Kjer K.M., Zhou X. 2014. Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346: 763–767.

Niehuis, O., Hartig, G., Grath, S., Pohl, H., Lehmann, J., Tafer, H., Donath, A., Krauss, V., Eisenhardt, C., Hertel, J., Petersen, M., Mayer, C., Meusemann, K., Peters, R.S., Stadler, P.F., Beutel, R.G., Bornberg-Bauer, E., McKenna, D.D., and Misof, B. 2012. Genomic and morphological evidence converge to resolve the enigma of Strepsiptera. *Current Biology* 22: 1309–1313.

O'Connor, B.A. 1959. The coconut treehopper, *Sexava* spp. and its parasites in the Madang district. *Papua New Guinea Agricultural Journal* 11: 121–125.

Pardi L. 1946. Ricerche sui Polistini. VI. La "dominazione" e il ciclo ovarico annuale in *Polistes gallicus* (L.). *Bollettino dell'Instituto di Entomologia dell'Università di Bologna* 15: 25–84.

Pierce, D.W. 1909. A monographic revision of the twisted winged insects comprising the order Strepsiptera Kirby. *Smithsonian Institution National Museum Bulletin* 66: i–xii, 1–232.

Pierce, D.W. 1964. The Strepsitpera are a true order, unrelated to Coleoptera. *Annales of the Entomological Society of America* 57: 603–605.

Pix, W., Nalbach, G., and Zeil, J. 1993. Strepsipteran forewings are halter-like organs of equilibrium. *Naturwissenschaften* 80: 371–374.

Pix, W., Zanker, J.M., and Zeil, J. 2000. The optimotor response and spatial resolution of the visual system in male *Xenos vesparum* (Strepsiptera). *The Journal of Experimental Biology* 203: 3397–3409.

Pohl, H. and Beutel, R.G. 2005. The phylogeny of Strepsiptera (Hexapoda). *Cladistics* 21: 328–374.

Pohl, H. and Beutel, R.G. 2008. The evolution of Strepsiptera (Hexapoda). *Zoology* 111: 318–338.

Pohl, H. and Beutel, R.G. 2013. The Strepsiptera-Odyssey: the history of the systematic placement of an enigmatic parasitic order. *Entomologia* 1: e4.

Rossi, P. 1793. Observations sur un nouveau genre d'Insecte, voisin des Ichneumons. *Bulletin de la Société Philomathique de Paris* 1: 49.

Smith F. 1859. A contribution to the history of *Stylops*, with an enumeration of such species of exotic Hyenoptera as have been found to be attacked by those parasites. *Transactions of the Entomological Society of London* 5: 127–133.

Tolasch, T., Kehl, S., and Dötterl, S. 2012. First sex pheromone of the order Strepsiptera: (3*R*,5*R*,9*R*)-3,5,9-Trimethyldodecanal in *Stylops melittae* Kirby, 1802. *Journal of Chemical Ecology* 38: 1493–1503.

Vaurie, P. 1983. A catalog of the Coleoptera of America north of Mexico. Family: Curculionidae, Subfamily: Rhynchophorinae. *American Museum of Natural History*. New York, NY.

Wheeler, W.M. 1919. The phoresy of *Antherophagus*. *Psyche* 26: 145–152.

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D., and Wheeler, W.C. 1997. The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18s and 28S ribosomal DNA sequences and morphology. *Systematic Biology* 46: 1–68.

Whiting, M.F. and Wheeler, W.C. 1994. Insect homeotic transformation. *Nature* 368(6473): 696–696.

Young, G.R. 1987. Notes on the life history of *Stichotrema dallatorreanum* (Strepsitpera: Myrmecolacidae) a parasite of *Segestes decoratus* Redtenbacher (Orthoptera: Tettigoniidae) from Papua New Guinnea. *General Applied Entomology* 19: 57–64.

Chapter 2.

New Findings on Life History Traits of *Xenos peckii* (Strepsiptera: Xenidae)[1]

2.1. Abstract

We studied life history traits of *Xenos peckii* Kirby (Strepsiptera: Xenidae), a little-known parasite of the paper wasp *Polistes fuscatus* (Fabricus) (Hymenoptera: Vespidae) in North America. We field-collected 24 wasp nests in early July 2012, isolated parasitised wasps, tracked life history events of *X. peckii*, and recorded such behaviour as emergence of males and mating by normal-speed and high-speed cinematography. To emerge, males first cut the puparium with their mandibles along an ecdysial suture line, and then push aside the pupal cap during emergence. The endoparasitic females engage in active calling (pheromone release) behaviour by slowly inflating their cephalothorax, and then extruding it even farther out of, and tilting it away from, the host wasp abdomen. Seasonal and diel (afternoon) emergence periods of males coincide with seasonal and diel receptivity and calling periods of females. Males approach calling females in a swaying flight with smooth turns. They typically land on the anterior portion of the host wasp's abdomen, and then step backward until they make contact with the cephalothorax of the female. As soon as their mesothoracic legs contact the female's cephalothorax, they curl around it, and the male initiates mating. Thereafter, the female fully retreats and never re-mates.

[1] This chapter has been published in very similar form: Michael Hrabar, Adela Danci, Sean McCann, Paul W. Schaefer, and Gerhard Gries (2012) New findings on life history traits of *Xenos peckii* (Strepsiptera: Xenidae). The Canadian Entomologist 146: 512–527.

2.2. Introduction

Strepsiptera are a peculiar and poorly understood insect order. As obligate endoparasites of other insects, they exhibit a high degree of adaptive specialisation. Most notable is the extreme dimorphism between male and female adults. The grub-like females never leave their host (except for females of the basal group Menengillidae) (Kathirithamby 1989), whereas males undergo an additional instar and pupate (Kinzelbach 1971; Kathirithamby 2005), metamorphosing into winged adults that exit their host and search for mates (Fig. 2.1).

Strepsiptera have an unusual lifecycle, characterised by heteromorphosis (Reynolds 2013). On a host nest (Fig. 2.1a) the larvae (Fig. 2.1b) crawl out of their mother's brood canal (also termed ventral canal) through which she was inseminated the previous season (Beani *et al*. 2005), and quickly seek new host larvae into which they burrow. Once inside their host, the first instar larvae moult into a grub-like immotile form. They then develop through three successive instars within the abdomen of their host, growing in size (Manfredini *et al.* 2007) (Fig. 2.1c), but not inhibiting their host's metamorphosis (Fig. 2.1d). They remain endoparasitic until a few days after eclosion of the adult host wasp, at which time the fourth instar larvae partially extrude (arrows in Fig. 2.1e) between two abdominal sclerites, forcing their way through the wasp's inter-segmental membrane (Hughes *et al*. 2004). It is at this point that the male and female life cycles diverge.

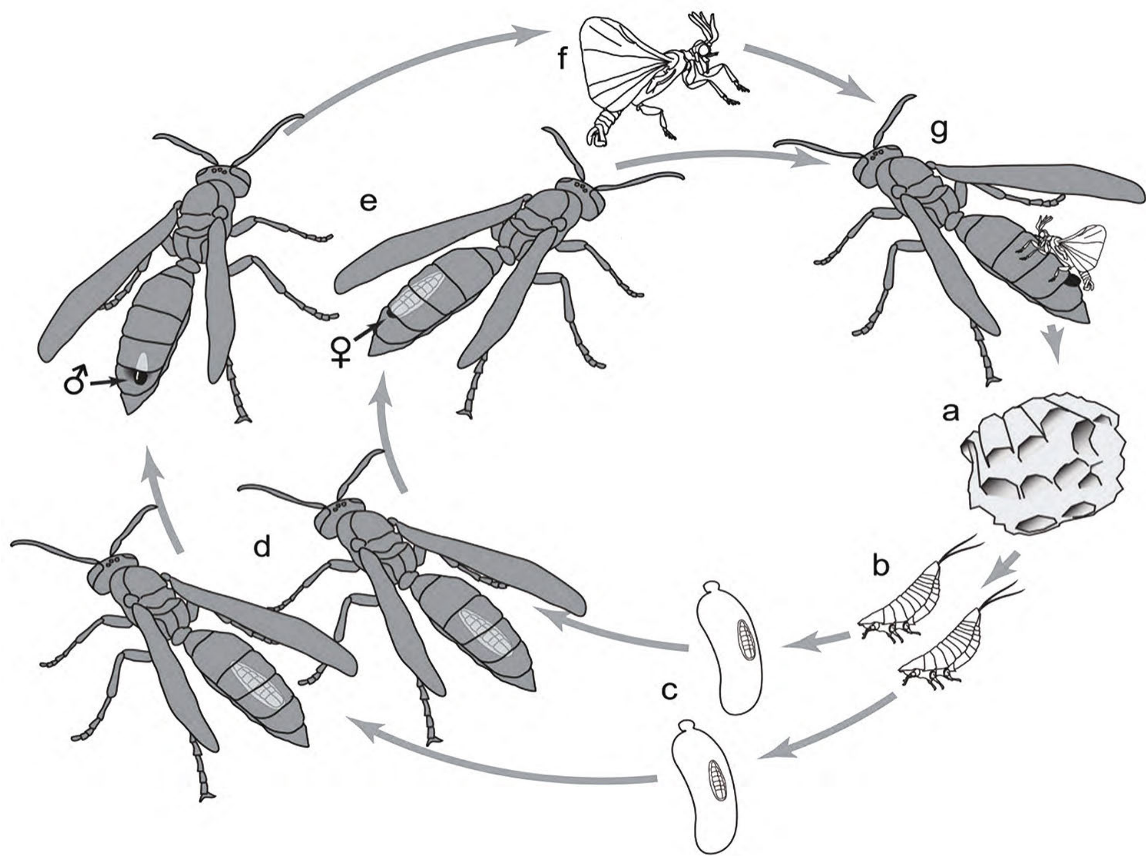

Figure 2-1. Illustration of the life cycle of Xenos peckii
(a) nest of host wasp *Polistes fuscatus* gets infested with (b) first-instar *X. peckii* that actively seek and burrow into (c) host wasp larvae where they moult into an apodous, grub-like, second instar and develop through three successive instars within the larvae of the host wasp; (d) adult wasps eclose with *X. peckii* larvae concealed within their abdomen; (e) male and female fourth instar larvae extrude from the abdomen of their host wasp; (e, left) the extruded structure of the male sclerotises and forms the cephalotheca (cap of the puparium); (e, right) the extruded structure of the female sclerotises to form the cephalothorax (fused head and prothorax), resulting in a neotenic adult female; (f–g) after a 10- to 15-day pupation period, the winged male (f) emerges, locates a receptive female (g), and mates. Note: drawings not to scale; drawing of first instar larvae adapted from a SEM in Osswald *et al*. (2010).

Females extrude their head and prothorax which sclerotises, fusing into a structure termed cephalothorax (Nassonow 1892). Once the cephalothorax has sclerotised, females undergo no further development and are considered neotenic adults, retaining larval features (Muir 1906). Adult females exhibit “specialisation by reduction” (Bohart 1941), lacking eyes, mouthparts, antennae, legs, wings, and even external genitalia. Once extruded, they are thought to remain motionless, release pheromone that attracts males,

overwinter as adults in the wasp's abdomen, and release their motile larvae the following spring (Schrader 1924).

Males, like females, also extrude their head and prothorax; their development differs in that they undergo an additional moult and pupation after they have extruded (Beani *et al.* 2005). The sclerotised portion comprising the shell of the previous larval instars serves as a puparium (Kinzelbach 1971; Kathirithamby 2005). The anterior (extruded) portion of a male's puparium is referred to as a cephalotheca (Kathirithamby 1983; Kathirithamby *et al*. 2010). Male pupation occurs in two stages, whereby a male sheds his pupal skin while still encased in his puparium. As an eclosed "pre-adult", the male remains within the puparium until his cuticle hardens and his wings expand (Kathirithamby 2005). As a consequence, the male is capable of immediate flight upon his emergence from the puparium (Fig. 2.1f), and quickly seeks females for mating (Fig. 2.1g). The winged adult males are short-lived (Kathirithamby 1989), adding to the challenge of locating a receptive female in a mobile host wasp.

Xenos peckii Kirby (Strepsiptera: Xenidae) is a parasite of the paper wasp *Polistes fuscatus* (Fabricius) (Hymenoptera: Vespidae). Many life history traits of *X. peckii* remain unknown, controversial, or hardly described. Our objectives were to investigate: (*i*) timing of sexual maturity in female and male *X. peckii*, (*ii*) the role of females during sexual communication and diel periodicity of communication; (*iii*) the emergence process of males and diel periodicity of emergence; and (*iv*) the mating sequence.

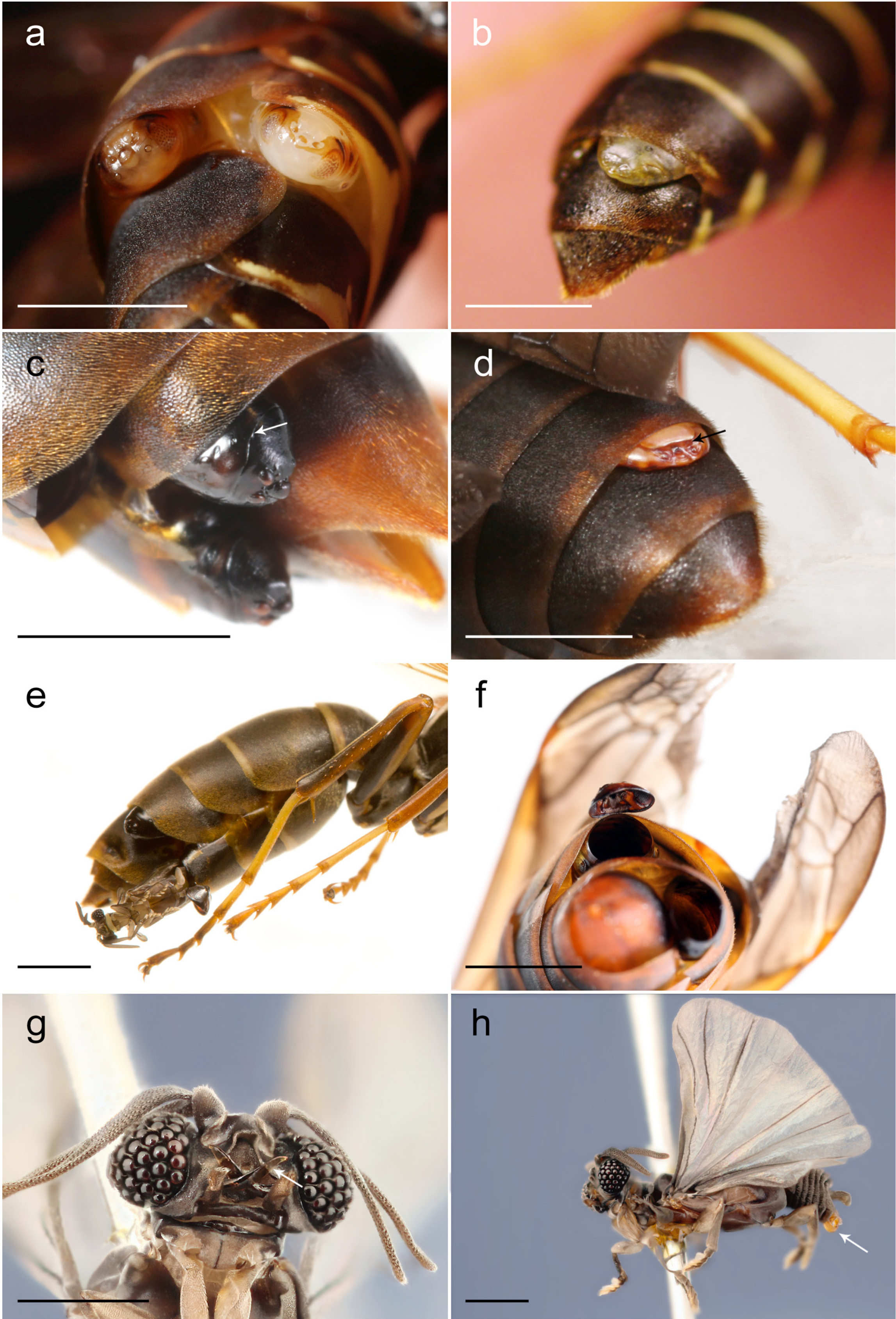
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Figure 2-2. Photographs of Strepsiptera life stages
(a) two male pupae 1.5 hours (left) or 0.5 hours (right) after extrusion; (b) an adult neotenic female (right) with incomplete sclerotisation 10 minutes after extrusion and her brood canal still closed, and (left) a partially sclerotised female 1.5–2.0 hours after extrusion; (c) two 14-day-old male pupae each with an adult male ready to emerge (arrow points to the ecdysial suture line); (d) a fully extruded, 20-day-old virgin female with her brood canal (arrow) open, photographed at 08:30 hours while she was not posturing (see Fig. 2.4); (e) a male *X. peckii* in the process of emergence from a pinned wasp; (f) a puparium cap (cephalotheca) still hinged to the puparium (left), and an empty puparium with the cephalotheca brushed off (right); (g) a male's head revealing well-developed mandibles (arrow); (h) an adult male *Xenos peckii* (arrow points to the hooked adeagus). Scale bars a–f = 2.0 mm; g, h = 500 µm.

