Reproductive strategies of plant-sap sucking insects with special focus on Thysanoptera

Dissertation

zur Erlangung des
Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftliche Fakultät I – Biowissenschaften –

der Martin-Luther-Universität
Halle-Wittenberg,

vorgelegt

von Frau Stephanie Krüger

geb. am 23.12.1986 in Leipzig

Gutachter:
Prof. Dr. Gerald Moritz
Prof. Dr. Rolf Beutel
Dr. Laurence Mound

Tag der Verteidigung:
03.05.2016

CONTENTS

ABBREVIATIONS

abd	abdomen
acc gl	accessory gland
aed	aedeagus
ao	aorta
AF-AB	aldehyde fuchsine-alcian blue staining
ATER	agranular tubular endoplasmatic reticulum
bc	bursa copulatrix
BFT	Bean Flower Thrips
bl	basal lobe
bsl	basal lamina
c	chitin capsule
cc	conducting canal
cer	cerci
cov	common oviduct
cr	cuticular ridge
dl	dorsal lobe
edth	endotheca
ejb	ejaculatory bulb
ejd	ejaculatory duct
ep1-3	epithelial layer 1-3
eppt	eppiproct
f	follicle epithelium
fc	fat cells
g	germarium
GC	gas chromatograph
glc	gland cell
gly	glycogen
gpr	gonopore
hc	haemocoel
hg	hind gut
i	intercellular system
IPM	integrated pest management
L:D	light: dark
lov	lateral oviduct
m	mitochondria
mb	mushroom bodies
mbb	microvilli brush border
met	metathorax
mg	midgut
mg I	fore part of mid gut
mg II	mid gut loop and forward-directed part of mid gut
mg III	hind part of mid gut
M l abd dors ext	Musculus longitudinalis abdominis dorsalis exterior
M l abd dors int	Musculus longitudinalis abdominis dorsalis interior
M l abd ventr	Musculus longitudinalis abdominis ventralis
M p-t	Musculus pleuro-tergalis
MS	mass spectrometer
ms	muscle
mt	malpighian tubule
M t-s	Musculus tergo-sternalis
n	nucleus
nc	nerve cord
nu	nutrition cord
o	oocyte
o-gl	orange gland
ov	ovary
ovp	ovipositor
p	paramere
paed	primitive aedeagus
papt	paraproct
par vag gl	Parietal- vaginal gland
PASH	periodic acid-Schiff`s reagent-haematoxylin staining
pbl	parabasal lobe
phb	phallobase
phl	phallus
phtc	phallotheca
phtr	phallotrema
pm	peritoneal membrane
pmr	primitive aedeagus

pph	paraphyses
ppl	pore plate (= areae porosae)
pX	paratergite X
r	reservoir
rER	rough endoplasmatic reticulum
recp	rectal papilla
rsb	rod-shaped bacteria
RH	relative humidity
RT	room temperature
sal gl	salivary gland
sb	spermathecal ball
sbu	spermathecal bulb
sc	secretory cell
sem v	seminal vesicle
sen	sensilla
smp	sigmoid process
spth	spermatheca
spth d	spermathecal duct
spth gl	spermathecal gland
sp	spermatozoa
stgl	sternal gland
sty	stylus
t	testes
tc	trophic core
tf	terminal filament
tph	tropharium
vag	vagina
vd	vas deferens
1-3	compartment 1-3
1vl	1st valvula
2vl	2nd valvula
3vl	3rd valvula
1vfl	1st valvifera
2vfl	2nd valvifera
arrow	region of special interest
asterisk (*)	extracellular cavity

LIST OF PUBLICATIONS

This thesis is based on the following manuscripts:

Chapter 3
Krüger S, Jilge M, Moritz G, (in prep.) Reproductive behavior of *Echinothrips americanus* (Thysanoptera: Thripidae).

Chapter 4
Krüger S, Mound LA, Moritz G, 2015. Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* MORGAN 1913 (Thysanoptera: Thripidae). Journal of Applied Entomology, doi: 10.1111/jen.12280

Section 5.1
Krüger S, Subramanian S, Saliou N, Moritz G, 2015. Sternal gland structures in males of bean flower thrips, *Megalurothrips sjostedti* and Poinsettia thrips *Echinothrips americanus* in comparison with those of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Arthropod Structure and Development 44, 455-467.

Section 5.2
Krüger S, Jilge M, Moritz G, (in prep.) Histochemical studies of sternal glands in economically important pest thrips.

Section 5.3
Krüger S, Hesse C, Moritz G, (in prep.) Correlation of sternal gland, body size and mating success in *Echinothrips americanus* (Thysanoptera: Thripidae)

Chapter 6
Krüger S, Radisch D, Lindemann P, Moritz G, Tschuch G (under rereview) Male pheromones influencing mating success of *Echinothrips americanus* (Insecta, Thysanoptera, Thripidae). Journal of Chemical Ecology.

CHAPTER 1

INTRODUCTION

1.1 General introduction

Each year, about 15 % of crop yield is lost to insects worldwide (Maxmen 2013), and in the years 2006 and 2007 over 5,200 million lbs of pesticides were used, of which about 900 million lbs were insecticides. This corresponds to expenditure on pesticides of about $US 38,000 million (insecticides $US 11,000 Mio) worldwide (Grube et al. 2011).

Several pest species belong to the superorder Paraneoptera. The included species are characterized by their special piercing and/or sucking mouthparts and corresponding diet. Additionally, some of them act as important vectors of phyto-, zoo- and human-pathogenic microorganisms. The Paraneoptera includes at least 4 orders: Hemiptera, Thysanoptera, Phthiraptera and Psocoptera, with about 100,000 described species.

Monophyly of Paraneoptera is widely accepted (e.g. Grimaldi and Engel 2005, Yoshizawa and Saigusa 2001), based on several autapomorphies: reduced number of tarsomeres, complete ganglion fusion, absence of cerci, partial reduction or absence of labial palpi, asymmetric mandibles, stylet-like laciniae, enlarged postclypeus, mostly six Malpighian tubules and the absence of sternum I (Kristensen 1991, Grimaldi and Engel 2005, Beutel et al. 2013).

The evolutionary history of paraneopterans is reflected in the structure and function of their mouthparts (Fig. 1). Basal paraneopterans (psocopterans and some basal thrips) are microbial surface feeders. The psocopterans have the most generalized “picking” mouthparts. Thysanoptera and anopluran lice developed probing and puncturing mouthparts, whereas Hemipterans possess a distinctive piercing and sucking rostrum or beak (Grimaldi and Engel 2005). Thysanoptera and Hemiptera evolved on a diet of plant fluids, despite some ancestral predatory species, which suck the hemolymph of their prey. Blood feeding by ectoparasitic lice arose independently (Grimaldi and Engel 2005) (Fig.1).

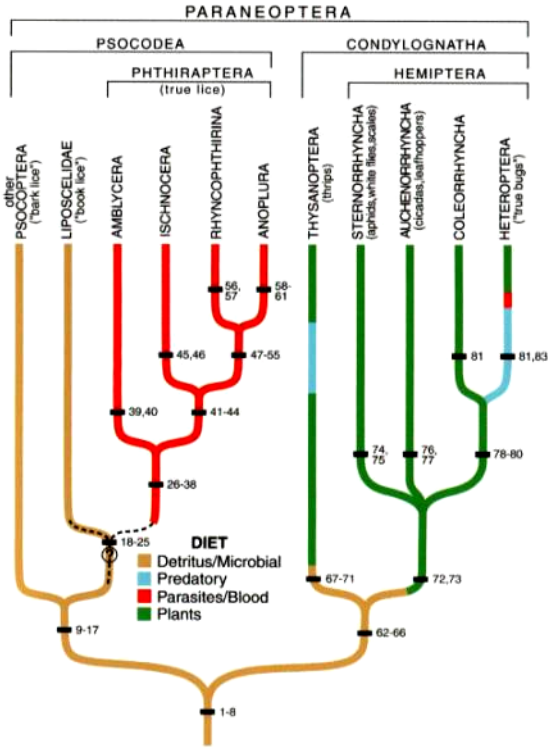


Fig. 1 Relationships among recent orders and suborders of the Paraneoptera, with their diets (Grimaldi and Engel 2005)

Nevertheless, the relationships between Thysanoptera, Hemiptera and Psocoptera are still under discussion (e.g. Buckman et al. 2013, Heming and Moritz 2013). Several authors assume Hemiptera to be sister group of the Thysanoptera (Condylognatha), which is supported by morphological and molecular data (Henning 1969, Boudreaux 1979, Lyal 1985, Whiting et al. 1997, Johnson et al. 2004, Yoshizawa and Johnson 2005, Ishiwata et al. 2011, Cui et al. 2013, Beutel et al. 2013, Wang et al. 2013).
Alternatively, Thysanoptera form a clade with the Psocodea (Micracercaria) based on their bi-flagellate sperm (Baccetti 1979, Jamieson 1987), the prominent dorsal cibarial dilator (Willmann 2005) and molecular analyses (Wheeler et al. 2001, Mound and Morris 2007, Buckman et al. 2013).