2.3. Methods

2.3.1. Collection of wasp nests

Active nests of *Polistes fuscatus* were collected in and around New Canaan, Maine, United States of America, during the first week of July 2012. Each nest, together with at least three adult resident female wasps tending larvae, was placed in a Rubbermaid 1.2 L disposable freezer container (Newell Rubbermaid, Winchester, Virginia, United States of America) with screen top (32-mesh, Saran Fabric, Synthetic Industries, Lumite Division, Norcross, Georgia, United States of America) for transportation to Canada. The nests were ultimately housed in the Global Forest Quarantine Facility at Simon Fraser University (Burnaby, British Columbia, Canada), where they were hot-glued into Plexiglas cages (24 cm long × 14 cm wide × 22 cm high) with a sliding front door for access and a 32-mesh back. Cages were kept at 20–30 ºC, 40–60% relative humidity, and a 16 light: 8 dark photoperiod. Wasps were provisioned with water delivered in cotton wicks (1 × 5 cm; Richmond Dental, Charlotte, North Carolina, United States of America), honey, and early instar larvae of wax moth (*Galleria mellonella* Linnaeus; Lepidoptera: Pyralidae) *ad libitum*. Voucher specimens of *P. fuscatus* and *X. peckii* have been deposited in the Spencer Entomological Collection at the Beaty Biodiversity Museum (University of British Columbia, Vancouver, British Columbia, Canada).

2.3.2. Timing of sexual maturity in females and males

Each wasp nest was examined daily. We marked the pronotum of each newly emerged wasp with a dot(s) using an oil-based paint pen (Sharpie®; Newell Rubbermaid, Illinois, United States of America), colour encoding the day of eclosion. In addition, we recorded the day a neotenic female or a male pupa of *X. peckii* appeared between the abdominal sclerites of a host wasp. Such parasitised wasps were isolated singly in a small Plexiglas cage (9 cm long × 6 cm wide × 9 cm high), and provided with a honey-water solution, allowing us to determine the day female *X. peckii* initiated calling (see below), and males emerged.

2.3.3. Role of females during sexual communication and diel periodicity of communication

To determine whether female *X. peckii* engage in active calling and assume a particular body posture during calling, we inspected females at 0.5–1-hour intervals between 06:00 and 22:00 hours. The extent (0–100%) of calling by a female was estimated based on the repleteness of her cephalothorax, its degree of additional protrusion, and its angle to the host's abdominal surface. A parallel study with a separate set of females that aimed at the identification of the female sex pheromone indicated that the pheromone was most abundant when it was extracted at the time the females' cephalothorax was most replete and extruded farthest from the host, and that females only in this calling posture attracted males (M.H., personal observation).

Focus-stacked images of calling females and of adult males were obtained with a Canon 5D Mark II digital SLR camera (Canon USA Inc., Mellville, New York, United States of America) through a Mitutoyo M-Plan Apo long working distance microscope objective (Fig. 2.2g: 40-image composite, 5× objective, Numerical Aperture (NA) = 0.14, 20-µm step; Fig. 2.2h: 36-image composite, 2× objective, NA = 0.055, 50-µm step; Fig. 2.8: 60-image composite, 10×, NA = 0.28 microscope objective, 10-µm step (Mitutoyo Canada, Mississauga, Ontario, Canada). Step increments were controlled with a

StackShot^{TM} controller and stepper motor (Cognisys, Kingsley, Michigan, United States of America), custom fitted to a micrometer-driven linear translation stage. Image composites were processed using Zerene Stacker software, version 1.04. (Zerene Systems LLC, Richland, Washington, United States of America).

2.3.4. Emergence process of males and diel periodicity of emerging

With preliminary evidence for eclosion of males in early afternoon, we recorded the time of day that 47 males emerged from live single-caged host wasps at 0.5–1-h intervals between 07:00 to 17:00 hours.

The emergence process of males was video recorded at 3840 × 2160 resolution, 30 frames/s, global shutter, using a Blackmagic 4K production camera (Blackmagic Design, Fremont, California, United States of America). Wasps with one or more pupae each housing a ready-to-emerge male *X. peckii* were cold-sedated prior to removing the abdomen by snipping the pedicel with microscissors (Fine Science Tools, North Vancouver, British Columbia, Canada). The abdomen was then immobilised by embedding the pedicel in modelling clay (Flair Leisure Products PLC, Surrey, United Kingdom). Video recordings of the restrained abdomen were run continuously until the adult male *X. peckii* had completely emerged.

2.3.5. Mating sequence

The mating sequence was filmed by both real-time and high-speed videography. Real-time footage was obtained using a Sony HDR-XR550 camera (Sony of Canada Ltd., Toronto, Ontario, Canada), and recorded at 1920 × 1080 resolution, 60 frames/s, interlaced. High-speed video was captured using the Fastec imaging camera IN1000M2GB, equipped with Fastec imaging software version 3.0.4 (Fastec Imaging, San Diego, California, United States of America). Footage was obtained at 1000 frames/s, at 320 × 240 pixel resolution.

Wasp abdomens with a sexually mature virgin female *X. peckii* were removed and mounted as described above, inside a glass enclosure (20 × 12 × 12 cm), with both the head and end section covered by fabric mesh, allowing a gentle, desk fan-driven airflow through the enclosure to aid in directional pheromone dissemination. For each recording, a male was introduced into the down-wind section of the enclosure.

2.4. Results

2.4.1. Timing of sexual maturity of females and males

The period of male extrusion preceded the period of female extrusion, with no overlap between the late-extruding males and the early-extruding females in our sample (Fig. 2.3). Males (Fig. 2.2a) and females (Fig. 2.2b) extruded 3–7 days and 9–17 days, respectively, after eclosion of the host wasp. After the early extrusion of males an additional moult and pupation period of 10–15 days ensued (Fig. 2.3). As a result, adult, mate-searching males emerged at a time shortly after females had reached sexual maturity, 2–3 days after extrusion.

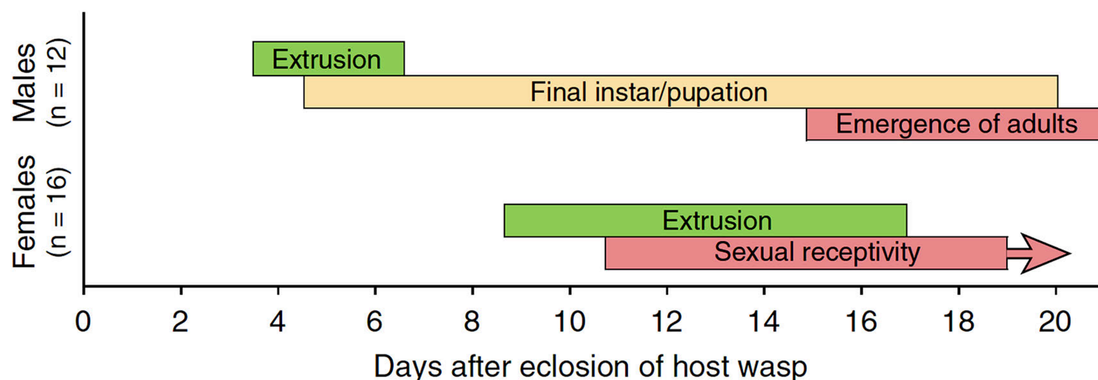

Figure 2-3. Timing of male and female sexual maturity
Days after eclosion of the host wasp at which male and female Xenos peckii extruded from their host, pupated and emerged (males), or attained sexual maturity and began posturing (females). One aberrant female that extruded after only two days following wasp eclosion is not depicted in this graph. Note: the emergence period of males overlaps with the onset of sexual receptivity in females.

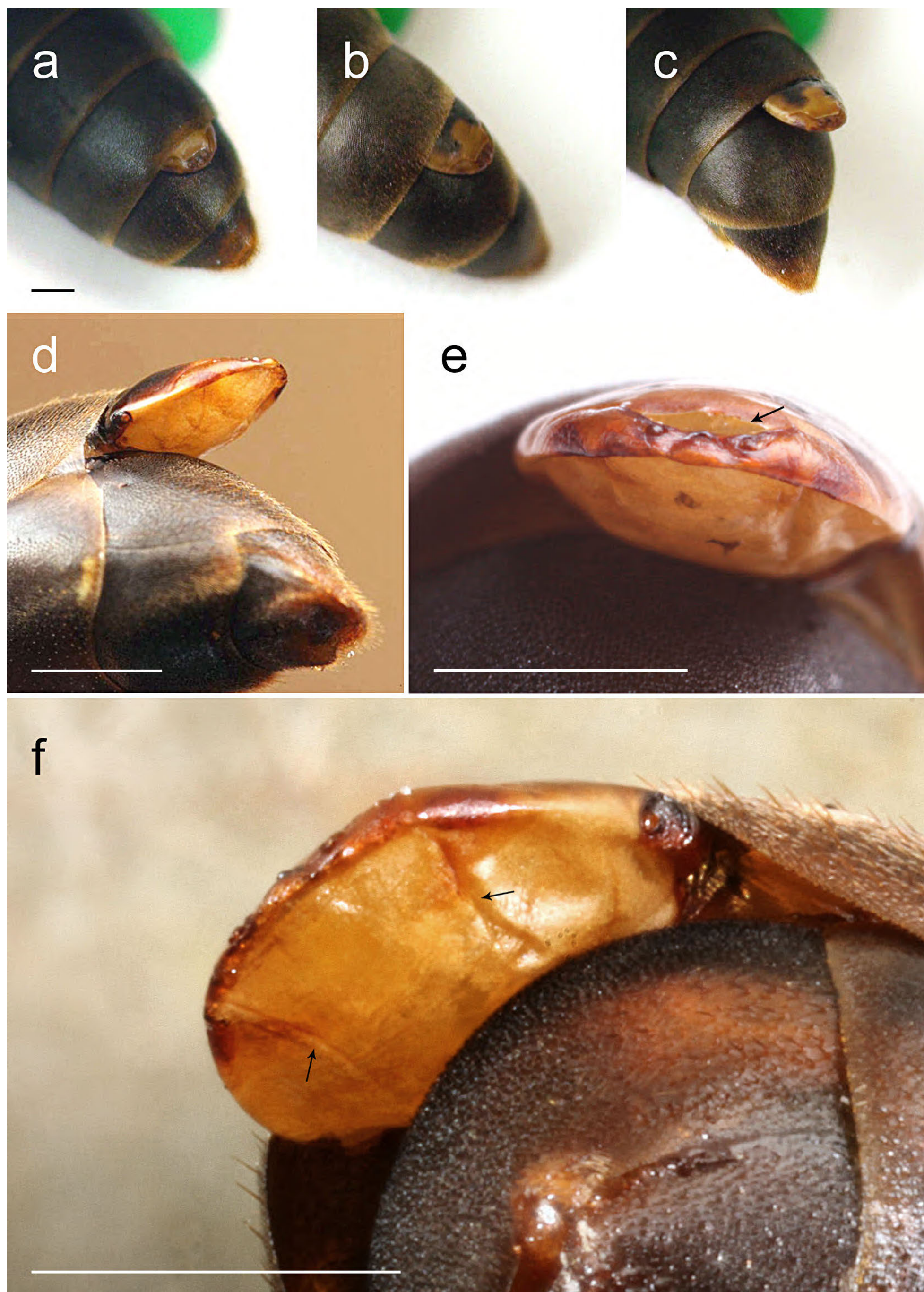

Figure 2-4. Superextrusion of *Xenos peckii* female

Representative photographs showing a female *Xenos peckii* in the process of assuming her calling (pheromone-release) posture. Calling is associated with a gradual inflation and a more rapid protrusion of the cephalothorax (see Results for details); (a–c) the female continues to inflate her cephalothorax and to protrude from the wasp's abdomen; (d) the female has assumed her final calling posture with her cephalothorax fully tilted away from the host's abdomen; (e) the cephalothorax is fully inflated, raising it off the wasp's abdomen (arrow points to the open brood canal); (f) underside of cephalothorax revealing fold lines (arrows) which allow inflation. Scale bars = 1.0 mm.

2.4.2. Role of females during sexual communication and diel periodicity of communication

Females engaged in active calling behaviour and assumed a particular calling position and posture. They initiated calling on the 3rd or 4th day following their extrusion from the host wasp and, if not mated, they continued to call for two to three weeks. Thereafter, they gradually reduced the extent and duration of calling, and ultimately ceased to call.

To call, females protruded their cephalothorax from the wasp's abdomen. Protrusion was mediated by, or accompanied with, inflation, "hyper/super-extrusion" (protruding farther out from the wasp), and tilting of the cephalothorax. Inflation typically occurred very slowly over the course of several minutes to more than an hour (Figs. 2.4d–f), and seemed to be facilitated by fold lines on the underside of the cephalothorax. Once the cephalothorax was nearly fully inflated (presumably by haemolymph pressure), females extended their cephalothorax in a comparatively more rapid motion occurring over seconds to minutes (Figs. 2.4a–c; supplemental video 2.1), and tilted their cephalothorax resulting in a gap between it and the wasp's abdominal surface (Figs. 2.4a–c; supplemental video 2.1).

Female calling behaviour followed a distinct diel periodicity (Fig. 2.5, top). It typically commenced between 10:00 and 10:30 hours, peaked between 13:00 and 14:00 hours, and gradually ceased during the late afternoon and early evening. Females exhibited slight variability in the time when they initiated calling and in how long it took them to reach the final calling posture. Even when multiple females occupied the same wasp, they varied in their onset and degree of calling.

2.4.3. Emergence process of males and diel periodicity of emergence

To emerge, males use their mandibles to cut open the puparium (Fig. 2.6), first piercing through it (Fig. 2.6a), and then cutting along a specific line (termed "ecdysial suture line" by Kathirithamby *et al.* (1990)) in a scissor-like fashion (Fig. 2.2c;

supplemental video 2.2), until the nearly severed cap (cephalotheca) just hinged to the puparium by a narrow thickening at the distal-most end. In our observations (n = 4), cutting always commenced at one side of the hinge point, proceeded radially around the ecdysial suture line, meeting the hinge point from the other side; however, the direction of cutting was not consistent clockwise or counter-clockwise between specimens. Once the cephalotheca was cut free, the male then pushed it open with his head (Fig. 2.6b; video 2.3), gradually emerging from the puparium ventral side out (Fig. 2.2e; Figs. 2.6c–e), and eventually rotated around his longitudinal body axis, bending ventrally to free himself (Fig. 2.6f). His wings were fully functional at this stage and he took flight as soon as he was free of the puparium. The cephalotheca (Fig. 2.2f) was eventually brushed off by the host wasp.

Similar to the calling cycle of virgin females, male emergence followed a diel periodicity. Most males emerged between 11:00 and 15:00 hours (Fig. 2.5, bottom), with peak emergence time between approximately 13:00 and 14:00 hours.