With regard to their agricultural importance, the main focus in this study is on the Thysanoptera, and partly on selected representatives of the Hemiptera.
The order Hemiptera is the largest group within the Paraneoptera, and four traditional subgroups are currently recognised: Sternorrhyncha, Auchenorrhyncha, Heteroptera and Coleorrhyncha (Grimaldi and Engel 2005). Within these, many phytophagous species are important horticultural and agricultural pests (O´Brien and Wilson 1985, Nielson 1985, Backus et al. 2006). Beside having a direct effect on plants through mechanical damage and sucking out plant sap, some species transmit diseases and viruses as contaminants on their mouthparts (Miles 1968, 1972).

The order Thysanoptera comprises approximately 6,100 species (ThripsWiki 2016) with an average size of 1-3 mm, with some Tubuliferan species considerable large (Haliday 1852, Mound 1971, Crespi et al. 2004, Moritz 2006). Thysanoptera are characterized by their fringed, band-like wings, their largely reduced right mandible, and their pretarsial arolium (Heming 1971, Mound et al. 1980, Moritz 1997), and most species have tropical and distributions (Lewis 1973, 1997). Two suborders can be distinguished: Terebrantia and Tubulifera (Haliday 1836, Priesner 1928). Females of the terebrantian suborder possess a distinct external ovipositor, whereas tubuliferan females have an inconspicuous, flexible internal structure (Heming 1970a, Bode 1975, Moritz 1989, 1997). Eight families are recognised in the suborder Terebrantia (Uzelothripidae, Merothripidae, Aeolothripidae, Melanthripidae, Adiheterothripidae, Fauriellidae, Heterothripidae, Thripidae), whereas the suborder Tubulifera comprises only one family, the Phlaeothripidae (Mound 1997, Moritz et al. 2001). The Phlaeothripidae and the Thripidae are the two major groups that together include more than 5,500 species (ThripsWiki 2016).

Approximately 87 Thysanoptera species are considered agricultural pests (Mound 1997), due to their feeding and oviposition injuries. Of these, about 10 species are propagative vectors of Tospoviruses, Machlomoviruses, Ilarvirus, Carmovirus, or Sobemovirus (Ullman et al. 1997, Mound 2001, Gitaitis et al. 2003, Jones 2005).

An enormous yield reduction in agricultural products is attributed to thrips feeding activity and virus transmission each year. In Africa, 63-68 % yield loss in French beans (Nyasani et al. 2012),

20 to 100 % loss in legumes (Singh and Allen 1980), up to 60 % yield loss in onions (Waiganjo et al. 2008), 80 to 100 % yield loss in tomatoes (Wangai et al. 2001) are reported.

Several thrips species develop new resistance mechanisms against insecticides (Lewis 1997). In Indonesia some farmers apply up to 16 sprays of quinalphos, endosulfan, mercaptodimethur, acephate, formentanate and cypermethrin at 3 to 4 day intervals throughout the growing season in order to control *Thrips tabaci, Thrips palmi* and *Thrips parvispinus* (Sastrosiswojo 1991). In attempts to control *T. palmi* and *Megalurothrips usitatus* in the Philippines, farmers put on two to four chemicals in succession applied at 3 to 14 day intervals. Nonetheless, yield losses are large and sometimes total (Bernardo 1991). Also in Thailand a similar excessive insecticide use is reported, with mixes of two to five chemicals applied to control thrips populations (Bansiddhi and Poonchaisri 1991).

In many developing countries programs to control exposure to pesticides are limited or non-existent (Alavanja 2009). Professional workers who spray pesticides have to certify their competence. Nevertheless, workers from developing countries, as well as seasonal workers, often have hardly any qualifications; 25 million agricultural workers from developing countries suffer unintentional pesticide poisoning each year (Jeyaratnam 1990). From 2006 to 2010 an average of 130,136 calls to poison control centers were reported in the USA, with 20,116 cases needing treatment in health care facilities (Langley and Mort 2012). The national cost associated with pesticide exposure was estimated at nearly $US 200 million, excluding cost from lost work time, hospital physican fees and pesticide-induced cancers (Langley and Mort 2012).

An efficient and targeted use of integrated pest management strategies (IPM) is needed, to control pest species, to avoid health problems and to enable an effective and economic use of insecticides.

Detailed knowledge of reproduction is critical to managing those species that compete with us in the field, forest and greenhouse (Heming 1995). Comprehensive knowledge of morphology, function, physiology and development of reproductive systems, germ cells and reproduction strategies within the Thysanoptera and other plant-sucking insects is missing. Significantly, complete analysis for any one species is rare, because each author provide information on a detailed part of the biology of their experimental animals, but rarely considers all aspects together, to enable a comprehensive understanding of the thrips and their reproductive strategies.

Therefore, the aim of this thesis is to demonstrate the functional connections between different aspects of the biology of several plant-sucking insects and their resulting life style. Special focus is given to the biology of a terebrantian thysanopteron, *Echinothrips americanus* Morgan 1913 (Thysanoptera: Thripidae), as an example. This species is an emerging pest worldwide, and is a possible vector of several pathogens. The biology of this species is presented in Chapter 2.

1.2 Scope of this thesis

This study is divided into chapters according to different aspects of the reproductive strategy. In the review of the basic morphology, species of Hemiptera and Thysanoptera are considered. The subsequent chapters focus mainly on *E. americanus* in order to give a comprehensive understanding of the reproduction of that species.

Basic morphology

Reproductive behavior cannot be understood without knowledge of the morphological structures of the inner and outer genitalia. Briefly, in Chapter 2 the basic morphological structures of the inner and outer reproductive organs of pterygote insects are described. Because of the unsolved problem of the phylogenetic relations of Thysanoptera and Hemiptera (see above), the detailed morphology of the reproductive system of *E. americanus* as a representative for Thysanoptera is described and compared with selected hemipteran species (*Pyrrhocoris apterus*: Heteroptera, Pyrrhocoridae; *Campyloneura virgula*: Heteroptera, Miridae and *Arboridia ribauti*: Auchenorrhyncha, Cicadellidae). Each species was selected for its special reproduction mode and availability. The analysis of reproductive morphology is accompanied by a short introduction to their biology.

Reproductive behavior

Most Thysanoptera have a haplo-diploid reproduction mode and, like bees, reproduce via arrhenotoky (Shull 1914). Diploid females arise from fertilized eggs, whereas unfertilized eggs produce haploid males. Only a few are diplo-diploid (thelytoky) with females developing from unfertilized eggs (Jordan 1888). While females can mature eggs successively throughout almost their entire life, in most terebrantian males, spermiogenesis is completed with eclosion of the adult (Bournier 1956, Heming 1970b, Bode 1983). In contrast, tubuliferan species possess spermiogenesis over their entire lifetime (*Suocerathrips linguis*: Kumm 2002, *Gynaikothrips ficorum*: Moritz 2006), with some species exhibiting an intermediate condition (*Haplothrips verbasci:* Heming 1970b*, Bactrothrips brevitubus:* Kumm 2002).

It is not clear whether the sperm limitation in Terebrantia has implications for their mating strategy and fitness. With the help of the analysis of the mating behavior and an examination of the possible effects of sperm limitation, new insights into the mating strategy of this species are described in Chapter 3.

Reproduction strategy

The costs of reproduction are almost unknown in thrips, and estimation of these costs requires an understanding of temporal and spatial life-history variations and sex ratios.

At present, our knowledge is scant and anecdotal; little is known of the real genetic background of the sex determination processes (Moritz 1997, 2006). In thrips and other insects with arrhenotokous parthenogenesis, females are able to control the sex ratio of their offsprings. Evidence for adaptive sex ratio strategies has been described in haplo-diploid species (Clausen 1939; Charnov 1982, King 1987, Antolin 1993, Wrensch and Ebbert 1993, Godfray 1994). As in many insects after mating, females store sperm in a spermatheca. Later they release the sperm to allow fertilization via the micropyle of each egg. Heming (1970a),

Moritz (1982, 1989) and Dallai et al. (1996) found muscle insertions on the spermatheca and spermathecal duct among Thysanoptera, suggesting that these muscles can control sperm flow.
Most studies on sex allocation have focused on the influence of different environmental factors on reproduction, for example host plants and temperature (e.g. Lublinkhof and Foster 1977, Nugaliyadde 1984, Anyango 1992, Lowry et al. 1992, Oetting and Beshear 1993, van Rijn et al. 1995, Hoddle et al. 2000, Murai 2000, Stacey and Fellows 2002, Premachandra et al. 2004, Chhagan and Stevens 2007, Zhang et al. 2007, Nielsen et al. 2010, Fathi et al. 2011), but never on reproduction strategies. In Chapter 4 the differences and consequences of reproduction strategy by *E. americanus* within different mating states were tested.