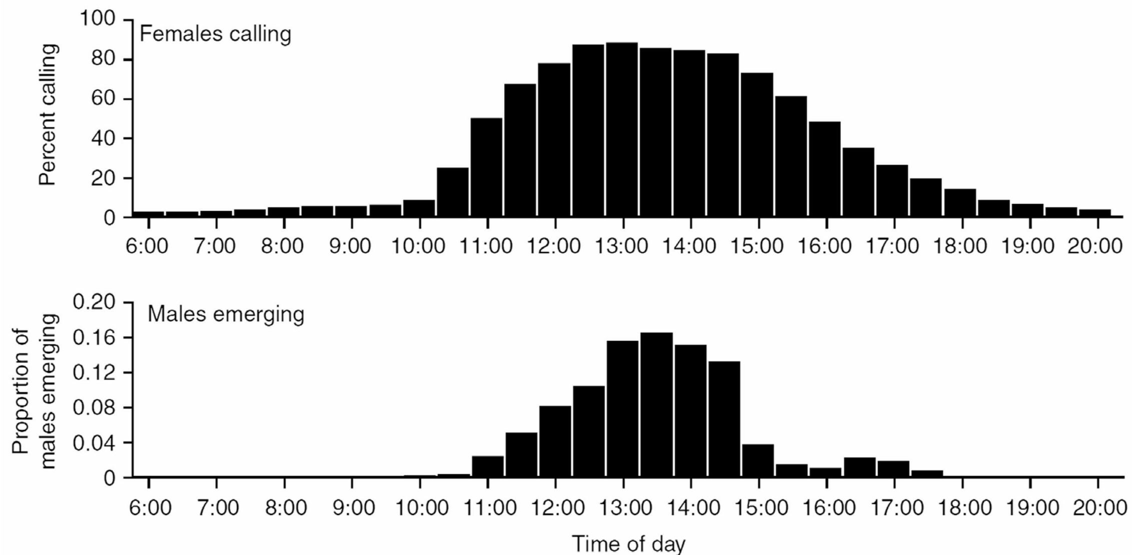

Figure 2-5. **Synchrony of female posturing with male emergence**
Comparison of (top) diel pheromone release (calling) periods of female *Xenos peckii* (final calling position assigned a value of 100%) (n = 5 averaged over 21 days) and (bottom) diel emergence period of conspecific males (n = 47).

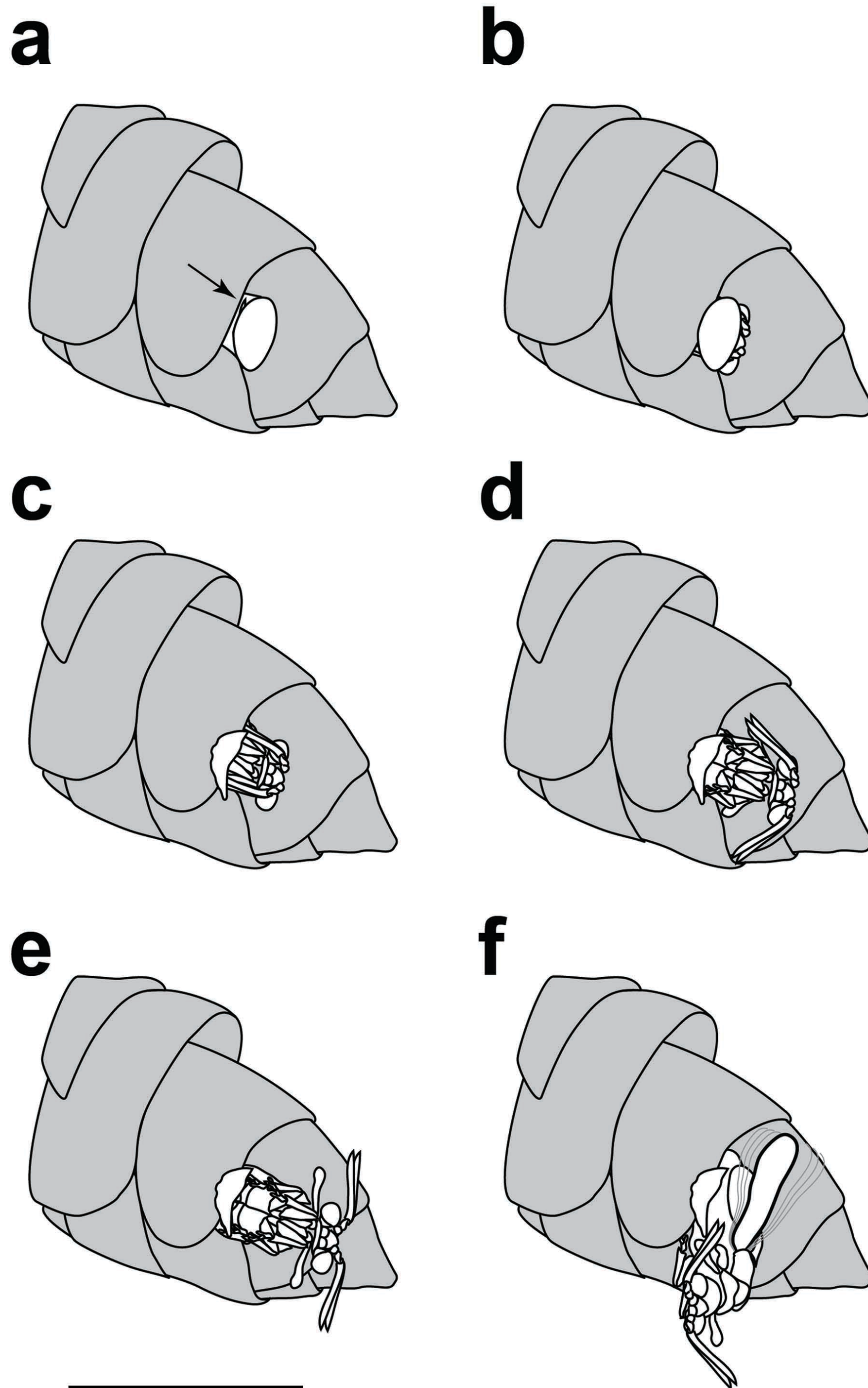

Figure 2-6. Emergence process of a male *Xenos peckii*
(a) a mandible pierces through the distal section of the puparium (arrow); (b) the nearly severed puparium cap (cephalotheca) hinges to puparium while the male uses his head to push open the cap; (c–e) the cap is completely pushed aside as the male gradually emerges from the puparium; (f) the male rotates around his longitudinal body axis, bends ventrally, and immediately takes flight in search for females. Scale bar = 2.0 mm.

2.4.4. Mating sequence

All *X. peckii* males that we observed in the laboratory ($n = 6$) approached calling females in a swaying flight with smooth turns and engaged in a similar sequence of mating behaviour. The sequence in Fig. 2.7, which was redrawn from a high-speed video recording (supplemental video 2.4), may serve as a representative example: The male approached a *P. fuscatus* host wasp in response to a calling conspecific female (Fig. 2.7a), landed on the anterior section of the wasp's abdomen (Fig. 2.7b), and then stepped backward (Figs. 2.7c–d) until he made contact with the female's cephalothorax (Fig. 2.7e). As soon as his mesothoracic legs touched the female's cephalothorax, they grasped it tightly (Fig. 2.7e). He then immediately curled his abdomen ventrally (Fig. 2.7f) and inserted his aedeagus into the female's brood canal (Fig. 2.7g). After a brief copulation (5 seconds), he detached from the female (Fig. 2.7h) and flew away. After copulation, the female immediately retreated back between the wasp's abdominal sclerites leaving only the very tip of her cephalothorax extruded and assuming the same position she exhibited during non-calling hours. Mated females remained in this retreated position, never to call again.

2.5. Discussion

Our study adds to the understanding of life history traits of *X. peckii*. Specifically, we reveal (*i*) synchrony of sexual maturity in males and females, (*ii*) active calling of females during sexual communication, (*iii*) emergence of males by cutting open the puparium with their mandibles; (*iv*) synchrony of female calling and male emergence periods; and (*v*) intricate details of the mating system. Below we will discuss our findings in the context of the current literature.

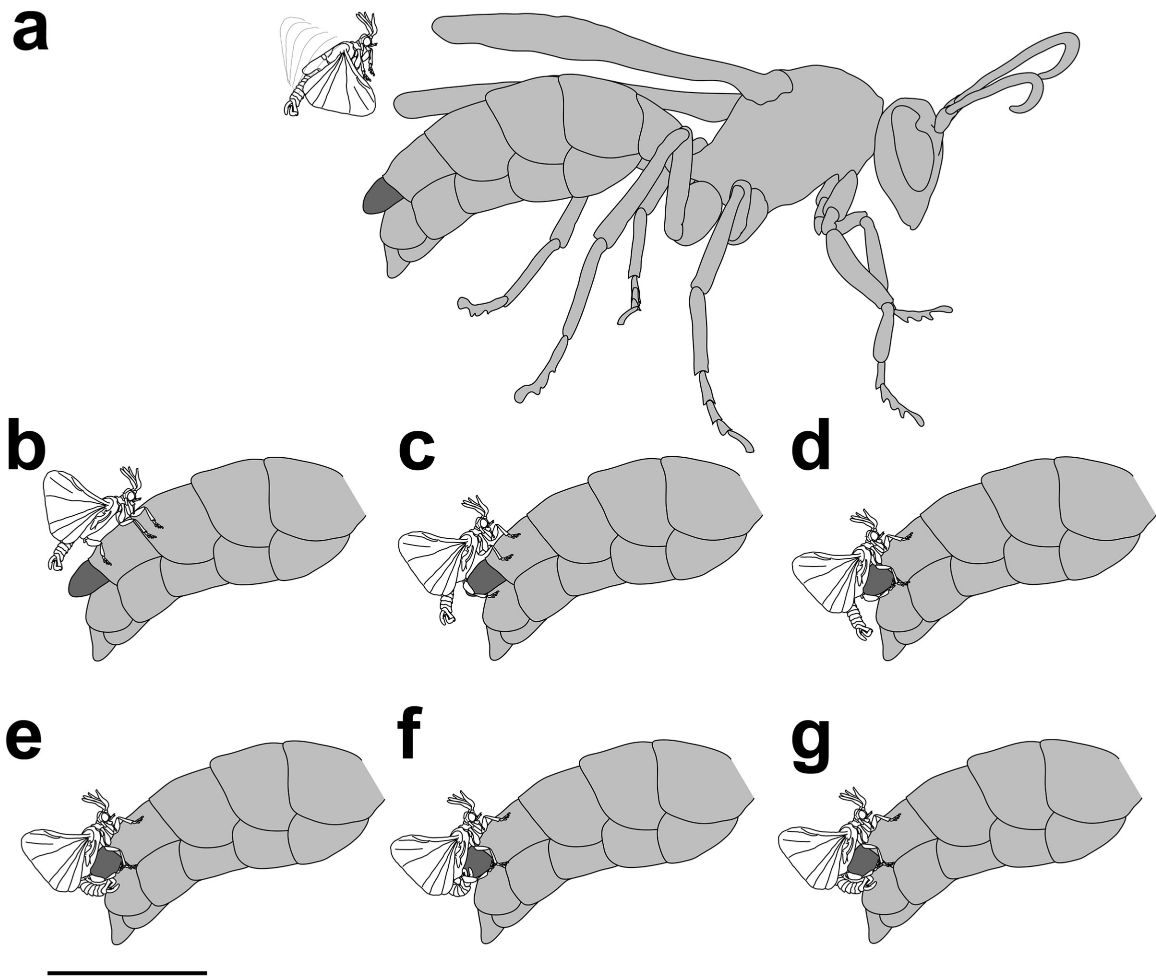

Figure 2-7. Mating sequence of *Xenos peckii*
(a) a flying male approaches a *Polistes fuscatus* host wasp in response to a calling conspecific female (arrow) protruded between abdominal sclerites of the wasp's abdomen; (b–c) he lands on the anterior section of the wasp's abdomen and steps backward; (d) his hindlegs make contact with the female's cephalothorax and curl around it; (e) as his mesothoracic legs touch the female's cephalothorax, they curl around it, and he immediately begins to bend his abdomen ventrally; (f) his abdomen is fully curved and copulation is about to commence; (g) 2-second copulation; (h) the male detaches from the female and is about to fly off. Scale bar = 5.0 mm.

We predicted sexual maturity of male and female *X. peckii* to be highly synchronised because emergent winged Strepsiptera males are typically short-lived and have merely 4–6 hours to find a female (Kathirithamby 1989). Male *X. peckii* in our study lived only 3–4 hours, leaving little time to locate and mate with a receptive female. Even though the period of male extrusion from the wasp abdomen preceded the period of female extrusion, with no overlap between late-extruding males and early-extruding

females in our sample (Fig. 2.3), sexually mature virgin females were available immediately when males emerged. This was the case because males entered a pupation period, and thus had a longer developmental time than the neotenic females that were sexually receptive shortly after extrusion; thus, despite their disparate developmental times, males and females reached sexual maturity in synchrony.

Our data support the conclusion that *X. peckii* females play an active role during sexual communication. Females engaged in active calling behaviour, assumed a particular calling position and posture (Fig. 2.4a–c; supplemental video 2.1), and followed a distinct diel periodicity of calling (Fig. 2.5, top). Early literature had considered female Strepsiptera immobile and largely passive participants in the sexual communication and mating process, their primary role in life being simply to provide an environment for the maturation of their ova. Females had been described as “a great sack full of eggs . . . (which have) lost all instinct” (Pierce 1909, p. 13), where the “helpless female must lie motionless in the body of its host with only the cephalothorax protruding and await the coming of the active, nervous male” (Pierce 1909, p. 45). Females are still considered to be inactive (Kathirithamby *et al*. 1990; Kathirithamby & Hamilton 1992), and to be immobile once fully extruded and sclerotized (Pohl and Beutel 2008). Similarly, studies on the sexual communication and sex pheromones of Strepsiptera do not report any evidence for an active role by females (Lauterbach 1954; Kinzelbach 1971; Dallai *et al.* 2004; Cvačka *et al*. 2012; Tolasch *et al.* 2012). Yet, many insects including nocturnal moths exhibit distinct periodicity of sexual communication and they assume a particular body posture or position during “calling” or pheromone release, which may either serve as a visual cue to a searching male (Ringo 1996) or aid in pheromone dissemination (McNeil 1991). Our data suggest that *X. peckii* females too engage in active and temporally discrete calling behaviour. Whether this is a widespread phenomenon in Strepsiptera is yet to be investigated but some information already implies that it may occur in species other than *X. peckii*. Studying mating of female *X. vesparum* (Rossi) in *Polistes dominula* (Christ) host wasps, Beani *et al*. (2005) noted the “cephalothorax fully extruded from the tergite”, and Waloff (1981) and Hans Henderickx

(pers. comm.) noted that a female *Halictophagus silewoodensis* Waloff "heaved" her cephalothorax towards the male during his attempts to inseminate her.

Video recordings of the emergence process revealed that males use their mandibles to cut open the puparium, nearly severing the entire cephalotheca (Fig. 2.2c; supplemental video 2.2). In studies with other Strepsiptera, males are reported to simply push off the pupal cap with their head (Williams 1957; Kinzelbach 1967), or with a ptilinum-like structure (Kathirithamby 1983), or to cut open the puparium with their mandibles (Kathirithamby 1989, and references therein). The discrepancy of accounts in the literature suggests that specific taxa may differ in the mode of male emergence, perhaps depending on the shape and structure of the mandibles (Kathirithamby, 1983). We show that *X. peckii* males use their sickle-shaped mandibles to systematically cut all the way around the ecdysial suture line of the puparium and then push the cephalotheca aside with their head during the emergence process.

Emergent, sexually mature males are faced with a mate-finding challenge that is unparalleled in the Insecta. Within the few hours of their short life, males must locate a receptive female, which is largely concealed within a mobile and defensive host wasp. The co-occurrence of the males' emergence period (Fig. 2.5, bottom) and the females' calling period (Fig. 2.5, top) may be an adaptation that mitigates this challenge. This does not exclude other potential adaptations including acute vision and olfaction of mate-seeking males (Strohm 1910; Rösch 1913; Wachmann 1972; Bushbeck *et al*. 1999, 2003; Beutel *et al.* 2005; Srdjan *et al.* 2007), the males' extraordinary flight ability and apparatus (Kinzelbach 1971; Pohl and Beutel 2008), as well as behavioural manipulation of the host wasp (Salt 1927, 1931, and references therein; Hughes *et al.* 2004; Beani 2006; Dapporto *et al.* 2007; Beani *et al.* 2011).

In light of reports (Brues 1905; Bohart 1941; Kinzelbach 1971; Beani *et al.* 2005, and references therein; PWS, personal observation) that male *Xenos* species in general, and male *X. peckii* in particular (Hubbard 1892; Schrader 1924), emerge early in the morning (but see Kathirithamby and Hughes 2006), we expected *X. peckii* males in this

study to emerge and mate in the morning. That males instead emerged, and females called, in the afternoon (Fig. 2.5) was surprising. The variation in diel emergence times may simply be due to phenotypic plasticity, or it may indicate the presence of cryptic species that may differ in their sex pheromone or the periodicity of sexual communication. For example, populations of the gall midge *Dasineura oxycoccana* Johnson (Diptera: Cecidomyiidae) on cranberry and blueberry were recently found to be cryptic species that look identical but produce and respond to different sex pheromone (Fitzpatrick *et al*. 2013). Recent molecular studies lend evidence to suggest that, due to a paucity of morphological features, cryptic lineages may be common in the Strepsiptera (Kathirithamby 2009; Hayward *et al*. 2011, and references therein; Nakase and Kato 2013).

The mating sequence of *X. peckii* appears stereotypic and proceeded in the following way each time we filmed it (Fig. 2.7): after landing on the anterior section of the wasp's abdomen, the male stepped backward until he made contact with the female's cephalothorax. A sensory patch on his mesothoracic legs (Dubitzky 2001; Pohl and Beutel 2004; Henderickx, 2008) (Fig. 2.8) seems to mediate sensory recognition of the female, because as soon as his mesothoracic legs touched the female's cephalothorax, they grasped it tightly, and he initiated copulation through the opening of the female's brood canal. Copulation through the brood canal was also observed in a mating study with *X. vesparum* (Beani *et al.* 2005). After copulation, female *X. peckii* immediately and fully retreated, assumed a non-calling position, and never re-mated. Similarly, recently mated female *X. vesparum* are ignored by conspecific males (Dallai *et al.* 2004).

Other direct observations of mating in Strepsiptera are rare. However, Schrader (1924) provide an early and most detailed account of copulation in *Xenos* species. She noted that the male maintains an erect position on the host wasp abdomen. Facing forward, and keeping his wings in constant vibration, the male recurves his abdomen ventrally, pushes his aedeagus downward and forward, and then inserts it into the female's brood chamber. The entire mating sequence, including the time from alighting on the host wasp, inseminating the female, and detaching from her, took 20–50 seconds

to complete. According to Schrader (1924), males mated only females that had extruded four or five days prior.

Figure 2-8. Mesothoracic leg of an adult male *Xenos peckii*
Note microtrichia (mt) and sensory patch (sp) on tarsomers. Scale bar = 250 µm.

In conclusion, our study expands the knowledge of life history traits of *X. peckii*. Many other aspects, however, remain to be studied in *X. peckii* and in the entire Strepsiptera to fully appreciate their phylogeny, the selection of and interaction with their host, and the complexity of their sexual communication system as well as the sensory receptors involved.

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2.7. References

Beani, L. 2006. Crazy wasps: when parasites manipulate the *Polistes* phenotype. Annales Zoologici Fennici, 43: 546–574.

Beani, L., Dallai, R., Mercati, D., Guisti, F., Cappa, and Manfredini, F. 2011. When a parasite breaks all the rules of a colony: morphology and fate of wasps infected by a strepsipteran endoparasite. Animal Behaviour, 82: 1305–1312.

Beani, L. Guisti, F., Mercati, D., Lupetti, P., Paccagnini, E., Turillazzi, S., and Dallai, R. 2005. Mating of *Xenos vesparum* (Rossi) (Strepsiptera, Insecta) revisited. Journal of Morphology, 265: 291–303.

Beutel, H.G., Pohl, H., and Hünfeld, F. 2005. Strepsipteran brains and effects of miniaturization (Insecta). Arthropod Structure & Development, 34: 305–313.

Bohart, R.M. 1941. A revision of the Strepsiptera with special reference to the species of North America. University of California Publications in Entomology, 7: 91–160.

Brues, C.T. 1905. Notes on the life history of the Stylopidae. Biological Bulletin, 8: 290–295.

Bushbeck, E., Ehmer, B., and Hoy, R. 1999. Chunk versus point sampling: visual imaging in a small insect. Science, 286: 1178–1180.

Bushbeck, E., Ehmer, B., and Hoy, R. 2003. The unusual visual system of the Strepsiptera: external eye and neuropils. Journal of Comparative Physiology A–Neuroethology, Sensory Neural, and Behavioral Physiology, 189: 617–630.

Cvačka, J., Jiroš, P., Kalinová, B., Straka, J., Černá, K., Šebesta, P., *et al*. 2012. Stylopsal: the first identified female-produced sex pheromone of Strepsiptera. Journal of Chemical Ecology, 38: 1483–1491.

Dallai, R., Lupetti, P., Giusti, F., Mercati, E., Paccagnini, E., Turillazzi, S., *et al*. 2004. Fine structure of the Nassonow's gland in the neotenic endoparasite of female *Xenos vesparum* (Rossi) (Strepsiptera, Insecta). Tissue and Cell, 36: 211–220.

Dapporto, L., Cini, A., Palagi, M., Simonti, A., and Turillazzi, S. 2007. Behaviour and chemical signature of pre-hibernating females of *Polistes dominulus* infected by the strepsipteran *Xenos vesparum*. Parasitology, 134: 545–552.

Dubitzky, A. 2001. Die äußere Morphologie von *Xenos vesparum* im REM (Strepsiptera, Stylopidae). Mitteilungen der Münchner Entomologischen Gesellschaft, 91: 71–76.

Fitzpatrick, S.M., Gries, R., Khaskin, G., Peach, D.A.H., Iwanski, J., and Gries, G. 2013. Populations of the gall midge *Dasineura oxycoccana* on cranberry and blueberry produce and respond to different sex pheromones. Journal of Chemical Ecology, 39: 37–49.

Hayward, A., McMahon, D.P., and Kathirithamby, J. 2011. Cryptic diversity and female host specificity in a parasitoid where the sexes utilize hosts from separate orders. Molecular Ecology, 20: 1508–1528.

Henderickx, H. 2008. Faunistische Bemerkingen over Strepsiptera met onderzoek van een populatie *Halictophagus silwoodensis* (Halictopagidae) in het Nationaal Park Hoge Kempen (Maasmechelen). Phegea, 36: 103–107.

Hubbard, H.G. 1892. The life history of *Xenos.* The Canadian Entomologist, 24: 257–262.

Hughes, H.G., Kathirithamby, J., Turillazzi, S., and Beani, L. 2004. Social wasps desert the colony and aggregate outside if parasitized: parasite manipulation? Behavioral Ecology, 6: 1037–1043.

Kathirithamby, J. 1983. The mode of emergence of the adult male *Elenchus tenuicornis* (Kirby) (Strepsiptera, Elenchidae) from its puparium. Zoological Journal of the Linnean Society, 77: 97–102.

Kathirithamby, J. 1989. Review of the order Strepsiptera. Systematic Entomology, 14: 41–92.

Kathirithamby, J. 2005. Further homage to Santa Rosalia: discovery at last of the elusive females of a species of Myrmecolacidae (Strepsiptera:Insecta). *In* Narrow roads of gene land: collected papers by W. D. Hamilton, Vol. III. *Edited by* M. Ridley. Oxford University Press, New York, United States of America. Pp. 117–134.

Kathirithamby, J. 2009. Host-parasitoid associations in Strepsiptera. Annual Review of Entomology, 54: 227–249.

Kathirithamby, J., Carcuypino, M., and Mazzini, M. 1990. Ovarian structure in the order Strepsiptera. Frustula Entomologica, 13: 1–8.

Kathirithamby, J. and Hamilton, W.D. 1992. More covert sex: the elusive females of Myrmecolacidae. Trends in Ecology & Evolution, 7: 349–351.

Kathirithamby, J., Hayward, A., McMahon, D.P., Ferreira, R.S., Andreazze, R., Almeida Andrade, H.T.D., and Fresneau, D. 2010. Conspecifics of a heterotrophic heteronomous species of Strepsiptera (Insecta) are matched by molecular characterization. Systematic Entomology, 35: 234–242.

Kathirithamby, J. and Hughes, D. 2006. Description and biological notes of the first species of *Xenos* (Strepsiptera: Stylopidae) parasitic in *Polistes carnifex* F. (Hymenoptera: Vespidae) in Mexico. Zootaxa, 1104: 35–45.

Kathirithamby, J., Luke, B.M., and Neville, C. 1990. The ultrastructure of the preformed ecdysial 'line of weakness' in the puparium cap of *Elenchus tenuicornis* (Kirby) (Insecta: Strepsiptera). Zoological Journal of the Linnean Society, 98: 229–236.

Kinzelbach, R.K. 1967. Zur Kopfmorphologie der Fächerflügler (Strepsiptera, Insecta). Zoologische Jahrbücher (Anatomie), 84: 559–684.

Kinzelbach, R.K. 1971. Comparative morphology of the Strepsiptera and its phylogenetic interpretation (Insecta, Strepsiptera). Zoologica (Stuttgart), 41 (119 parts 1 and 2): 1–256.

Lauterbach, G. 1954. Begattung und Larvengeburt bei den Strepsipteren. Zugleich ein Beitrag zur Anatomie der Stylops-Weibchen. Zeitschrift für Parasitenkunde, 16: 255–297.

Manfredini, F., Guisti, F., Beani, L., and Dallai, L. 2007. Developmental strategy of the endoparasite *Xenos vesparum* (Strepsiptera: Insecta): invasion into the host and elusion of its defense reaction. Journal of Morphology, 268: 588–601.

McNeil, J.N. 1991. Behavioral ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. Annual Review of Entomology, 36: 407–430.

Muir, F. 1906. Notes on some Fijian insects. Honolulu Experiment Station Hawaiian Sugar Planters' Association. Division of Entomology. Bulletin, 2: 1–11.

Nakase, Y. and Kato, M. 2013. Cryptic diversity and host specificity in giant *Xenos* strepsipterans parasitic in large *Vespa* hornets. Zoological Science, 30: 331–336.

Nassonow, N.V. 1892. Development of *Xenos rossii* (systematic position of the group). Congress of Zoology, 1: 174–184.

Osswald, J., Pohl, H., and Beutel, R.G. 2010. Extremely miniaturized and highly complex: the thoracic morphology of the first instar larva of *Mengenilla chbouti* (Insecta, Strepsiptera). Arthropod Structure and Development, 39: 287–304.

Pierce, W.D. 1909. A monographic revision of the twisted winged insects comprising the order Strepsiptera Kirby. Bulletin of the United States National Museum, 66: 1–232.

Pohl, H. and Beutel, G. 2004. Fine structure of adhesive devices of Strepsiptera (Insecta). Arthropod Structure & Development, 33: 31–43.

Pohl, H. and Beutel, G. 2008. The evolution of Strepsiptera. Zoology, 111: 318–338.

Reynolds, S. 2013. Postembryonic development. *In* The insects: structure and function, 5th ed. *Edited by*. S. J. Simpson and A. E. Douglas. Cambridge University Press, Cambridge. Pp. 298–454.

Ringo, J. 1996. Sexual receptivity in insects. Annual Review of Entomology, 41: 473–494.

Rösch, P. 1913. Beiträge zur Kenntnis der Entwicklungsgeschichte der Strepsipteren. Jenaische Zeitschrift für Naturwissenchaften, 50: 97–146.

Salt, G. 1927. The effects of stylopization on Aculeate Hymenoptera. Journal of Experimental Zoology, 48: 223–331.

Salt, G. 1931. A further study of the effects of stylopization. Journal of Experimental Zoology, 59: 133–166.

Schrader, S.H. 1924. Reproduction of *Achroschismus wheeleri* Pierce. Journal of Morphology and Physiology, 39: 157–197.

Srdjan, M., Layne, J.E., and Bushbeck, E.K. 2007. Behavioral evidence for within-eyelet resolution in twisted-winged insects (Strepsiptera). Journal of Experimental Biology, 210: 2819–2828.

Strohm, K. 1910. Die zusammengesetzten Augen der Männchen von *Xenos rossi.* Zoologischer Anzeiger, 36: 156–159.

Tolasch, T., Kehl, S., and Dötterl. 2012. First sex pheromone of the order Strepsiptera: (*3R,5R,9R*)-3,5,9-trimethyldodecanal in *Stylops melittae* Kirby, 1802. Journal of Chemical Ecology, 38: 1493–1503.

Wachmann, E. 1972. Zum Feinbau des Komplexauges von *Stylops* spec. (Insecta, Strepsiptera). Zeitschrift für Zellforschung und Mikroskopische Anatomie, 123: 411–424.

Waloff, N. 1981. The life history and descriptions of *Halictophagus silwoodensis* sp. n. (Strepsiptera) and its host *Ulopa reticulata* (Cicadellidae) in Britain. Systematic Entomology, 6: 103–113.

Williams, J.R. 1957. The sugar-cane Delphacidae and their natural enemies in Mauritius. Transactions of the Royal Entomological Society of London, 109: 65–110.

Chapter 3.

(7*E*,11*E*)-3,5,9,11-Tetramethyltridecadienal: sex pheromone of the strepsipteran *Xenos peckii* [2]

3.1. Abstract

Xenos peckii is a strepsipteran parasitoid of the common North American paper wasp *Polistes fuscatus*. Mate-seeking *X. peckii* males respond to a long-range sex pheromone from the female that remains permanently embedded within the abdomen of a mobile host wasp. During peak pheromone signalling, we excised the female from her host, severed the cephalothorax containing the pheromone gland, extracted it in hexane, and analyzed aliquots of combined extracts by coupled gas chromatographic-electroantennographic detection (GC-EAD). These analyses revealed a candidate pheromone component (CPC) that consistently elicited strong responses from male antennae. We identified CPC as (7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal based on its (*i*) retention indices (RI) on three GC-columns, (*ii*) RI inter-column differentials, (*iii*) mass and NMR spectra, and (*iv*) the synthesis of an authentic standard that matched the GC-retention and spectrometric characteristics of CPC. For a field experiment, we prepared 3,5,9*R*,11-tetramethyltridecadienal [(*R*)-9] and 3,5,9*S*,11-tetramethyltridecadienal [(*S*)-9)], baited traps with (*R*)-9, (*S*)-9 or both, and attracted *X. peckii* males to all three treatments, but not to unbaited control traps. The sex pheromone of *X. peckii* resembles that reported for the strepsipterans *Stylops mellittae* and *S. muelleri*

[2] A very similar version of this chapter was under review by the Journal of Chemical Ecology. Minor revisions have been invited and a revised manuscript has been re-submitted: Michael Hrabar, Huimin Zhai, Regine Gries, Paul W. Schaefer, Jason Draper, Robert Britton, and Gerhard Gries (2015) (7*E*,11*E*)-3,5,9,11-Tetramethyltridecadienal: sex pheromone of the strepsipteraen *Xenos peckii*

[(*R*,*R*,*R*)-3,5,9-trimethyldodecanal], suggesting a common biosynthetic pathway across taxonomic genera.

3.2. Introduction

Xenos peckii (*S*trepsiptera: Xenidae) is an obligate endoparasitoid of the North American paper wasp *Polistes fuscatus,* and of at least seven other species of paper wasps (Krombein and Hurd, 1979). Male and female *Xenos peckii* exhibit extreme dimorphism. The male ecloses as a free-living albeit short-lived adult (Fig. 3.1c), whereas the female undergoes extreme neoteny (Fig. 3.1a,b), maintaining her larval form throughout adulthood, and never leaving her host wasp.