Sternal glands in Thysanoptera
In many thrips species, especially within the subfamily Thripinae, typical sexually dimorphic structures, taxonomically referred to as area porosa (= pore plates after Heming 1970b), are present on sternites III or IV to VII or VIII of males. Because of the absence of sternal glands in most females, Klocke (1926) and Mound (2009) postulated a sexually determined function for these structures. Additionally, based on behavioral studies on *Taeniothrips dianthi* (=*Pezothrips dianthi*), Pelikan (1951) concluded that the sternal glands secrete a pheromonal substance. Subsequent, ultrastructural and functional investigations indicated that the sternal glands are structurally consistent with pheromone release as initially inferred by Pelikan (Bode 1978, Moritz 1997, 2006, Sudo and Tsutsumi 2002, 2003, Shitatani and Tsutsumi 2005, 2006, El-Ghariani and Kirk 2008, Olaniran et al. 2013). Functionally, the sternal pore plates, observed externally as a porous cover, are associated internally with specialized epidermal gland cells and an extracellular, subcuticular cavity that functions as a reservoir for glandular secretions. Their differences in shape, size, number and arrangement (even within one genus) made these structures an important character state for identification of male thrips (Stannard 1968, zur Strassen 2003, Mound 2009). In addition to the lack of information about gland morphology within the different pore plate arrangements, no reliable evidence of their function is available.
In Chapter 5 the morphology of the sternal glands of three different economically important species is described in detail. Section 5.2 provides histochemical analyses of the produced secretions and components of the gland cells. In Section 5.3 possible functions in mate assessment and pheromone production were tested and discussed further.

Male emitted pheromones
The remarkable presence of the sternal glands and the former assumption of a pheromonal function prompted me to test male *E. americanus* for the presence of such active substances.
Currently, males of three thrips species are known to produce pheromones; *Frankliniella intonsa* (Hamilton et al. 2005, Zhang et al. 2011), *Frankliniella occidentalis* (De Kogel and van Deventer 2003, Hamilton et al. 2005, Kirk and Hamilton 2010, Olaniran et al. 2013) and *Thrips palmi* (Kirk and Hamilton 2010, Akella et al. 2014). Recently, active aggregation behavior of male Bean Flower Thrips (BFT) on cowpea leaves has been observed, indicating a semiochemical- or behavior-mediated aggregation (Niassy et al. 2016).

Chapter 6 provides evidence for a short-range male recognition pheromone and an anti-aphrodisiac.

All results are discussed and interpreted in general in order to place their reproductive biology and established strategies within the context of the existing knowledge about the biology of Thysanoptera.
Chapters 2, 3, 5 and 6 possess additional information of the methods used and more detailed results, which are given in the Supplementary Material on the attached CD ROM.

1.3 References

Akella SVS, Kirk WDJ, Lu YB, Murai T, Walters KFA, Hamilton JGC, Renou M, 2014. Identification of the aggregation pheromone of the melon thrips, *Thrips palmi*. PLOS ONE 9, e103315.

Alavanja MCR, 2009. Pesticides Use and Exposure Extensive Worldwide. Rev Environ Health 24, 303-309.

Anyango JJ, 1992. The life history of chrysanthemum thrips, *Thrips nigropilosus* Uzel (Thripidae: Thysanoptera) and an evaluation of the effects of temperature on its life cycle. Int J Trop Insect Sci 13, 41-44.

Antolin MF, 1993. Genetics of biased sex ratios in subdivided populations: models, assumptions and evidence. Oxf Surv Evol Biol 9, 239-281.

Baccetti B, 1979. Ultrastructure of sperm and its bearing on arthropod phylogeny. In: Arthropod Phylogeny. Ed. by Gupta AP, Van Nostrand, New York, USA, 609-644.

Backus E, Dugravot S, Readon B, Shugart H, Chen J, Miller T, Ruzin S, Morgan D, 2006. Where, when and how do ingestion and other feeding behaviours of the glassy winged sharpshooter allow inoculation in *Xyella fastidiosa*? Proceedings 2006 Pierce`s Disease Research Symposium, 252-255.

Banshiddhi K, Poonchaisri S, 1991. Thrips in vegetables and other commercially important crops in Thailand. In: Thrips in South East Asia. Ed. by Talekar NS, AVRDC Publications, Bangkok, Indonesia, 34-39.

Bernando EN, 1991. Thrips on vegetable crops in the Philippines. In: Thrips in South East Asia. Ed. by Talekar NS, AVRDC Publications, Bangkok, Indonesia, 5-11.

Beutel R, Friedrich F, Yang X-K, Ge S-Q, 2013. Insect Morphology and Phylogeny: A textbook for students of entomology. De Gryter, Berlin, Germany.

Bode W, 1975. Der Ovipositor und die weiblichen Geschlechtswege der Thripiden (Thysanoptera, Terebrantia). Z Morphol Ökol Tiere 81, 1-53.

Bode W, 1978. Ultrastructure of the sternal glands in *Thrips validus* Uzel (Thysanoptera, Terebrantia). Zoomorphologie 90, 53-65.

Bode W, 1983. Spermienstruktur und Spermiohistogenese bei *Thrips validus* Uzel (Insecta, Thysanoptera). Zool Jb Anat 109, 301-318.

Boudreaux HB, 1979. Arthropod phylogeny with special reference to insects. Wiley, New York, USA.

Bournier A, 1956. Contribution a L´ètude de la parthènogèse des Thysanopteres et de sa cytology. Arch Zool exp gen 93, 219-318.

Buckman R, Mound LA, Whiting MF, 2013. Phylogeny of thrips (Insecta: Thysanoptera) based on five molecular loci. Syst Entomol 38, 123-133.

Charnov EL, 1982. The theory of sex allocation, Princeton University Press, New York, USA.

Chhagan A, Stevens P, 2007. Effect of temperature on the development, longevity and oviposition of greenhouse thrips (*Heliothrips haemorhoidalis*) on lemon fruit. N Z Plant Prot 60, 50-55.

Clausen C, 1939. The effect of host size upon the sex ratio of hymenopterous parasites and its relation to methods or rearing and colonization. J NY Entomol Soc 47, 1-9.

Crespi BJ, Morris DC, Mound LA, 2004. Evolution of Ecological and Behavioural Diversity: Australians Acacia Thrips as Model Organisms. Australian Biological Resources Study and Australian National Insect Collection, Canberra, Australia.

Cui Y, Xie Q, Hua J, Dang K, Zhou J, Liu X, Wang G, Yu X, Bu W, 2013. Phylogenomics of Hemiptera (Insecta: Paraneoptera) based on mitochondrial genomes. Syst Entomol 38, 233-245.

Dallai R, Del Bene G, Lupetti P, 1996. Fine structure of spermatheca and accessory gland of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Int J Insect Morphol Embryol 25, 317-330.

De Kogel WJ, van Deventer P, 2003. Intraspecific attraction in the western flower thrips *Frankliniella occidentalis*, indications for a male sex pheromone. Entomol Exp Appl 107, 87-89.

El-Ghariani IM, Kirk WDJ, 2008. The structure of the male sternal glands of the western flower thrips, *Frankliniella occidentalis* (Pergande). Acta Phytopathol Entomol Hung 43, 257-266.

Fathi SAA, Gholami F, Nouri-Ganbalani G, Mohiseni A, 2011. Life history parameters of *Thrips tabaci* (Thysanoptera: Thripidae) on six commercial cultivars of canola. Appl Entomol Zool 46, 505-510.

Gitaitis RD, Walcott RR, Wells ML, Diaz Perez JC, Sanders FH, 2003. Transmissions of Pantoea ananatis, causal agent of center rot of onion, by tobacco thrips, *Frankliniella fusca*. Plant Dis 87, 675–678.

Godfray HCJ, 1994. Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, Princeton, USA.

Grimaldi DA, Engel MS, 2005. Evolution of the Insects. Cambridge University Press, Cambridge, USA.

Grube A, Donaldson D, Kiely T, Wu L, 2011. Pesticides Industry Sales and Usage- 2006 and 2007 Market Estimates. Biological and Economic Analysis Division Office of Pesticide Programs, US Environmental Protection Agency. available from: http://www2.epa.gov/sites/production/files/2015-10/documents/market_estimates2007.pdf

Haliday AH, 1836. An epitome of the British genera in the Order Thysanoptera with indications of a few of the species. Entomol Mag 3, 439-451

Haliday AH, 1852. Order III Physapoda. In: List of the Homopterous insects in the British Museum Part IV. Ed. by Walker F, British Museum, London, 1094-1118.

Hamilton JGC, Hall DR, Kirk WDJ, 2005. Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem Ecol 31, 1369-1379.

Heming BS, 1970a. Postembryonic development of the female reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc Publs Entomol Soc Amer 7, 197-234.

Heming BS, 1970b. Postembryonic development of the male reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc Publs Entomol Soc Amer 7, 237-272.

Heming BS, 1971. Functional morphology of the thysanopteran pretarsus. Can J Zool 49, 91-108.

Heming BS, 1995. History of the Germ Line in Male and Female Thrips. In: Thrips Biology and Management. Ed. by Parker BL, Skinner M, Lewis T, Plenum Press, New York, USA, 505-535.

Heming BS, Moritz GM, 2013. The thrips in their paraneopteran context. 6th Dresden Meeting on Insect Phylogeny, 26-27.

Henning W, 1969. Die Stammesgeschichte der Insekten. Senckenbergische Naturforschende Gesellschaft, Kramer, Frankfurt a.M., Germany.

Hoddle MS, Robinson L, Drescher K, Jones J, 2000. Developmental and reproductive biology of a predatory *Franklinothrips n. sp*. (Thysanoptera: Aeolothripidae). Biol Control 18, 27-38.