In contrast to the holometabolous male, the simple "cigar shaped" body plan of the female (Fig. 3.1a) may be viewed as an extraordinary adaptation for housing thousands of ova from which free living first instar larvae hatch and then emerge from their mother the following spring. This simple body plan and its extreme specialization have inspired the moniker "viviparous reproductive machine" (Kathirithamby, 2009), emphasizing the female's primary function as a repository for eggs.

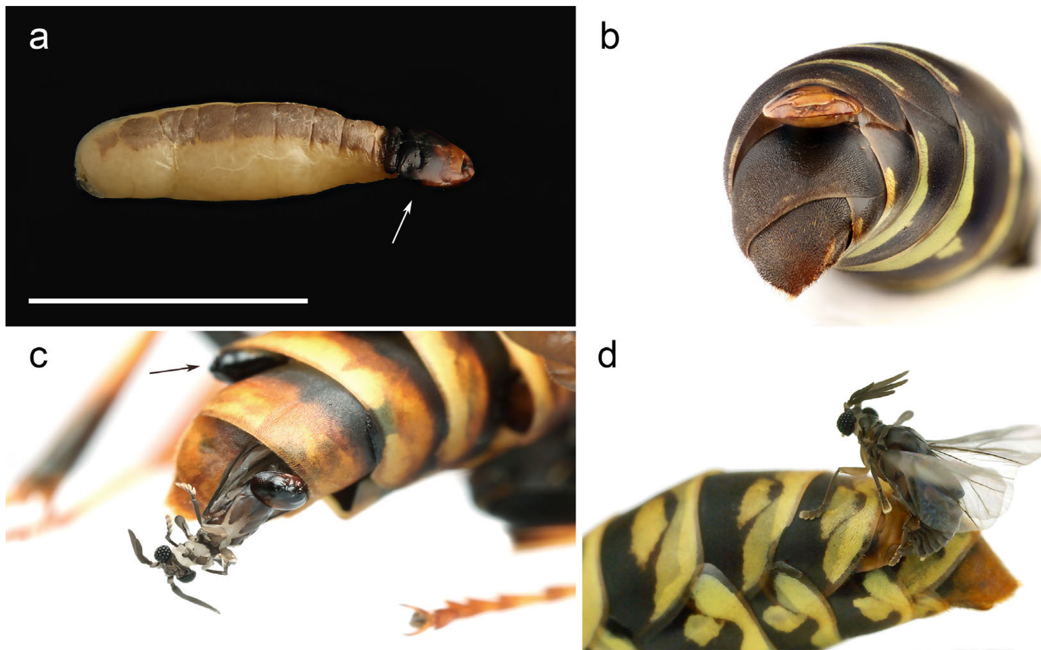

Figure 3-1. Images of male and female *Xenos peckii*
(a) a *Xenos peckii* female (with cephalothorax marked by arrow) excised from her paper wasp host; (b) a *X. peckii* female super-extruding her cephalothorax from the abdomen of her paper wasp host during peak calling activity; (c) a *X. peckii* male eclosing from a puparium lodged between abdominal sclerites of a paper wasp host (note a second puparium marked by arrow); and (d) a *X. peckii* male mating with a female through the brood canal of her cephalothorax. Scalebar (a – d) = 5 mm.

Mate-seeking *X. peckii* males respond to a long-range sex pheromone emitted by the female that remains permanently embedded within the abdomen of a mobile host wasp (Fig. 3.1b). The diel periodicity of calling behavior by females coincides with the diel emergence period of males (Hrabar *et al*., 2014). The receptive *X. peckii* female extrudes her cephalothorax (head and first thoracic segment) (Fig. 3.1b), and disseminates a potent sex pheromone that attracts free-living males (Fig. 3.1d; Supplementary Video) that must find her within their extremely short lifespan of 1 to 3 hours.

Little is known about the molecular structure of sex pheromones of the Strepsiptera. In 2010, Tolasch and colleagues reported the identification, synthesis, and field-testing of the *Xenos vesparum* (Xenidae) pheromone at the annual meeting of the International Society of Chemical Ecology in Tours (France) but the authors neither revealed the pheromone structure nor subsequently published their results. More recently,

Tolasch *et al*. (2012) identified the sex pheromone of the bee parasitoid *Stylops mellittae* (Strepsiptera: Stylopidae) as (*R*,*R*,*R*)-3,5,9-trimethyldodecanal and Cvačka *et al*. (2012) reported the sex pheromone of *Styplops muelleri* as (9*R*)-3,5-*syn*-3,5,9-trimethyldodecanal which was later shown to also have the (*R,R,R*)-configuration. As the *Stylops* parasitoids investigated in these studies not only came from the same host bee (*Andrena vaga*) but also produced the same absolute configuration of the same pheromone, it is conceivable that the reported two *Stylops* species are indeed synonomous.

Here we report the identification and field-testing of the sex pheromone of *X. peckii.*

3.3. Methods and materials

3.3.1. Collection and maintenance of experimental insects

During the first week of July 2012, we field-collected 28 nests of *P. fuscatus* in and around Canaan, Maine, USA. For transportation to Canada under permit (P-2012-02204) from the Canadian Food Inspection Agency, we placed each nest together with at least three larvae-tending female wasps into separate disposable 1.2-L Rubbermaid freezer containers (Newell Rubbermaid, Winchester, VA, USA) fitted with a screen top (32-mesh, Saran Fabric, Synthetic Industries, Lumite Division, Norcross, GA, USA). In the Global Forest Quarantine Facility at Simon Fraser University (SFU, Burnaby, BC, Canada), we removed nests from the Rubbermaid containers, and hot-glued the nest-bearing pedicel of each nest to the ceiling of a Plexiglas cage (24 cm long × 14 cm wide × 22 cm high) with a sliding front door for access and a screen back (32-mesh, Saran Fabric, Synthetic Industries, Lumite Division, Norcross, GA, USA) for air circulation. We kept Plexiglas cages at 20–30 °C, a 40–60 % RH, and a 16L:8D photoperiod. We provisioned adult wasps with water-soaked cotton wicks (1 × 5 cm; Richmond Dental, Charlotte, NC, USA) and honey, and wasp larvae with early instar larvae of the noctuid

moth *Trichoplusia ni* brought to them by brood-tending adult wasps. We deposited voucher specimens of *P. fuscatus* and *X. peckii* in the Spencer Entomological Collection of the Beaty Biodiversity Museum (University of British Columbia, Vancouver, BC, Canada), and deposited *X. peckii* males captured in pheromone-baited traps in the departmental collection of Entomology and Applied Ecology at the University of Delaware (Newark, Delaware, USA).

3.3.2. Acquisition of volatiles

To survey for newly extruded *X. peckii*, we daily removed the wasps from their nests, placed them individually into Petri dishes, and examined them closely under flashlight illumination. New specimens within the first two to three hours post-extrusion from the host wasp are not fully sclerotized (Schraeder, 1924; Hrabar *et al*., 2014) and as such readily recognized. We separated host wasps with a newly extruded *X. peckii* female from their nests and kept them individually in smaller Plexiglas cages (14 cm long × 7 cm wide × 12 cm high) to prevent mating of the *X. peckii* female with a male from another host wasp.

3.3.3. Extraction of pheromone

During peak calling (posturing) activity four to six days post-extrusion (Hrabar *et al*., 2014) when females were fully extruded from their host wasps in the afternoon hours, we excised a total of 21 *X. peckii* females, severed the cephalothorax of each female, placed it in ~50 µL of HPLC-grade hexane (Fischer Scientific, Fair Lawn, New-Jersey, USA), and macerated it with a stainless steel rod to facilitate pheromone extraction from the Nassonov (pheromone) gland, located in the cephalothorax (Dallai *et al*., 2004). We combined the supernatant from several extracts, and evaporated it under a stream of nitrogen until 20 µl of extract = 1 female equivalent.

3.3.4. Coupled gas chromatographic–electroantennographic detection (GC-EAD) analysis of pheromone gland extracts

We analyzed aliquots of Nassonov gland extract by GC-EAD (Arn *et al*., 1975; Gries *et al*., 2002), using a Hewlett Packard 5890A gas chromatograph fitted with either a DB-5, DB-23, or DB-210 GC column (30 m × 0.25 or 0.32 mm ID) (J&W Scientific, Folsom, CA, USA), helium as carrier gas (35 cm s^{-1}), and the following temperature program: 50 °C (1 min), then 20 °C min^{-1} to 280 °C (20 min). For GC-EAD recordings, we placed a severed cephalothorax (with both antennae intact) into the opening of one glass capillary electrode (1.0 × 0.58 × 101 mm) (A-M Systems, Inc., Carlsborg, Washington, USA) filled with saline solution (Staddon and Everton, 1980), and one branch of one antenna with its tip removed by spring microscissors (Fine Science Tools Inc., North Vancouver, British Columbia, Canada) into the second capillary electrode.

Retention Indices (RI), GC-Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR) Spectroscopy of the Candidate Pheromone Component (CPC) We calculated the retention indices (Van den Dool and Kratz, 1963) of the CPC on each of three GC columns (DB-5, DB-23, DB-210; 30 m × 0.25 mm ID each), obtained a mass spectrum of the CPC using a Saturn 2000 Ion Trap (Varian Instrument, Millcreek Dr., Mississauga, ON, L5N-5M4: now subsidiary of Agilent Technologies Inc.) fitted with a DB-5 MS column (30 m × 0.25 mm ID), and isolated the CPC from five extracts with the greatest pheromone titre (150 to 300 ng per female for a total of 1.2 µg) by flash chromatography for analysis by nuclear magnetic resonance (NMR) spectroscopy. To isolate the CPC by flash chromatography, we used a glass column (14 cm long × 0.5 cm inner diameter) filled with silica gel (0.6 g), pre-rinsed it with pentane, and then eluted the pheromone extract with five consecutive rinses (2 ml each) of pentane/ether, with increasing proportions of ether [(1) 100:0; (2) 90:10; (3) 80:20; (4) 50:50; (5) 0:100]. We recorded the nuclear magnetic resonance (NMR) spectrum of the insect-produced and the synthetic CPC, and of other synthetic compounds, on a Bruker 400 (400 MHz), a Bruker 500 (500 MHz) or a Bruker 600 (600 MHz), using either deuterochloroform ($CDCl_3$) or deuterobenzene (C_6D_6, 99.96 atom % D) as the solvent. Signal positions (δ) are given in

parts per million from tetramethylsilane (δ 0) and are measured relative to the signal of the solvent (^{1}H NMR: $CDCl_3$: δ 7.26; ^{13}C NMR: $CDCl_3$: δ 77.0). Coupling constants (J values) are given in Hertz (Hz) and are reported to the nearest 0.1 Hz. ^{1}H NMR spectral data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; sept, septet; m, multiplet; app apparent; br broad), coupling constants, number of protons.

3.3.5. Syntheses

The details of all syntheses are described in the Supplementary Information.

3.3.6. Field testing of the synthetic candidate pheromone component

We ran a field trapping experiment in Canaan (Somerset County, Maine, USA) at four locations within 3 km of one another [replicates (reps.) 1–3: 1815 Hill Road, 44°45'08"N 69°32'34"W; rep. 4: 32 Henshaw Road, 44°44'48"N 69°32'26"W; reps. 5–7: 1833 Hill Road, 44°45'08"N 69°32'51"W; reps: 8–10: 29 Main Street, 44°45'50"N 69°35'07"W]. At all locations, we observed *P. fuscatus* nests on weathered wooden buildings. From two of the four locations (29 Main Street and 1815 Hill Road), we had previously (14–18 July, 2013) collected active *P. fuscatus* nests for rearing purposes in SFU's Quarantine Facility and had noticed parasitism of some wasps by *X. peckii.* We ran the field trapping experiment (n = 10; 14 August to 06 September 2014) using a randomized block design. We baited each trap with a gray sleeve stopper (The West Co., Lionville, PA, USA) impregnated with test chemical(s) in HPLC-grade hexane (300 µl). We had four randomly assigned treatments in each block: (1) (*R*)-9 (150 µg); (2) (*S*)-9 (150 µg); (3) (*R*)-9 (150 µg) + (*S*)-9 (150 µg); and (4) solvent control (300 µl). We prepared the delta-like traps from juice cartons (29.5 × 18.3 cm) (Elopak Co., Oslo, Norway), coated the inner surface with adhesive Tanglefoot™ (Tanglefoot Co., Grand Rapids, MI, USA; now owned by Scotts, Maryville OH, USA), and painted the outer surface green for uniformity (Schaefer *et al*., 2013). We suspended the four traps in each replicate 2.0–2.5 m above ground from the sides of buildings below overhanging eves, except for replicate 5 where we affixed traps to a beam on the inner wall of a large barn.

Given the diversity of trapping sites, inter-trap spacings within replicates varied from 2–6 m. Without replacing lures, we monitored trap captures at noon (12:00 h) at 1- to 10-day intervals, and on 23 and 24 August at 12:00 h and 17:00 h (both +/- 1 h). Trap catch data were too low to warrant any statistical analyses.

3.4. Results and discussion

GC-EAD analyses of Nassonov gland extract revealed one component (CPC in Fig. 3.2) that consistently elicited strong responses from male *X. peckii* antennae. Retention indices (RI) (Van den Dool and Kratz, 1963) of CPC (DB-5: 1712; DB-23: 2133; DB-210: 2070) and RI inter-column differentials (for description of the analytical technique see Gries et al. 2002, 2005) suggested that the CPC had an aldehyde functional group. The mass spectrum of the CPC (Fig. 3.3) indicated a molecular weight (MW) of *m*/*z* 250, four mass units lower than a saturated heptadecanal (MW *m*/*z* 254), indicating that the CPC had two double bonds (or less likely had two rings or an alkyne functionality). Moreover, the RIs of the CPC on each of the three GC columns were approximately 200 units lower than the RIs of heptadecanal (DB-5: 1925; DB-23: 2350; DB-210: 2270), implying that the CPC had four methyl branches, each branch accounting for about 50 RI units. Despite all the above information, we could not infer the position of the two double bonds and the four methyl branches, and thus isolated the CPC (~2 µg in combined extracts) for NMR spectroscopic analysis. Using normal phase silica flash chromatography with a pentane/ether gradient, the CPC eluted in the 10% ether fraction.

To elucidate the structure of CPC, we analyzed this fraction by ^{1}H NMR spectroscopy using a Bruker TCI CryoProbe. As indicated in Table 3.1, the ^{1}H NMR spectra recorded on the pheromone (600 MHz, C_6D_6, 10,000 scans) confirmed the presence of an aldehyde functionality (δ = 9.39 ppm) as well as two vinyl methyl groups (δ = 1.57 and 1.55 ppm). Notably, the vinyl methyl group that resonated at δ = 1.57 ppm at C13 (Table 1, Fig. 4) displayed a coupling constant of 6.7 Hz, indicating that this methyl group represents the terminus of the carbon chain. We assigned three additional

methyl groups to resonances at δ = 1.01, 0.81 and 0.75 ppm, all of which were doublets and consistent with three methyl branches on the carbon chain. Additional information regarding the CPC structure was gleaned from the analysis of a COSY spectrum, that permitted assignment of the three spin systems 1–3 depicted in Figure 3.4. Considering that there remained only a single vinyl methyl group to connect to the structure, and that the olefinic resonance at δ = 5.27 ppm coupled only with the terminal methyl resonance at δ = 1.57, the remaining vinyl methyl group was attached at C11, as indicated in the gross structure for the CPC (4 in Fig. 3.4). Unfortunately, the resonances of protons at C7 and C8 overlapped in the ^{1}H NMR spectrum in C_6D_6 and, as a consequence, we were unable to confidently assign the configuration of the $\Delta^{7,8}$ olefin. Owing to insufficient amounts of material, we were also unable to observe NOE correlations between H12 and protons at C10 or the methyl group C17. Consequently, we also did not assign the configuration of the $\Delta^{11,12}$ olefin.