Ishiwata K, Sasaki G, Ogawa J, Miyata T, Su ZH, 2011. Phylogenetic relationship among insect orders based on three nuclear protein-coding gene sequences. Mol Phylogenet Evol 58, 169-180.

Jamieson BDGM, 1987. The Ultrastructure and Phylogeny of Insect Spermatozoa. Cambridge University Press, Cambridge, USA.

Jeyaratnam J, 1990. Acute pesticide poisoning: a major global health problem. World Health Stat 43, 139-144.

Johnson KP, Yoshizawa K, Smith VS, 2004. Multiple origins of parasitism in lice. Proc Roy Soc B 271, 1771-1776.

Jones DR, 2005. Plant viruses transmitted by thrips. Eur J Plant Pathol 113, 119–157

Jordan K, 1888. Anatomie und Biologie der Physapoda. Z wiss Zool 47, 542-620.

King B, 1987. Offspring sex ratios in parasitoid wasps. Q Rev Biol 62, 367-396.

Kirk WDJ, Hamilton JGC, 2010. The aggregation pheromones in thrips. In: IXth International Symposium on Thysanoptera and Tospoviruses. Ed. by Persley D, Wilson C, Thomas J, Sharman M, Tree D, J Insect Sci 10, 166.

Klocke F, 1926. Beiträge zur Anatomie und Histologie der Thysanopteren. Z wiss Zool Abt A 128, 1-36.

Kristensen NP, 1991. Phylogeny of extant hexapods. In: The Insects of Australia. A Textbook for Students and Research Workers. Ed by Naumann ID, Carne PB, Lawrence JF, Nielsen ES, Spradbery JP, Taylor RW, Whitten MJ, Littlejohn MJ. Cornell University Press, Ithaca, USA, 125-140.

Kumm S, 2002. Reproduction, progenesis, and embryogenesis of thrips (Thysanoptera, Insecta). PhD-thesis, Martin-Luther University Halle-Wittenberg, Halle, Germany.

Langley RL, Mort SA, 2012. Human exposure to pesticides in the United States. J Agromedicine 17, 300-315.

Lewis T, 1973. Thrips: Their Biology, Ecology and Economic Importance. Academic Press London, New York, USA.

Lewis T, 1997. Chemical control. In: Thrips as Crop Pests. Ed. by Lewis T, CAB International, Oxon, UK, 567-593.

Lowry V, Smith J, Mitchell F, 1992. Life-fertility tables of *Frankliniella fusca* (Hinds) and *F. occidentalis* (Pergande) (Thysanoptera: Thripidae) on peanut. Ann Entomol Soc Am 85, 744-754.

Lublinkhof J, Foster D, 1977. Development and reproductive capacity of *Frankliniella occidentalis*. J Kans Entomol Soc 50, 313-316.

Lyal CHC, 1985. Phylogeny and classification of the Psocodea, with particular reference to lice (Psocodea: Phthiraptera). Syst Entomol 10, 145-165.

Maxmen A, 2013. Crop pests: Under attack. Nature 501, 15-17.

Miles PW, 1968. Insect secretions in plants. Ann Rev Phytopathol 6, 137-164.

Miles PW, 1972. The Saliva of Hemiptera. Adv Insect Physiol 9, 183-225.

Moritz G, 1982. Zur Morphologie und Anatomie des Fransenflüglers *Aeolothrips intermedius* Bagnall. 3. Mitteilung: Das Abdomen. Zool Jb Anat 108, 293-340.

Moritz G, 1989. Die Ontogenese des Thysanoptera (Insecta) unter besonderer Berücksichtigung des Fransenflüglers *Hercinothrips femoralis* (O.M. Reuter 1891) (Thysanoptera, Thripidae, Panchaetothripinae). V. Mitteilung: Imago, Abdomen. Zool Jb Anat 119, 157-217.

Moritz G, 1997. Structure, growth and development. In: Thrips as crop pests. Ed. by Lewis T, CAB International, Oxon, UK, 15-63.

Moritz G, 2006. Thripse - Fransenflügler, Thysanoptera. Westarp-Verlag, Hohenwarsleben, Germany.

Moritz G, Morris DC, Mound LA, 2001. Thrips*ID*-Pest thrips of the world. An interactive identification and information system. CSIRO Publishing, Collingwood, Australia. online available: http://thripsnet.zoologie.uni-halle.de/key-server-neu/data/09070302-040a-4006-8c08-0e0105060801/media/Html/index.html

Mound LA, 1971. Gall-forming thrips and allied species (Thysanoptera: Phlaeothripinae) from Acacia trees in Australia. Bull br Mus nat Hist 25, 387-466.

Mound LA, 1997. Biological diversity. In: Thrips as crop pests. Ed. by Lewis T, CAB International, Oxon, UK, 197-216.

Mound LA, 2001. So many thrips-so few tospoviruses? In: Thrips and Tospoviruses: Proceedings of the 7th international symposium on Thysanoptera. Ed. by Marullo R, Mound LA, Australian National Insect Collection, Canberra, Australia, 15-18.

Mound, LA, 2009. Sternal pore plates (glandular areas) of male Thripidae (Thysanoptera). Zootaxa 2129, 29-46.

Mound LA, Heming BS, Palmer JM, 1980. Phylogenetic relationship between the families of recent Thysanoptera (Insecta). Zool J Linnean Soc 69, 111-141.

Mound LA, Morris DC, 2007. The insect order Thysanoptera: Classification versus Systematics. Zootaxa 1668, 395-411.

Murai T, 2000. Effect of temperature on development and reproduction of the onion thrips, *Thrips tabaci* Lindemann (Thysanoptera: Thripidae), on pollen and honey solution. Appl Entomol Zool 35, 499-504.

Niassy S, Ekesi S, Maniania NK, Moritz GB, Kogel WJ de, Subramanian S, 2016. Active aggregation among sexes in bean flower thrips (*Megalurothrips sjostedti*) on cowpea (*Vigna unguiculata*). Entomol Exp Appl 158, 17-24.

Nielsen MC, Teulon D, Chapman R, Butler RC, Drayton GM, Philipsen H, 2010. Comparison of life history parameters of two *Frankliniella occidentalis* (Thysanoptera: Thripidae) strains in New Zealand. Environ Entomol 39, 303-311.

Nielson MW, 1985. Leafhopper Systematics. In: The leafhoppers and planthoppers. Ed. by Nault LR, Rodriguez JG, John Wiley and Sons, New York, USA, 11-40.

Nugaliyadde L, 1984. Biology of rice thrips, *Stenchaetothrips biformis* (Bagnall) (Thysanoptera: Thripidae) and a greenhouse rearing technique. J Econ Entomol 77, 1171-1175.

Nyasani JO, Meyhöfer R, Subramanian S, Poehling HM, 2012. Effect of intercrops on thrips species composition and population abundance on French beans in Kenya. Entomol Ex Appl 142, 236-246.

O´Brien LB, Wilson SW, 1985. Planthoppers systematic and external morphology. In: The leafhoppers and planthoppers. Ed. by Nault LR, Rodriguez JG, John Wiley and Sons, New York, USA, 61-102.

Oetting RD, Beshear RJ, 1993. Biology of the greenhouse pest *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Int J Pure Appl Zool 4, 307-315.

Olaniran OA, Sudhakar AVS, Drijfhout FP, Dublon IAN, Hall DR, Hamilton JGC, Kirk WDJ, 2013. A male-predominant cuticular hydrocarbon, 7-methyltricosane, is used as a contact pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem Ecol 39, 559-568.

Pelikan J, 1951. On carnation thrips, *Taeniothrips dianthi* Priesner. Folia Entomol 14, 5-38.

Premachandra W, Borgemeister C, Chabi-Olaye A, Poehling HM, 2004. Influence of temperature on the development, reproduction and longevity of *Ceratothripoides claratris* (Thysanoptera: Thripidae) on tomatoes. B Entomol Res 94, 377-384.

Priesner H, 1928. Die Thysanopteren Europas. Wagner-Verlag, Wien, Austria.

Sastrosiswojo S, 1991. Thrips in vegetables in Indonesia. In: Thrips in South East Asia. Ed. by Talekar NS, AVRDC Publications, Bangkok, Indonesia, 12-17.

Shitatani S, Tsutsumi T, 2005. Ultrastructure of sternal glands in *Tenothrips frici* (Uzel) (Thysanoptera: Terebrantia). Proc Arthropod Embryol Soc Jpn 40, 35-39.

Shitatani S, Tsutsumi T, 2006. Ultrastructure of sternal glands in *Hydatothrips* (*Hydatothrips*) *abdominalis* (Kurosawa) and *H.* (*Neohydatothrips*) *gracilicornis* (Williams) (Thysanoptera: Terebrantia). Proc Arthropod Embryol Soc Jpn 41, 59-65.

Shull AF, 1914. Biology of the Thysanoptera. II. Sex and life cycle. Am Nat 48, 236-247.

Singh SR, Allen DJ, 1980. Pests, diseases, resistance and protection of (*Vigna unguiculata* (L.) Walp. In: Advances in Legume Science. Ed. by Summerfield RJ, Bunting AHK, Kew Publishing, London, UK, 419-443.

Stacey AD, Fellows D, 2002. Temperature and development rates of thrips: Evidence for a constraint on local adaption? Eur J Entomol, 399-404.

Stannard L, 1968. The thrips, or Thysanoptera, of Illinois. Bull Ill Nat Hist Surv 29, 215-552.

Sudo M, Tsutsumi T, 2002. Ultrastructure of sternal glands in two thripine thrips and one plaeothripinae thrips (Thysanoptera: Insecta). Proc Arthropod Embryol Soc Jpn 37, 35-41.

Sudo M, Tsutsumi T, 2003. Ultrastructure of the primordia of sternal glands in the second-instar pupal stage of *Frankliniella intonsa* (Thysanoptera: Insecta). Proc Arthropod Embryol Soc Jpn 38, 27-29.

ThripsWiki (2016). *ThripsWiki - providing information on the World's thrips*. <http://thrips.info/wiki/Main_Page> [Retrieved January 25, 2016]

Ullman D, Sherwood JL, German TL, 1997. Thrips as vectors of plant pathogens. In: Thrips as Crop Pests. Ed. by Lewis T, CAB International, Oxon, UK, 539–565.

van Rijn PCJ, Mollema C, Steenhuis-Broers GM, 1995. Comparative life-history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. Bull Entomol Res 85, 285-297.

Waiganjo MM, Mueke JM, Gitonga LM, 2008. Susceptible onion growth stages for selective and economic protection from onion thrips infestations. In: Proceedings of the international Symposium on Sustainability through Integrated and Organic horticulture. Ed. by Prange RK, Bishop SD, International Society of Horticultural Science, Leuven, Belgium,193-199.

Wang Y, Engel MS, Rafael JA, Dang K, Wu H, Wang Y, Xie Q, Bu W, 2013. A Unique Box in 28S rRNA Is Shared by the Enigmatic Insect Order Zoraptera and Dictyoptera. PLOS ONE 8, e53679.

Wangai AW, Mandal B, Pappu HR, Kilonzo S, 2001. Outbreak of *Tomato spotted wilt virus* in Tomato in Kenya. Plant Dis 85, 1123.

Wheeler WC, Whiting M, Wheeler QD, Carpenter JM, 2001. The Phylogeny of Exant Hexapod Orders. Cladistics 24, 113-169.

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC, 1997. The Strepsitera Problem: Phylogeny of the Holometabolous Insect Orders Inferred from 18S and 28S Ribosomal DNA Sequences and Morphology. Syst Biol 46, 1-68.

Willmann R, 2005. Phylogenese und System der Insecta. In: Lehrbuch der Speziellen Zoologie, Band 1 Wirbellose Tiere, Teil 5 Insecta. Ed. by Dathe HH. Spektrum Akademischer Verlag, Heidelberg, Germany, 1-65.

Wrensch DL, Ebbert MA, 1993. Evolution and diversity of sex ratio in insects and mites. Chapman and Hall, New York, USA.

Yoshizawa K, Saigusa T, 2001. Phylogenetic analysis of paraneopteran orders (Insecta: Neoptera) based on forewing base structure, with comments on monophyly of Auchenorrhyncha (Hemiptera). Syst Entomol 26, 1-13.

Yoshizawa K, Johnson KP, 2005. Aligned 18S for Zoraptera (Insecta): Phylogenetic position and molecular evolution. Mol Phylogenet Evol 37, 572-580.

Zhang ZJ, Wu QJ, Li XF, Zhang YJ, Xu BY, Zhu GR, 2007. Life history of western flower thrips, *Frankliniella occidentalis* (Thysan., Thripidae), on five different vegetable leaves. J Appl Entomol 131, 347-354.

Zhang P-J, Zhu X-Y, Lu Y-B, 2011. Behavioural and chemical evidence of a male-produced aggregation pheromone in the flower thrips *Frankliniella intonsa*. Physiol Entomol 36, 317-320.

zur Strassen R, 2003. Die terebranten Thysanopteren Europas. Goecke and Evers, Keltern, Germany.

CHAPTER 2

MORPHOLOGY OF THE EXTERNAL AND INTERNAL REPRODUCTIVE ORGANS OF SELECTED INSECT SPECIES, WITH SPECIAL FOCUS ON THYSANOPTERA

2.1 Summary

To understand the functional connection between the different aspects of reproduction, a detailed knowledge is needed of the internal and external morphology. This chapter provides an introduction to morphological characteristics associated with reproduction, and on the biology of selected species, rather than a presentation of new insights. For that reason, after a brief summary of the general morphology of structures associated with reproduction in pterygote insects, a detailed description of the biology and reproductive system of the selected species is given.

Abbreviations:

abd-abdomen, acc gl-accessory gland, aed-aedeagus, bc-bursa copulatrix, bl-basal lobe, c-chitin capsule, cc-conducting canal, cer-cerci, cov-common oviduct, dl-dorsal lobe, edth-endotheca, ejb-ejaculatory bulb, ejd-ejaculatory duct, ep 1-3-epithelilal layer 1-3, eppt-eppiproct, f-follicle epithel, fc-fat cells, g-germarium, glc-gland cells, gpr-gonopore, lov-lateral oviduct, mb-mushroom bodies, met-metathorax, mg-midgut, M l abd ventr- Musculus longitudinalis abdominis ventralis, ms-muscle, mt-malpighian tubules, n-nucleus, nu-nutrition cord, o-oocyte, o gl-orange gland, ov-ovary, ovp-ovipositor, p-paramere, paed-primitive aedeagus, papt-paraproct, par vag gl-parietovaginal gland, pbl-parabasal lobe, phb-phallobase, phl-phallus, phtc-phallotheca, phtr-phallotrema, pm-peritoneal membrane, pmr-primitive aedeagus, pph-paraphyses, pIX-paratergit IX, r- reservoir, sbu-spermathecal bulb, sc-secretory cell, sem v- seminal vesicle, sen-sensilla, smp-sigmoid process, spth-spermatheca, spth d-spermathecal duct, spth gl-spermathecal gland, sp-spermatozoa, stgl-sternal gland, sty-stylus, t-testes, tc-trophic core, tf-terminal filament, tph-tropharium, vag-vagina, vd-vas deferens, 1-3-compartment 1-3, 1vl-1st valvulae, 2vl-2nd valvulae, 3vl-3rd valvulae, 1vfl-1st valvifer, 2vfl-2nd valvifer

2.2 General morphology of external and internal reproductive system in pterygote insects

The 10th abdominal segment is the location of the primary male external genitalia, and therefore also termed the genital segment (Fig. 1A). The median intromittent apparatus, the phallic organs, are derived from the 9th segment, and function in coition. Accessory genital structures may be derived from the genital segment or from adjacent ones, and comprise the periphallic organs that function in grasping or clasping during copulation.

Generally, the phallus consists of more or less distinct parts and may be provided with accessory structures. The phallobase is the basal part of the phallus. An aedeagus, a more or less slender terminal part is located on the phallobase. The outer walls of both parts constitute the ectophallus, whereas the inner walls form the endophallus, which is usually invaginated at the end of the organ. In some species the endophallus is eversible, but it is sometimes distinct as a permanent internal structure.

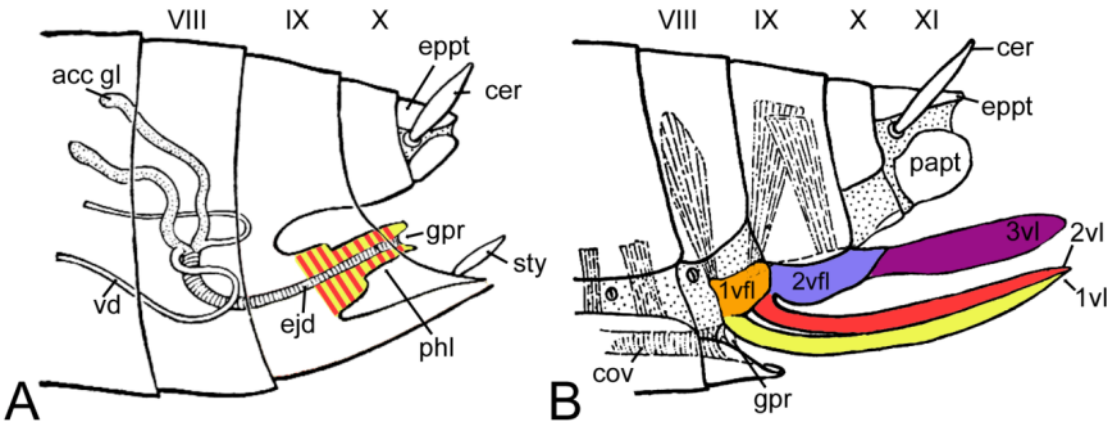


Fig. 1 Scheme of outer male (A) and female (B) genitalia in insects, colors indicate for presumed homology, adapted after Snodgrass 1935, Scudder 1971, Grimaldi and Engel 2005

The basic form described above is commonly modified in some way. When the distal phallobase develops a fold about the base of the aedeagus, this fold may be produced into a tubular sheath that encloses the aedeagus and is called the phallotheca. In this case, the aedeagus is reduced or completely suppressed. The inner lining of the phallotheca is termed the endotheca. Lobes or other processes derived from the phallobase are frequent, and are commonly named parameres (reviewed in Snodgrass 1935, Seifert 1970, Weber 1974, Grimaldi and Engel 2005).