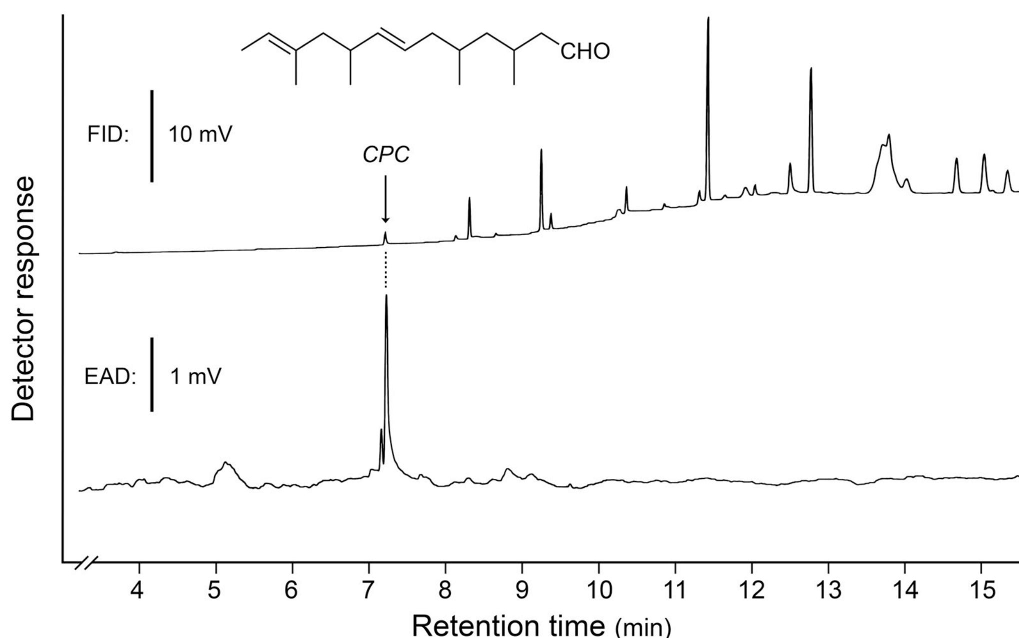

Figure 3-2. Coupled GC-FID & GC-EAD recordings
Representative (n = 6) recording of flame ionization detector (FID) and electroantennographic detector (EAD: male *Xenos peckii* antenna) responses to < 0.5 female equivalents (FE) of female *X. peckii* pheromone gland extract. Pheromone extract was obtained by (*i*) excising virgin females during peak calling activity (Fig. 3.1b) from their paper wasp host, (*ii*) severing the cephalothorax of each female (Fig. 3.1a, arrow), (*iii*) macerating it while submerged in hexane, and (*iv*) withdrawing the supernatant after 10 min of pheromone extraction at room temperature. The candidate pheromone component (CPC) was identified as (7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal. Chromatography: DB-5 column; splitless injection; temperature of injection port and FID: 240 °C; temperature program: 1 min at 50 °C, then 10 °C min^{-1} to 280 °C

In an attempt to simplify the stereochemical quandary, we analyzed the chemical shift of the methylene protons between the methyl branches at C3 and C5 using the empirical rules developed by Schmidt *et al*. (2012). As indicated in the inset of Figure 3.4, the difference in the chemical shift (Δδ)) of the methylene resonances should be <0.1 ppm if the adjacent methyl groups have a 1,3-*anti* relationship, and between 0.2–0.3 ppm if the methyl groups possess a 1,3-*syn* relationship. From the COSY spectrum recorded on the CPC, common correlations between H3 and H5 indicated that the methylene protons at C4 resonate at δ 0.82 and δ 1.08 ppm (Δδ = 0.26), consistent with a 1,3-*syn* relationship between the methyl branches at C3 and C5. It is notable that this 1,3-*syn* relationship is also consistent with the preponderance of examples of naturally occurring deoxypropionates (Hanessian *et al*., 2004).

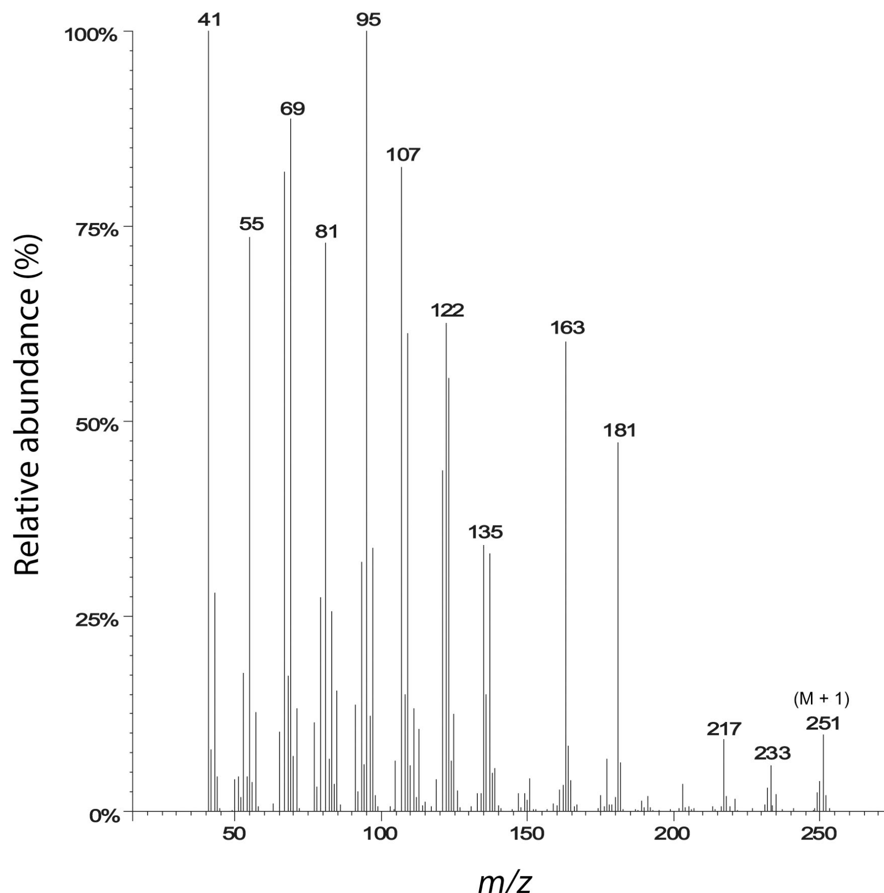

Figure 3-3. Ion trap mass spectrum of candidate pheromone component (7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal (See methods and materials for technical details.); note: Ion trap mass spectra of ketones and aldehydes commonly show a M+1 fragment ion.

In order to confirm and further refine the structure of CPC (4), we initiated synthetic campaigns aimed at addressing the configuration of the $\Delta^{7,8}$ and $\Delta^{11,12}$ olefin functions as well as the stereochemical relationship between the remote stereocenter at C9 and the 1,3-*syn*-deoxypropionate fragment (C3–C5). As summarized in Figure 3.5, starting with the known racemic iodoester 5 (Rodstein *et al*., 2009), reduction with DIBAL followed by protection of the resulting primary alcohol as the corresponding THP ether afforded the iodide 6 in good yield. A one-carbon homologation then involved displacement of the iodide with cyanide and sequential reductions with DIBAL and $NaBH_4$, affording the alcohol 7 in 77% overall yield. Finally, an Appel reaction (Appel

1975) gave the corresponding alkyl bromide, which was converted into the phosphonium salt 8 with concomitant removal of the THP protecting group. We prepared the racemic aldehyde 9 from tiglic acid following a recently reported procedure (Amorelli *et al*., 2015). Treatment of this aldehyde with the phosphorus ylide derived from the reaction of 8 with 2 equivalents of *n*-BuLi provided a 15:1 (*Z*:*E*) mixture of the dienols 10ab and 11ab (Fig. 3.5). Finally, oxidation of the primary alcohol function afforded the desired dienals 12ab and 13ab (Fig. 3.5). The retention time and mass spectrum of the minor compound each matched that of the insect-produced CPC in pheromone extracts.

As the dienals 12ab and 13ab proved difficult to separate by flash chromatography, we attained a sample of the dienol 11ab (*Z*:*E* ~ 1:1) by repetitive chromatography using $AgNO_3$-impregnated silica gel (20% $AgNO_3$) eluted with 1% EtOAc in hexane. Oxidation of this mixture of alcohols provided a ~1:1 mixture of the dienals 12ab and 13ab, from which the desired *E*-isomer 13ab could be purified by preparative HPLC. The ^{1}H NMR spectrum of 13ab was in close agreement with that of the insect-product CPC (see overlay, Fig. 3.6), as were their respective GC retention times (DB-5 MS) and mass spectra. Notably, the resonances in the ^{1}H NMR spectrum of 13ab assigned to the methyl groups at C13, C14, C15, and C17 were identical in both chemical shift and coupling patterns to those in the spectrum of insect-produced CPC. Likewise, the resonances assigned to the $\Delta^{7,8}$ olefinic protons (δ 5.35 ppm) in both the synthetic and natural material were identical. These data confirm the relative configuration of the C3–C5 deoxypropionate fragment as 1,3-*syn*, and allow configurational assignment of the olefin functions in the CPC (4) as 7*E* and 11*E*.

To gain further insight into the absolute configuration of the remote stereocenter at C9, we repeated the synthesis described in Figure 3.5 with enantiomerically enriched (~85% ee) samples of the aldehyde (*R*)-9 and (*S*)-9, each prepared using Evan's auxiliary (Evans *et al*., 1982) following established synthetic routes (Fotiadou *et al*., 2011).

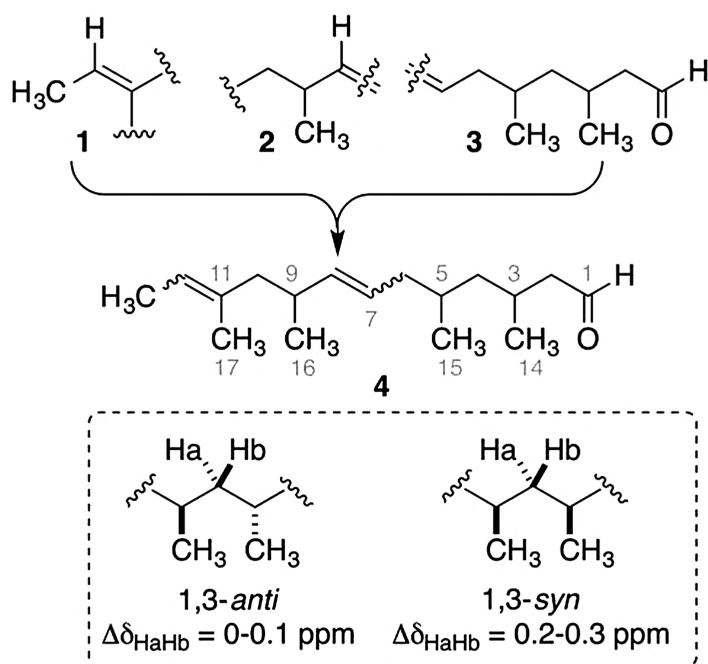

Figure 3-4. Fragments of CPC assigned by analysis of ^{1}H NMR and COSY spectra, and of the proposed structure 4.

In a field experiment, traps baited with (*R*)-9, (*S*)-9 or with both isomers captured four, three and nine males, respectively, whereas unbaited control traps had no captures. The 16 males were captured in eight different replicates, indicating that *X. peckii* populations were present in each of the four widely-spaced trap locations, or that males had been attracted over a long distance from elsewhere. When we recorded trap captures twice a day (23 & 24 August 2014), one male had been captured before 12:00 h and four males between 12:00 and 17:00 h, suggesting that males seek virgin females primarily in the afternoon during the warmest time of day. This conclusion is supported by laboratory observations of field-collected *P. fuscatus* nests with parasitized wasps, revealing that *X. peckii* males eclosed from puparia in the early afternoon coincident with the peak calling activity of virgin *X. peckii* females (Hrabar *et al*., 2014). Extracting the cephalothorax of a super-extruded, actively calling female (Hrabar *et al*., 2014), yielded 5–300 ng of the CPC, significantly more than from a non-calling female which had only trace amounts of the CPC.

With evidence that the *R,R,R*-isomer of 3,5,9-trimethyldodecanal as a single pheromone component effectively attracts males of *Stylops melittae* (Tolash *et al*., 2012) and (synonymous ?) *Stylops muelleri* (Cvačka *et al*., 2012; Lagoutte *et al*., 2013), we had predicted that only one isomer, (*R*)-9, would attract males of *X. peckii*. Therefore, captures of males in traps baited with (*R*)-9 or (*S*)-9 were surprising. In light of the moderate enantiomeric excess (85% ee) of each compound, it is conceivable that each isomer contained the pheromone component in an amount sufficient to attract males. That traps baited with both (*R*)-9 and (*S*)-9 captured twice as many males as traps baited with either (*R*)-9 or (*S*)-9 could not be shown statistically due to insufficient data, and may have been merely coincidental. Alternatively, *X. peckii* females may signal with two or more optical isomers of (7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal, termed here peckidienal, that may have additive attractive pheromonal properties.

The possibility that *X. peckii* females produce a two- or multiple-component pheromone blend is supported by evidence that antennae of *X. peckii* males respond to a second compound in female pheromone gland extracts (Fig. 3.2), which could be a diastereomer of (7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal. Additive or even synergistic effects of pheromone enantiomers have been reported in several insect taxa, including moths (e.g., Millar *et al*., 1990, 1991; Gries *et al*., 1999, 2003) and beetles (e.g., Borden *et al*., 1976, 1980; Payne *et al*., 1982; reviewed in Wood, 1982), the latter close phylogenetic relatives of the Strepsiptera (Boussau et al., 2014).

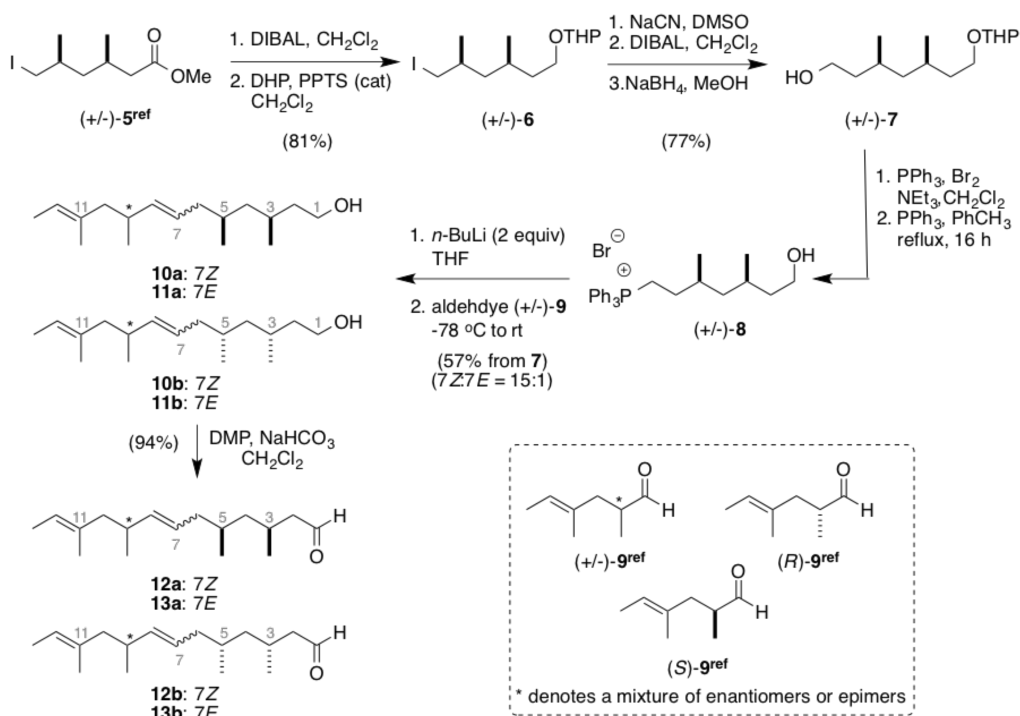

Figure 3-5. Scheme for the synthesis of the dienals 12ab and 13ab
Abbrevitions as follows: DIBALH: diisobutylaluminium hydride; DHP: 3,4-dihydro-2H-pyran; PPTS: pyridinium *p*-toluenesulfonate; DMSO: dimethyl sulfoxide, DMP: Dess-Martin periodinane; CH_2Cl_2: dichloromethane; NaCN: sodium cyanide; $NaBH_4$: sodium borohydride; PPh_3: triphenylphosphine; NEt_3: triethylamine; $PhCH_3$: toluene; $NaHCO_3$: sodium bicarbonate.