The typical ovipositor of female pterygote insects consists of a shaft, a basal apparatus and a pair of accessory lobes (Fig. 1B). The shaft comprises two pairs of elongate processes: ventrally the first valvulae and dorsally the second valvulae (valve). The 2nd valvulae are often united to form a single median dorsal piece. Both valvulae are fixed at the base to their corresponding 1st and 2nd valvifers. An accessory lobe (3rd valvulae) arises on the posterior ends of the 2nd valvifers, with a function that varies among taxonomic groups. Usually it ensheates the distal

part of the shaft, but in Orthoptera it develops a third pair of blades (reviewed in Snodgrass 1935, Seifert 1970, Weber 1974, Grimaldi and Engel 2005).

Determination of homologies of male and female genitalic structures is difficult because of the great structural diversity among male genitalia (Snodgrass 1935). Presumably the phallus of pterygote insects is in part homologous with the female 1st and 2nd valvulae (Fig. 1), whereas the parameres correspond to the 2nd valvifers (reviewed in Scudder 1971, Weber 1974).

The internal reproductive organs host the germ cell line, providing their nutrition, and additional structures involved in their development and fertilization, as well as different gland structures that ensure their functionality (Snodgrass 1935, Gullan and Cranston 2010).

The male internal organs correspond to the female structures: a pair of testes, each consisting of a distinct number of testis- tubules opening into the mesodermally derived vas deferens (sperm duct). Posteriorly, the sperm duct may be expanded to form a sperm storage organ (seminal vesicle). Secretions of mesodermal and/or ectodermal derived glands assist sperm transfer, sperm activation, and sperm maintenance, also induction of desired female behavior after sperm transfer (Simmons 2001).

The female reproductive organs consist of a pair of ovaries, two lateral oviducts, and a median oviduct that receives the lateral oviducts anteriorly and opens to the gonopore posteriorly. Additional structures may include a spermatheca for storing received sperm, and accessory glands with diverse possible functions such as secretions for egg protection or egg adhesion (Gullan and Cranston 2010). The exit duct from the median oviduct may be tubular (vagina) or modified to form a copulatory pouch (genital chamber) (Snodgrass 1935).
Each ovary comprises a distinct number of egg- tubes or ovarioles, this number depending on the taxonomic position, as well as the lifestyle and size of the insect (Chapman 2013). Some viviparous aphids possess only one ovariole per ovary, whereas queens of *Eutermes* have over 2,000 (Chapman 2013). The ovarioles lead via a pedicel into the lateral oviducts, which unite to form the common oviduct. These connecting ducts consist typically of columnar or cuboid cells on a basal lamina, surrounding by a muscle layer. Being ectodermal in origin, the median oviduct is lined with cuticle (Chapman 2013).The opening of the median oviduct is usually concealed within an inflection of the body wall. This inflection typically forms a cavity, the genital chamber, and this chamber often has a specialized function during mating and is termed the bursa copulatrix (Gullan and Cranston 2010).

Within the different orders examined, various authors have used different structural nomenclature, depending on their personal background and history. To ensure greater comparability between species, I have used the definitions given by Snodgrass (1935), because these refer more to function than to homology. The terms are described in the following glossary. Synonyms used by other authors are given in the corresponding tables in the text.

Glossary:

Table 1 Glossary of male reproductive organs, adapted after Snodgrass (1935)

Male organs	Definition
Accessory gland	mucous glands opening into the ejaculatory duct
Aedeagus	distal part of the phallus, usually the principal part of the intromittent organ, typically a sclerotic tube
Basal plates	sclerites of the phallobase
Ectophallus	outer phallic wall in distinction to the endophallus
Ejaculatory duct	ectodermal outlet duct of the male genital system
Endophallus	inner chamber of the phallus invaginated at the end of the aedeagus, into which the ejaculatory duct opens, typically a eversible sac or tube, but sometimes a permanently internal phallic structure
Endotheca	inner wall of the phallotheca
Epimere	a dorsal process of the phallobase
Genital chamber	ventral invagination of the conjunctival membrane between the ninth and tenth abdominal segment containing the phallic organs
Genitalia	the genital organs collectively, but usually applied only to the external genitalia
Gonopore	in the male the external opening of the median ejaculatory duct, usually concealed in the endophallus, or one of the apertures of paired exit ducts
Parameres	lateral processes or lobes of the phallobase
Penis	one of the paired intromittent organs of certain insects, or usual median phallic organ
Phallobase	proximal part of the phallus, highly variable in its development, sometimes a large structure supporting the aedeagus, often produced into a thecal fold or sheath about the aedeagus, sometimes represented only by basal phallic sclerites in the wall of the genital chamber
Phallomeres	genital lobes formed at the sides of the gonopore in ontogeny of some insects, in most cases they unite to form the phallus, but in Blattidae and Mantidae they develop separately into complex genital organs of the adult
Phallotheca	a fold or tubular extension of the phallobase about the aedeagus
Phallotreme	distal opening of the endophallus, usually at the end of the aedeagus
Phallus	unpaired penis, or median intromittent organ, including the phallobase, the aedeagus, the endophallus, and various processes of the phallobase and the aedeagus if present
Sperm cyst	one of the cellular capsules in the testes containing the spermatocytes
Subgenital plate	usually the ninth abdominal sternum of the male extended beneath the genital chamber, but sometimes the eighth or seventh sternum
Testicular tubes	one of the secondary divisions of the testis
Vas deferens	one of the lateral ducts of the male reproductive system
Vas efferens	one of the short ducts connecting sperm tubes of the testis with the vas deferens, corresponding to the pedicel of an ovariole
Vesicular seminalis	a dilation of the vas deferens in which the spermatozoa may be retained

Table 2 Glossary of female reproductive organs, adapted after Snodgrass (1935)

Female organs	Definition
Accessory gland	a pair of glands opening primarily on the venter of the ninth abdominal segment, secreting an adhesive substance or material forming a covering or a case for the eggs
Bursa copulatrix	a copulatory pouch of the female, usually the genital chamber or a part of the latter
Common oviduct	median ectodermal outlet duct of the female genital system, usually opening in the genital chamber, or vagina
Follicel cells	inner epithelial cells, which surrounds the egg cells
Genital chamber	in the female a copulatory invagination cavity behind or above the eight abdominal sternum containing the gonopore and the orifice of the spermatheca, often narrowed to form a pouch like or tubular vagina
Germarium	end chamber of an ovarial or testicular tube, containing the primary oögonia or spermatogonia
Gonapophyses	mesal process of the bases of the gonopods, perhaps endites, forming the first and second valvulae of the ovipositor
Gonopore	in the female either one of the paired primitive openings of the lateral oviducts, or the median opening of the oviductus communis
Intervalvulae	sternal sclerites in the venter of the ninth abdominal segment between the second valvifers
Lancets	first valvulae
Lateral oviduct	one of the paired lateral ducts of the female system connected with the ovary, mesodermal origin, but sometimes partly or entirely replaced by an ectodermal branch of the median duct
Ovarioles	one of the secondary divisions of the ovary, composed of terminal filament, egg tube and pedicel
Ovary	female reproductive organ, containing the egg cells
Ovipositor	the egg-laying organ formed of the gonopods of the eighth and ninth abdominal segments, or also, in a functional sense, the egg-laying tube of some insects formed of the protractile terminal segments of the abdomen
Spermatheca	sperm receptacle of the female
Stylet	a median dorsal element in the shaft of the ovipositor formed of the united second valvulae
Subgenital plate	in the female the eight abdominal sternum, or the seventh when the eighth is reduced or obliterated
Vagina	a part of the definitive egg passage in many insects posterior to the true common oviduct, derived from the genital chamber
Valvifers	basal plate of the ovipositor, probably derived from the coxopodites of the gonopods, carrying the valvulae, including first valvifers of the eighth abdominal segment, and second valvifers of the ninth segment
Valvulae	three pairs of processes forming the blades and ensheathing lobes of ovipositor; the first and second valvulae are gonapophyses of the gonopods, the third valvulae are distal outgrowths of the coxopodites of the ninth abdominal segment
Vulva	external opening of the genital chamber or vagina serving in most cases for both copulation and the discharge of the eggs, sometimes on the eighth abdominal segment, sometimes on the ninth.

2.3 Biology and morphology of the individual species

As mentioned in Chapter 1, the order Thysanoptera belongs to the superorder Paraneoptera. Some authors consider Hemiptera as sister group to Thysanoptera, a second economically important group. Therefore, species were selected from this group and their reproductive systems were analysed using light- and electron microscopy in order to show the various forms. Detailed descriptions of methods are given in Supplementary Material S2 on the attached CD.

2.3.1 Thysanoptera

2.3.1.1 Biology of *Echinothrips americanus*

Systematic position:

Arthropoda
Insecta
Thysanoptera
Terebrantia
Thripidae
Echinothrips

The genus *Echinothrips* consists of 7 species, with an original distribution in North-, Central- and South America (Mound and Marullo 1996) (Fig. 2), but knowledge of these is sparse. Two species are known only from single specimens (*E. asperatus* and *E. pinneatus* in southern Brazil, Mound and Marullo 1996). Much more frequent are *E. caribbeanus* from the Caribbean area and *E. mexicanus* from Mexico, Honduras, Guatemala and El Salvador. The more northern *E. subflavus* can be found from Quebec to Tennessee on *Tsuga*. *Echinothrips americanus* (Poinsettia thrips) originates from the eastern parts of the United States (Stannard 1968).