The molecular structures of the two strepsipteran pheromones identified in the genera *Stylops* (3,5,9-trimethyldodecanal) (Cvačka *et al*., 2012; Tolash *et al*., 2012; Lagoutte *et al*., 2013) and *Xenos* [(7*E*,11*E*)-3,5,9,11-tetramethyltridecadienal] (this study) bear resemblance, and possess the same methyl-branch patterns as some beetle pheromones (Francke and Dettner, 2005; Rodstein *et al*., 2011), and are likely biosynthesized involving propanoate and acetate units (Francke and Dettner, 2005; Tolash *et al*., 2012; Lagoutte *et al*., 2013).

While our present study reveals the basic molecular structure of the *X. peckii* pheromone, the absolute configuration of the pheromone, or that of several pheromone components, will be reported in a follow-up study.

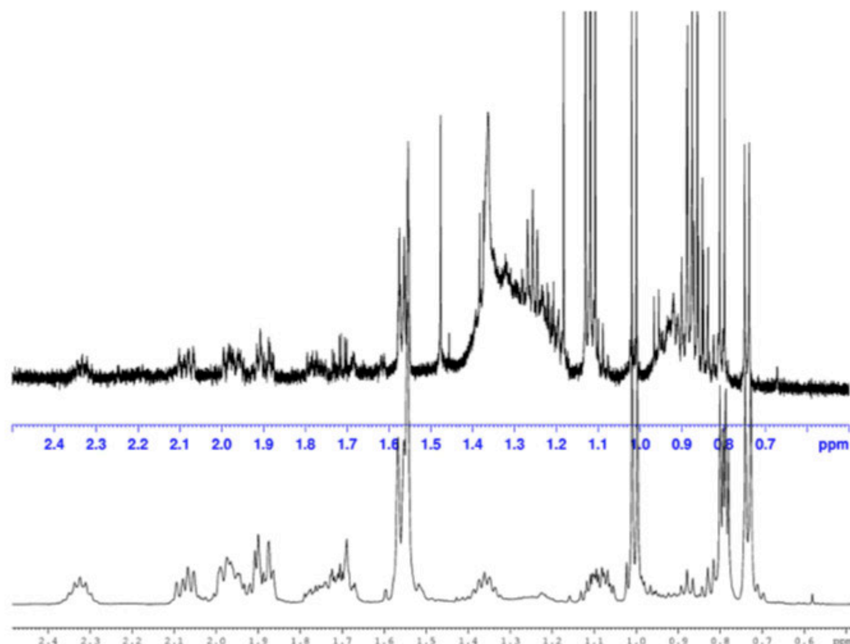

Figure 3-6. Overlay of an expanded region (0.6–2.5 ppm) of the ^{1}H NMR spectra (C_6D_6, 600MHz) recorded on CPC (4, top) and the synthetic dienal 13ab (bottom)

3.5. Acknowledgements

We thank Pilar Cepeda for assistance with rearing wax moths to feed wasps; Jim Henshaw, Dan Long, Dianne Pratt, Parker Nelson, Christine Payne, Ralph Payne, and Dan Ring for permission to collect wasp nests from their properties or to hang pheromone-baited traps; Stephen DeMuth for graphical illustrations; Laurence Packer for help in locating stylopised hymenoptera populations; Caesar Inducil and Anna Löppmann for help with field collections; Rodney Bushway, Francis Drummond, Eleanor Groden, and Tamara Levitsky (University of Maine) for providing laboratory space and logistical support. This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) – Discovery Grant to GG, and by an NSERC Discovery Grant and a MSFHR Career Investigator Award to RB.

3.6. References

Amorelli B, Arruda ME, Belko RP, Cai T, Closson AP, Giffin NL, Mertz GJ, Monteleone MG (2015) Novel organoleptic compounds. U.S. Pat. Appl. Publ. US 20150057207

Appel R (1975) Tertiary phosphane/tetrachloromethane, a versatile reagent for chlorination, dehydration, and P—N linkage. Angew Chem Int Ed Engl 14(12):801–811

Arn H, Städler E, Rausher, S (1975) The electroantennographic detector – a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. Z Naturforsch 30c:722–725

Borden JH, Chong L, McLean JA, Slessor KN, Mori K (1976) *Gnathotrichus sulcatus*: synergistic response to enantiomers of the aggregation pheromone sulcatol. Science 192(4242):894–896

Borden JH, Handley JR, McLean JA, Silverstein RM, Chong L, Slessor KN, Johnston BD, Shuler HR (1980) Enantiomer-based specificity in pheromone communication by two sympatric *Gnathotrichus* species (Coleoptera: Scolytidae). J Chem Ecol 6:445–456

Boussau B, Walton Z, Delgado JA, Collantes F, Beani L, Stewart IJ, Cameron SA, Whitfield JB, Johnston S, Holland PWH, Bachtrog D, Kathirithamby J, Huelsenbeck JP (2014) Strepsiptera, phylogeomics and the long branch attraction problem. *PLoS ONE*. 9(10) e107709

Cvačka J, Jiroš P, Kalinová B, Straka J, Černá K, Šebesta P, Tomčala A, Vašíčková K, Jahn U, Šobotník J (2012) Stylopsal: the first identified female-produced sex pheromone of Strepsiptera. J Chem Ecol 38:1483–1491

Dallai R, Lupetti P, Guisti F, Mercati D, Paccagnini E, Turillazzi S, Beani L, Kathirithamby J (2004) Fine structure of the Nassonow's gland in the neotenic endoparasitic female *Xenos vesparum* (Rossi) (Strepsiptera, Insecta). Tissue Cell 36:211–220

Evans DA, Ennis MD, Mathre DJ (1982) Asymmetric alkylation reactions of chiral imide enolates. A practical approach to the enantioselective synthesis of α-substituted carboxylic acid derivatives. J Am Chem Soc 104:1737–1739

Fotiadou AD, Zografos AL (2011) Accessing the structural diversity of pyridine alkaloids: concise total synthesis of rac-citridone A. Org Let 13:4592–4595

Francke W, Dettner K (2005) Chemical signalling in beetles. In: Schulz S (ed) The Chemistry of Pheromones and Other Semiochemicals II: Topics in Current Chemistry 240. Springer, Berlin, Heidelberg, New York, pp 85–166

Gries G, Gries R, Schaefer PW, Gotoh T, Higashiura Y (1999) Sex pheromone components of pink gypsy moth, *Lymantria mathura*. Naturwissenschaften 86:235–238

Gries R, Khashkin G, Gries G, Bennett RG, King S, Morewood P, Slessor KN, Morewood WD (2002) (*Z*,*Z*)-4,7-tridecadien-(*S*)-2-yl acetate: sex pheromone of douglas-fir cone gall midge *Contarinia oregonensis*. J Chem Ecol 28:2283–2297

Gries R, Khaskin G, Khaskin E, Foltz JL, Schaefer PW, Gries G (2003) Enantiomers of (*Z*,*Z*)-6,9-heneicosadien-11-ol: sex pheromone components of *Orgyia detrita.* J Chem Ecol 29:2201–2212

Gries R, Khaskin G, Schaefer PW, Hahn R, Gotoh T, Gries G (2005) (7*R*,8*S*)-*cis*-7,8-Epoxy-2-methyloctadec-17-ene: a novel trace component from the sex pheromone gland of gypsy moth, *Lymentria dispar*. J Chem Ecol 31:49–62

Hanessian S, Mascitti V, Giroux S (2004) Total synthesis of the cytotoxic cyclodepsipeptide (-)-doliculide: the ''ester'' effect in acyclic 1,3-induction of deoxypropionates. Proc Natl Acad Sci USA 101:11996–12001

Hrabar M, Danci A, McCann S, Schaefer P, Gries G (2014) New findings on life history traits of *Xenos peckii* (Strepsiptera, Xenidae). Can Entomol 146(5):512–527

Hubbard HG (1892) The life History of *Xenos.* Can Entomol 24:257–262

Kathirithamby J (1989) Review of the order Strepsiptera. Syst Entomol 14:41–92

Kathirithamby J (2009) Host-parasitoid associations in Strepsiptera. Ann Rev Entomol 54:227–249

Krombein KV, Hurd PD Jr. (1979) Catalog of Hymenoptera in America North of Mexico. Volume 2 Apocrita (Aculeata). Smithsonian Institution Press. Washington DC

Lagoutte R, Šebesta P, Jiroš P, Kalinová B, Jirošová A, Straka J, Černá K, Šobotník J, Cvačka J, Jahn U (2013). Total Synthesis, proof of absolute configuration, and biosynthetic origin of stylopsal, the first isolated sex pheromone of *Strepsiptera*. Chem Eur J 19:8515–8524

Millar JG, Giblin M, Barton D, Morrison A, Underhill EW (1990) Synthesis and field testing of enantiomers of 6*Z*,9*Z*-*cis*-epoxydienes as sex attractants for geometrid moths. J Chem Ecol 16:2317–2339

Millar JG, Giblin M, Barton D, Underhill EW (1991) Chiral lepidopteran sex attractants: Blends of optically active C20 and C21 diene epoxides as sex attractants for geometrid and noctuid moths (Lepidoptera). Environ Entomol 20:450–457

Payne TL, Richerson JV, Dickens JC, West JR, Mori K, Berisford CW, Hedden RL, Vité JP, Blum MS (1982) Southern pine beetle: olfactory receptor and behaviour discrimination of enantiomers of the attractant pheromone frontalin. J Chem Ecol 8:873–881

Reynolds S (2013) Postembryonic development. In: Simpson SJ, Douglas AE (eds) The insects: structure and function. 5th edition. Cambridge University Press, Cambridge, UK, pp 298–454

Rodstein J, Millar JG, Barbour J D, McElfresh JS, Wright IM, Barbour KS, Ray AM, Hanks LM (2011) Determination of the relative and absolute configurations of the female-produced sex pheromone of the cerambycid beetle *Prionus californicus*. J Chem Ecol 37:114–124

Schraeder SH (1924) Reproduction in *Achroschismus wheeleri* Pierce. J Morphol Physiol 39:157–205

Schaefer PW, Jiang M, Gries R, Gries G, Wu J (2013) Attraction of male *Lymantria schaeferi* Schintlmeister (Lepidoptera: Erebidae: Lymantriinae) to traps baited with (+)-xylinalure in Jiangxi Province, China. Life: The Excitement of Biology 1(2):95–99

Schmidt Y, Lehr K, Colas L, Breit B (2012) Assignment of relative configuration of desoxypropionates by ^{1}H NMR spectroscopy: method development, proof of principle by asymmetric total synthesis of xylarinic acid A and applications. Chem Eur J 18:7071–7081

Staddon BW, Everton IJ (1980) Haemolymph of milkweed bug. *Oncopeltus fasciatus* (Heteroptera: Lygaeidae): inorganic constituents and amino acids. Comp Biochem Physiol 65A:371–374

Tolasch T, Kehl S, Dötterl S (2010) Sex pheromone of a twisted-wing parasite. Lecture on the 26th Annual Meeting of the ISCE, August 2nd 2010, Tours, France

Tolasch T, Kehl S, Dötterl S (2012) First sex pheromone of the order Strepsiptera: (3*R*,5*R*,9*R*)-3,5,9-Trimethyldodecanal in *Stylops melittae* Kirby, 1802. J Chem Ecol 38:1493–1503

Van den Dool H, Kratz PD (1963) A generalization of the retention index system including linear temperature-programmed gas-liquid partition chromatography. J Chromatogr 2:463–471

Wood DL (1982) The role of pheromones, kairomones, and allomones in the host selection and colonization behaviour of bark beetles. Ann Rev Entomol 27:411–446

Table 3.1. ^{1}**H NMR data for the candidate pheromone component (CPC in figure 3.2; 4 in figure 3.4).**

position	^{1}H (ppm)	multiplicity (*J*)
1	9.39	dd, (*J* = 1.5, 2.1 Hz)
2	1.71	m
	1.89	m
3	1.89	m
4[a]	0.82	m
	1.08	m
5[a]	1.38	m
6	1.97	m
	1.77	m
7	5.35	m
8	5.35	m
9	2.33	m
10	1.97	m
	2.09	dd (*J* = 7.7, 13.8 Hz)
12	5.27	dq (*J* = 0.8, 6.7 Hz)
13	1.57	d (*J* = 6.7 Hz)
14	0.75	d (*J* = 7.0 Hz)
15	0.81	d (*J* = 6.6 Hz)
16	1.01	d (*J* = 7.0 Hz)
17	1.56	br s

[a] Assignment from analysis of COSY spectrum.

Chapter 4. Concluding Summary

Based on my data and observations, I draw the following conclusions:

My findings are summarized as follows:

1. *Xenos peckii* exhibits developmental synchrony. Fourth instar males extrude from their paper wasp host prior to females but undergo an extra moult and pupation. Thus, the time adult males emerge coincides with the period of sexual maturity of females.

2. The *X. peckii* male uses his sharp mandibles during emergence from his puparium, cutting along the ecdysial suture line in a scissor-like fashion, and then popping the cephalotheca open like a cap.

3. The *X. peckii* female, previously thought to be totally inactive, does engage in active pheromone signalling. She "superextrudes", inflating and tilting her cephalothorax. This posturing occurs with a diel periodicity.

4. Peak pheromone signalling of *X. peckii* females and emergence of conspecific males occur during afternoon hours.

5. During times of superextrusion (peak signalling), the pheromone is most abundant in the pheromone gland.

6. The *X. peckii* male typically approaches a calling female in a swaying flight with smooth turns. He lands on the anterior portion of the host wasp's abdomen, and then steps backward until he makes contact with the cephalothorax of the female. As soon as his mesothoracic legs contact the female's cephalothorax, they curl around it, and he initiates mating.The mated *X. peckii* female fully retreats, loses her attractiveness to males shortly thereafter, and never re-mates.

7. Males die within a few hours of emerging from their host and mating, whereas females overwinter embedded in the abdomen of their paper wasp host.

8. Female *X. peckii* produce (7*E*,11*E*)-3,5,9,11-tetramethyl tridecadienal is a sex pheromone component. A synthetic replica of this compound attracts males in the field. The absolute configuration of this pheromone component is yet to be determined.

9. The sex pheromone component of female *X. peckii* resembles that of *Stylops melittae* and *S. muelleri* [(*R*,*R*,*R*)-3,5,9-trimethyldodecanal], suggesting a common biosynthetic pathway across genera.

Appendix A.

Experimental Section

All reactions described were performed at ambient temperature and atmosphere unless otherwise specified. Column chromatography was carried out with 230–400 mesh silica gel (E. Merck, Silica Gel 60). Concentration and removal of trace solvents was done via a Buchi rotary evaporator using an acetone-dry-ice condenser and a Welch vacuum pump.

Some compounds (see below) were purified by preparative high performance liquid chromatography (HPLC), using a Waters 600 instrument equipped with the dual absorbance detector model 2487 and fitted with a synergy hydro reverse phase column (250 mm × 4.6 mm × 4 microns; Phenomenex, Torrance, CA 90501, USA); samples were analyzed using a 1 ml/min flow of acetonitrile. Nuclear Magnetic Resonance (NMR) spectra were recorded using deuterochloroform ($CDCl_3$) or deuterobenzene (C_6D_6) as the solvent. Signal positions (δ) are given in parts per million from tetramethylsilane (δ 0) and were measured relative to the signal of the solvent (^1H NMR: $CDCl_3$: δ 7.26; C_6D_6: δ 7.16; ^{13}C NMR: $CDCl_3$: δ 77.0; C_6D_6: δ 128.1). Coupling constants (*J* values) are given in Hertz (Hz) and are reported to the nearest 0.1 Hz. ^1H NMR spectral data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; sept, septet; m, multiplet; app apparent; br broad), coupling constants, number of protons. NMR spectra were recorded on a Bruker 400 (400 MHz), a Bruker 500 (500 MHz) or a Bruker 600 (600 MHz).

Preparation of (3*R*,5*S*/3*S*,5*R*)-3,5-dimethyl-6-iodohexan-1-ol. A solution of methyl 6-iodo-3,5-dimethylhexanoate (Rodstein *et al*. 2009) (5) (5.0 g, 17.6 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (100 ml) was cooled to −78 °C, and DIBAL (1.0 M in hexanes,

36.0 ml, 36.0 mmol, 2.0 eq) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h, then quenched with a 15% aqueous solution of Rochelle salt. The aqueous layer was separated and extracted with CH_2Cl_2 (30 mL). The combined organic layers were washed sequentially with water, and brine, then dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (hexane-EtOAc = 5:1 then 3:1) to provide 4.05 g (90%) of 3,5-dimethyl-6-iodohexan-1-ol as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.65-3.79 (m, 2H), 3.27 (dd, J = 4.0, 9.6 Hz, 1H), 3.19 (dd, J = 5.6, 9.6 Hz, 1H), 1.48-1.70 (m, 4H), 1.33-1.46 (m, 2H), 1.02-1.10 (m, 1H), 0.99 (d, J = 6.4 Hz, 3H), 0.93 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 60.8, 43.9, 39.7, 31.5, 26.8, 21.4, 19.8, 18.3. HRMS: *m/z* calcd for $C_8H_{17}IO$: 256.1244. Found: 256.1248.

Preparation of (2*R*,4*S*/2*S*,4*R*)-1-iodo-2,4-dimethyl-6-((tetrahydro-2*H*-pyran-2-yl)oxy hexane (6). A solution of 3,5-dimethyl-6-iodohexan-1-ol (1.83 g, 7.15 mmol, 1.0 eq) in CH_2Cl_2 was added to 3,4-dihydro-2*H*-pyran (1.76 g, 21.0 mmol, 3.0 eq) and pyridinium *p*-toluenesulfonate (181 mg, 0.72 mmol, 0.1 eq). The mixture was stirred at ambient temperature for 16 h and was then washed sequentially with 10% of aqueous $NaHCO_3$, and brine, then dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (hexane-EtOAc = 10:1 to provide 2.18 g (90%) of the tetrahydropyranyl ether 6 as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.54-4.61 (m, 1H), 3.73-3.92 (m, 2H), 3.36-3.54 (m, 2H), 3.21-3.28 (m, 2H), 3.07-3.16 (m, 2H), 1.76-1.88 (m, 1H), 1.46-1.74 (m, 8H), 1.30-1.43 (m, 2H), 1.00-1.09 (m, 1H), 0.97 (diastereomer, d, J = 6.4 Hz, 3H), 0.96 (diasteromer, d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 99.0, 98.8, 65.8, 65.4, 62.4, 62.3, 44.1, 44.0, 36.6, 36.5, 31.9, 31.7, 30.8, 27.5, 27.2, 25.5, 21.3, 21.2, 20.0, 19.9, 19.7, 19.6, 18.1, 18.0. HRMS: *m/z* calcd for $C_{13}H_{25}IO_2$: 340.2409. Found: 340.2404.

Preparation of (3*R*,5*S*/3*S*,5*R*)-3,5-dimethyl-7-((tetrahydro-2H-pyran-2-yl)oxy)hept-anenitrile. To a solution of the tetrahydropyranyl ether 6 (1.02 g, 3.0 mmol, 1.0 eq) in DMSO (20 ml) was added NaCN (294 mg, 6.0 mmol, 2.0 eq). The mixture was then heated to 50 °C and maintained at this temperature for 18 h. After this time, the reaction mixture was diluted with H_2O (20 mL) and EtOAc (55 mL). The organic layer

was separated and washed sequentially with H_2O (3 × 20 mL) and brine. The organic layer was dried over Na_2SO_4 and concentrated to provide 700 mg (98%) of the desired nitrile as a colorless oil that was used directly in the subsequent reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.53-4.60 (m, 1H), 3.73-3.91 (m, 2H), 3.35-3.54 (m, 2H), 2.28-2.37 (m, 1H), 2.16-2.24 (m, 2H), 1.92-2.02 (m, 1H), 1.76-1.87 (m, 1H), 1.46-1.73 (m, 7H), 1.34-1.44 (m, 2H), 1.11-1.20 (m, 1H), 1.08 (d, J = 6.4 Hz, 3H), 0.94 (diastereomer, d, J = 6.4 Hz, 3H), 0.93 (diastereomer, d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 118.8, 118.7, 99.1, 98.9, 65.5, 65.2, 62.5, 62.4, 43.6, 43.4, 36.4, 36.3, 30.8, 27.9, 27.8, 27.3, 27.2, 25.4, 24.3, 24.2, 20.0, 19.9, 19.8, 19.7. HRMS: *m/z* calcd for $C_{14}H_{25}NO_2$: 240.1964 (M+H). Found: 240.1967 (M+H).

Preparation of (3*R*,5*S*/3*S*,5*R*)-3,5-dimethyl-7-((tetrahydro-2*H*-pyran-2-yl)oxy)heptanal. A solution of the nitrile (700 mg, 2.9 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (40 ml) was cooled to −78 °C, and DIBAL (1.0 M in hexanes, 6.1 ml, 6.1 mmol, 2.1 eq) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h, then quenched with a 15% aqueous solution of Rochelle's salt. The aqueous layer was separated and extracted with CH_2Cl_2 (30 mL). The combined organic layers were washed sequentially with H_2O and brine, then dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (hexane-EtOAc = 6:1) to provide 625 mg (89%) of the desired aldehyde as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.75 (t, J = 2.4 Hz, 1H), 4.53-4.60 (m, 1H), 3.73-3.90 (m, 2H), 3.34-3.54 (m, 2H), 2.36-2.44 (m, 1H), 2.14-2.24 (m, 2H), 1.76-1.86 (m, 1H), 1.60-1.73 (m, 3H), 1.47-1.59 (m, 4H), 1.24-1.42 (m, 2H), 1.06-1.15 (m, 1H), 0.95 (d, J = 6.0 Hz, 3H), 0.91 (diastereomer, d, J = 6.4 Hz, 3H), 0.90 (diastereomer, d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 202.9, 99.0, 98.7, 65.6, 65.4, 62.3, 50.8, 44.7, 36.4, 36.2, 30.7, 27.3, 27.2, 25.5, 25.4, 20.4, 20.0, 19.6. HRMS: *m/z* calcd for $C_{14}H_{26}O_3$: 242.3544. Found: 242.3549.

Preparation of (3*R*,5*S*/3*S*,5*R*)-3,5-dimethyl-7-((tetrahydro-2*H*-pyran-2-yl)oxy)heptan-1-ol (7). To a solution of the above-mentioned aldehyde (496 mg, 2.02 mmol, 1.0 eq) in MeOH (15 ml) was added $NaBH_4$ (308 mg, 8.1 mmol, 4.0 eq) portionwise. Following complete addition of the $NaBH_4$, the reaction mixture was stirred

at ambient temperature for 3 h, then quenched with an aqueous solution of $NaHCO_3$ (10%). The reaction mixture was concentrated and the remaining aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed sequentially with H_2O and brine, then dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (hexane-EtOAc = 4:1) to provide 437 mg (88%) of the alcohol 7 as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.52-4.59 (m, 1H), 3.70-3.90 (m, 2H), 3.58-3.70 (m, 2H), 3.32-3.53 (m, 2H), 1.88-2.13 (br, OH), 1.44-1.83 (m, 10H), 1.20-1.41 (m, 3H), 0.94-1.05 (m, 1H), 0.88 (d, J = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 99.0, 98.7, 65.9, 65.7, 62.4, 62.3, 60.9, 45.1, 39.7, 36.4, 36.2, 30.7, 27.3, 27.2, 26.8, 25.4, 20.3, 20.2, 20.1, 19.6. HRMS: *m/z* calcd for $C_{14}H_{28}O_3$: 244.3703. Found: 244.3726.

Preparation of (3*R*,5*S*/3*S*,5*R*)-1-bromo-3,5-dimethyl-7-((tetrahydro-2*H*-pyran-2-yl)oxy)heptane. A solution of triphenylphosphine (330 mg, 1.26 mmol, 1.5 eq) in anhydrous CH_2Cl_2 (20 ml) was cooled to 0 °C, and bromine (202 mg, 1.26 mmol, 1.5 eq) was added dropwise. The light yellow reaction mixture was stirred at 0 °C for 30 min, then a solution of the alcohol 7 (208 mg, 0.84 mmol, 1.0 eq) and NEt_3 (170 mg, 1.68 mmol, 2.0 eq) in CH_2Cl_2 (10 ml) was added dropwise and the resulting reaction mixture was stirred at ambient temperature overnight. The crude reaction mixture was then concentrated and the residue was purified by flash chromatography (hexane-EtOAc = 6:1) to provide 218 mg (83%) of the desired bromide as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.54-4.61 (m, 1H), 3.72-3.90 (m, 2H), 3.33-3.53 (m, 4H), 1.76-1.95 (m, 2H), 1.48-1.76 (m, 9H), 1.31-1.43 (m, 1H), 1.23-1.31 (m, 2H), 0.96-1.06 (m, 1H), 0.90 (d, J = 6.0 Hz, 6H), 0.88 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 99.0, 98.8, 65.8, 65.6, 62.3, 44.6, 39.8, 36.5, 36.3, 32.0, 30.8, 29.0, 27.2, 25.5, 20.2, 19.7, 19.5, 19.4. HRMS: *m/z* calcd for $C_{14}H_{27}BrO_2$: 307.1273. Found: 307.1271.

Preparation of (3*R*,5*S*/3*S*,5*R*)-1-bromotriphenylphosphonium-3,5-dimethylheptan-7-ol (8). To a solution of the bromide (138 mg, 0.45 mmol, 1.0 eq) in toluene (10 mL) was added triphenylphosphine (236 mg, 0.90 mmol, 2.0 eq) and the resulting mixture was heated at reflux for 16 h. The reaction mixture was then concentrated and the residue was

washed with diethyl ether (2 × 10 mL) and dried under vacuum to provide 223 mg (>95%) of the phosphonium salt 8 as a white foam. This material was used directly in the subsequent reaction without further purification.

Preparation of (3*R*,5*S*,7*E*,9*RS*,11*E*/3*S*,5*R*,7*E*,9*RS*,11*E*)-*3,5,9,11*-tetramethyltrideca-7,11-dien-1-ol (10ab) and (3*R*,5*S*,7*Z*,9*RS*,11*E*/3*S*,5*R*,7*Z*,9*RS*,11*E*)-*3,5,9,11*-tetramethyltrideca-7,11-dien-1-ol (11ab). A solution of the phosphonium salt 8 (223 mg, 0.46 mmol, 1.0 eq) in anhydrous THF (15 ml) was cooled to −78 °C, and *n*-BuLi (1.6 M in hexanes, 0.76 ml, 0.92 mmol, 2.0 eq) was added dropwise. The reaction mixture was stirred at −78 °C for 1 h, then the aldehyde (+/-)-9 (Amorelli *et al*., 2015) (58 mg, 0.46 mmol, 1.0 eq) was added. The reaction mixture was stirred at −78 °C for 1 h, then slowly warmed to ambient temperature and stirred for an additional 1 h before treatment with saturated aqueous NH_4Cl (15 mL). The aqueous layer was separated and extracted with EtOAc (15 mL). The combined organic layers were washed sequentially with H_2O and brine, then dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (hexane-EtOAc = 10:1) to provide 116 mg (69%) of a 15:1 (7*Z*:7*E*) mixture of the dienols 10ab:11ab. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.15-5.29 (m, 3H), 3.63-3.75 (m, 2H), 2.55-2.65 (m, 1H), 1.78-2.10 (m, 4H), 1.51-1.71 (m, 11H), 1.22-1.38 (m, 3H), 0.96-1.05 (m, 1H), 0.91 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 137.1, 134.3, 126.5, 126.3, 119.8, 61.2, 47.7, 44.8, 39.8, 34.4, 30.7, 30.6, 30.0, 27.0, 20.6, 20.2, 20.1, 15.8, 13.3. HRMS: *m/z* calcd for $C_{17}H_{32}O$: 253.2526 (M+H). Found: 253.2519 (M+H). A sample (18 mg) containing a ~1:1 mixture of 7*E*:7*Z* dienols was available following repetitive chromatography using $AgNO_3$ (20%) impregnated silica gel.

Preparation of (3*R*,5*S*,7*Z*,9*RS*,11*E*/3*S*,5*R*,7*Z*,9*RS*,11*E*)-*3,5,9,11*-tetramethyltrideca-7,11-dienal (13ab). A sample containing a ~1:1 mixture of the dienols 10ab:11ab (18 mg, 0.07 mmol, 1.0 eq) was dissolved in dry CH_2Cl_2 (8 mL), and $NaHCO_3$ (9.4 mg, 0.11 mmol, 1.5 eq) was added followed by Dess-Martin periodinane (47 mg, 0.11 mmol, 1.5 eq). After stirring he reaction mixture at ambient temperature for 2 h, it was treated with a 10 % aqueous solution of $Na_2S_2O_3$ (5 mL) and a saturated aqueous

solution of $NaHCO_3$ (5 mL) and stirred for an additional 20 min. The aqueous layer was separated and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (8 mL), dried over $MgSO_4$, and concentrated to provide 17 mg of a mixture of the aldehydes 12ab and 13ab as a colorless oil (94%). Purification of 13ab by preparative HPLC (see above) afforded 3.1 mg of *7E,11E*-aldehydes 13ab. ^{1}H NMR (500 MHz, C_6D_6) δ: 9.37 (s, 1H), 5.30-5.39 (m, 2H), 5.20-5.29 (m, 1H), 2.27-2.38 (m, 1H), 2.03-2.11 (m, 1H), 1.93-2.02 (m, 2H), 1.85-1.93 (m, 2H), 1.66-1.79 (m, 2H), 1.56 (d, J = 7.0 Hz, 3H), 1.55 (s, 3H), 1.32-1.39 (m, 1H), 1.04-1.13 (m, 1H), 1.01 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.80 (diasteromer, d, J = 7.0 Hz, 3H), 0.79 (diastereomer, d, J = 6.5 Hz, 3H), 0.73 (d, J = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, C_6D_6) δ: 201.4, 138.9, 135.1, 127.0, 126.9, 120.9, 51.5, 48.8, 44.9, 44.7, 40.6, 40.4, 35.80, 31.2, 26.2, 21.3, 21.2, 21.1, 20.5, 16.3, 14.1. HRMS: *m/z* calcd for $C_{17}H_{30}O$: 251.2369 (M+H). Found: 251.2381 (M+H).

4.1. References to appendix A

Amorelli B, Arruda ME, Belko RP, Cai T, Closson AP, Giffin NL, Mertz GJ, Monteleone MG (2015) Novel organoleptic compounds. U.S. Pat. Appl. Publ. US 20150057207

Rodstein J, McElfresh SJ, Barbour JD, Ray AM, Hanks LM, Millar JG (2009) Identification and synthesis of a female-produced sex pheromone for the cerambycid beetle *Prionus californicus*. J Chem Ecol 35:590–600

Appendix B.

Supplemental Videos

Video 2.1. A female *Xenos peckii* assuming her mate-calling posture, sped up 16×. Note that she commenced to inflate her cephalothorax approximately 30 minutes prior to the start of the video recording, and that that she continued to inflate it for several minutes after the video sequence, at which time she had reached her final calling posture.

Video 2.2. A male using his mandibles to cut in a scissor-like fashion through the ecdysial suture line of his puparium. Note: the male began cutting adjacent to the hinge-point of the cephalotheca, and then continued cutting radially around the puparium until he reached the hinge point at the other side.

Video 2.3. A male *Xenos peckii* emerging from his puparium. The mandibles can be seen cutting the puparium in a scissor-like fashion.

Video 2.4. Mating sequence of *Xenos peckii*, filmed at 1000 fps (played back at ½ real time speed).

Video 3.1. Mating sequence of *Xenos peckii*, filmed at 30 fps (real time). The male copulated twice with the female, through the brood canal opening.