Since 2002, *E. americanus* has spread to the whole of North-Eastern USA and Canada (Shipp et al. 2001, Ferguson and Shipp 2002). In 1989 it was intercepted in a British glasshouse (Collins 1998) and subsequently spread rapidly over 20 European countries (Vierbergen et al. 2006, Varga and Fedor 2008, Andjus et al. 2009) (Fig. 2). Recently it has been recorded from Asia and Australia (Thailand: Mound 2000, Japan: Itoh et al. 2003, Thailand and Java: Mound and Ng 2009, China: Mirab-Balou et al. 2010, Northern Australia: Mound et al. 2013) (Fig. 2).

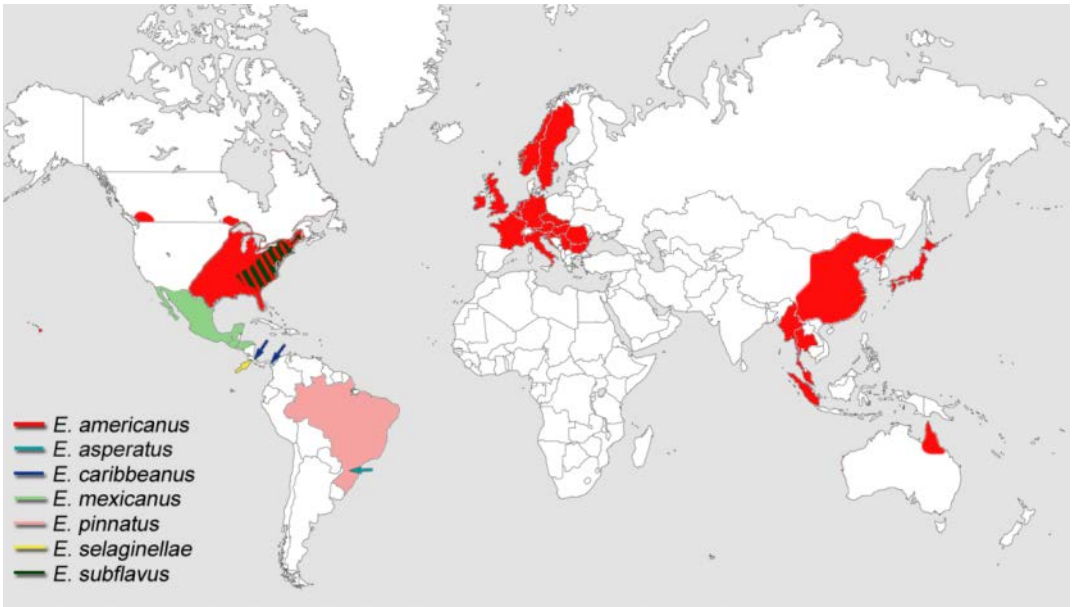


Fig. 2 Worldwide distribution of the family of *Echinothrips*, drawn after published findings mentioned in text

This species is phytophagous with hosts in about 24 plant families (Vierbergen 1998). In the USA, *E. americanus* is confirmed to reproduce on 40 cultivated and 59 wild plant species (Oetting et al. 1993, Plant Health Australia 2010). Their feeding causes small chlorotic areas and shallow feeding punctures, which cause the plant to look shrivel (Oetting and Beshear 1993, Oetting et al. 1993, Trdan et al. 2003) (Fig. 3).

Fig. 3 feeding injury of *Echinothrips americanus* on a leaf of *Phaseolus vulgaris* (left) and a not- infested leaf (right)

E. americanus reproduces via arrhenotokous haplo-diploidy. Females arise from diploid eggs, males from haploid eggs. Whereas Kumm (2002) and Li et al. (2012) observed production of purely male-biased offspring from virgin females, Oetting and Beshear (1993) reported some females developing from unmated females. Under normal lab conditions, the male-sex ratio is 0.3 (Kumm 2002). Females lay their eggs into plant tissue, separately, but details of embryonic development are unknown. Development from first larval-instar to adult takes 7 to 8 days at 23 °C, RH 75 %, L:D 16: 8 (Kumm 2002), during which they pass through two larval instars and two quiescent instars (propupa and pupa) prior to adult eclosion.

Because of the severe host damage, the wide host range and rapid spread, this thrips has the potential to become a major pest, needing further attention and control.

2.3.1.2 Morphology of *Echinothrips americanus*

External male genitalia

The structure of the external male reproductive organs was investigated using SEM, and consist of a phallobase, two parameres, a primitive aedeagus, a phallotheca, an endotheca and an aedeagus surrounded by paraphyses (Fig. 4A-C). Sternite IX serves as a subgenital plate.

The most proximal part of the phallus is the phallobase. This fully sclerotized component opens caudad and turns into the phallotheca (Fig. 4A, C). Two lateral parameres and a ventral primitive aedeagus arise from the phallobase (Fig. 4A).

The two parameres are sclerotized, slighty curved dorsally and about 60 µm long. They taper from about 8 µm at the base to 4 µm at the apex. There are 13 campaniform sensilla on the outer side of each paramere (Fig. 4B, D). Three sensilla are located on the tip of each paramere, four are arranged in a row on the ventral side, two are in a second row beneath the first row, and another four are on the base of each paramere.

Additionally, two trichoid sensilla are located on the outside of each paramere, where the ventrad angulation occurs (Fig. 4D). The inner side does not bear any sensilla.

The primitive aedeagus is a tapering sclerotized extension of the phallobase, located between both parameres. As both primitive aedeagus and parameres can be moved independently of one another, the connection of the primitive aedeagus and the phallobase appears to be membranous and flexible. No additional structures were apparent.

From the phallobase, the phallotheca emerges caudad. The two sclerotized parts of the phallotheca are connected via a membranous structure. Both parts form a cavity, which contains the retracted endotheca and the aedeagus.

The membranous endotheca is an eversible sac, which merges into the aedeagus at the tip.

The aedeagus is characterized by a short sclerotized plate, lined by two chitinous hooks (so called paraphyses) of about 5 µm length. In no samples was the terminal opening of the ejaculatory duct visible; presumably it was concealed by the membrane of the endotheca.

When the phallus is retracted into the genital chamber, the phallotheca, endotheca and parameres are partly placed into the ventral chamber of tergite X.

The postgenital segments are situated dorsally to the phallus. While the 10^{th} segment lacks a corresponding sternite, the sclerites of the 11^{th} segment are extremely reduced.

Internal male genitalia

The inner male reproductive organs consist of two testes, two associated vasa deferentia with seminal vesicles, a pair of accessory glands and an ejaculatory duct (Fig. 4G, H).

Each of the oval testes (70 µm x 20 µm) is located in the 6^{th} and/ or 7^{th} segment. They are surrounded by a thin orange-colored pigment layer, a thin muscle layer and an inner epithelium (Fig. 4E, F). The testes contain only one cyst. In adults only mature sperm are visible (Fig. 4E). The filamentous spermatozoa are distributed randomly (Fig. 4E), and were 75.3 ± 6.7 µm (n = 122) in length and without any distinct head region.

The testes join the vasa deferentia, which have the same structural organization as the testes. These ducts dilate and posteriorly form a small seminal vesicle, which leads to an ejaculatory bulb (Fig. 4H) where the accessory gland material is released (Fig. 4G). From the ejaculatory

bulb, the ejaculatory duct arises and provides the connection to the external genitalia (Fig. 4G, H).
The accessory gland consists of a pair of elongate tubes, surrounded by a single-layer epithelium. These glands extend from the 5th-6th to the 7th-8th segment and are up to 40 µm in diameter (Fig. 4G).

External female genitalia
The ovipositor is formed by appendages of the 8th and 9th segment (Fig. 5A). This egg-laying organ is composed of the basal apparatus and the shaft. The basal apparatus consists of the 1st and 2nd valvifers and the basalvalvulae. The shaft is provided by the 160 µm long 1st and 2nd valvulae (Fig. 5B). The 1st valvulae are toothed ventrally, whereas the 2nd valvulae are toothed dorsally (Fig. 5B). Three campaniform sensilla are located on the outside of the 1st valvulae (Fig. 5B, C). A third valvula is missing. When the ovipositor is retracted, it lies in a cavity derived from the 9th and 10th segments, and is protected by the ventral margins of the 9th segment.

Internal female genitalia
The inner genital system comprises a pair of ovaries, two lateral oviducts, common oviduct, vagina and a spermatheca (Fig. 5D,E). Each ovary consists of 4 secondary panoistic ovarioles (Fig. 5E). The ovaries are connected via a ligament to the thorax and extend up to the 6th segment. In segment 7 the lateral oviducts connect the ovarioles with the vagina in segment 7 and 8 via the common oviduct (Fig. 5D). The common oviduct and vagina form a tube, which can greatly expand during copulation (see Chapter 3). The spherical spermatheca is located dorsally on the vagina (Fig. 5D). In mated females a circular sperm ball, about 20 µm in diameter, is visible inside the spermatheca (Fig. 5D). Females possess an accessory gland dorsal in segments 7 and 8, which is formed by an oval bladder of secretory cell (Fig. 5D). The efferent duct is connected with the median ovipositor.

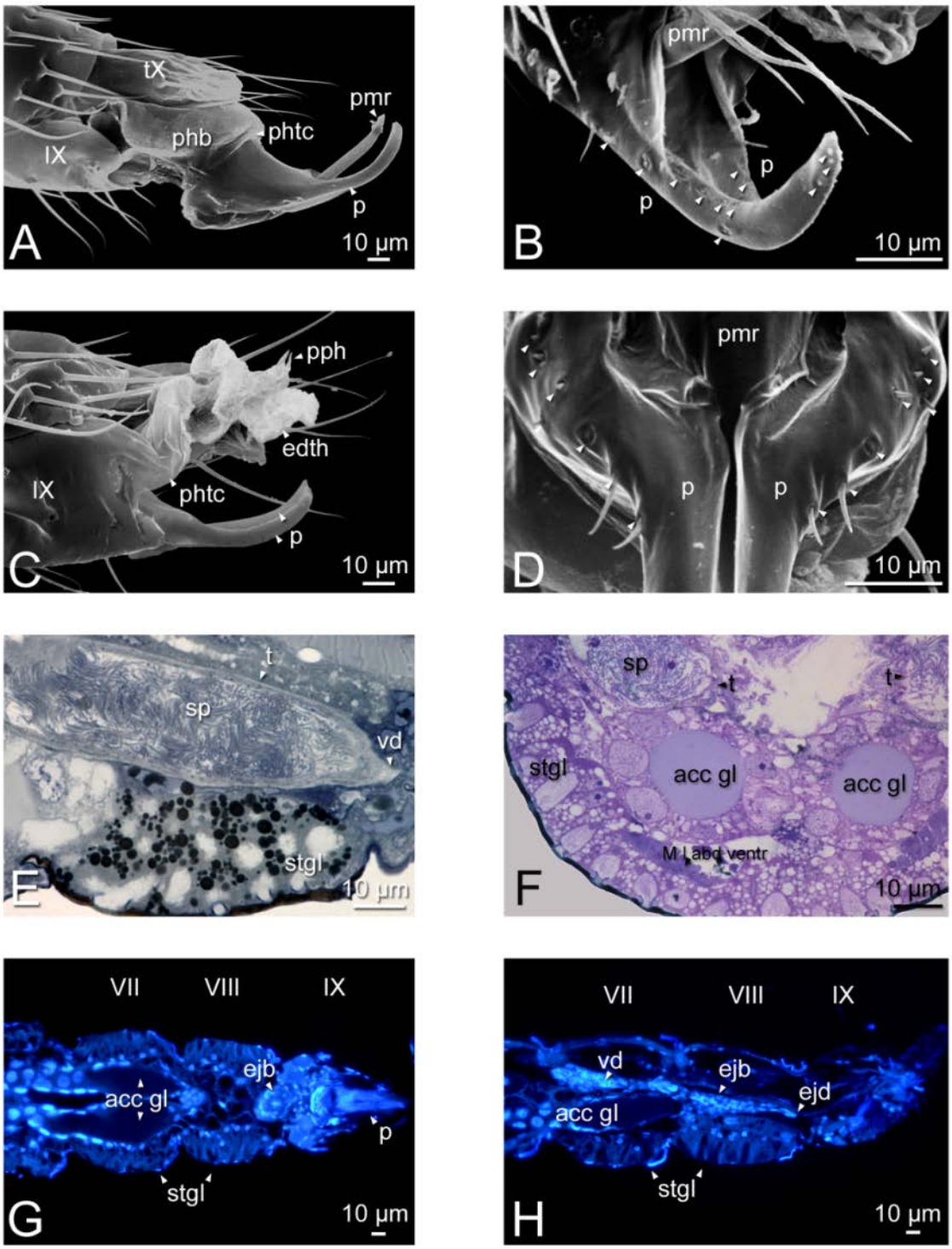


Fig. 4 External and internal reproductive organs of male *Echinothrips americanus*

A Partly everted external male genitalia with parameres, primitive aedeagus and phallobase, lateral, SEM

B Detailed dorsal view on parameres with sensilla on the outer side, SEM

C External male genitalia with evaginated endotheca and visible paraphyses, lateral, SEM

D Ventral view on phallobase and origin of parameres and primitive aedeagus, SEM

E Testes with randomly distributed spermatozoa within the 6th segment, sagittal, toluidine-blue, semithin section

F Accessory glands and testes in 7th segment, transversal section, toluidine blue, semithin section

G Segment 7 to 9 with accessory glands and ejaculatory bulb, frontal, DAPI, paraplast section

H Segment 7 to 9 with transition of accessory gland, vas deferens, ejaculatory bulb to the ejaculatory duct, sagittal, DAPI, paraplast-section

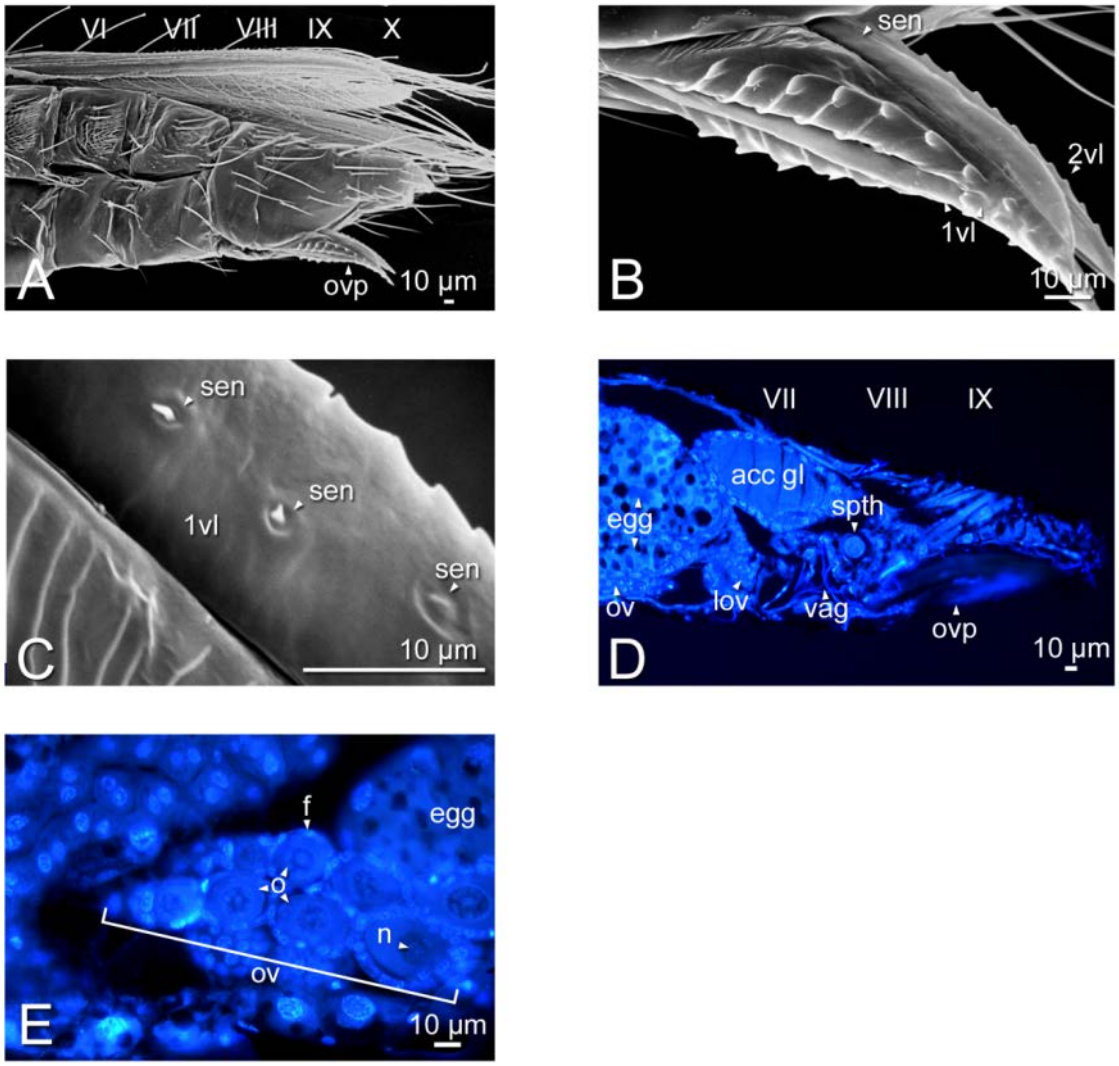


Fig. 5 External and internal reproductive organs of female *Echinothrips americanus*

A Everted ovipositor, lateral, SEM

B Detailed view of ovipositor with 1st and 2nd valvulae, note the location of the sensilla near the base of the 1st valvulae, lateral, SEM

C Detailed view of sensilla campaniformes on the outside of the 2nd valvulae, lateral, SEM

D Internal genitalia with ovary, lateral oviduct, filled spermatheca and accessory gland, note the ready-to-lay-eggs after vitellogenesis within the ovary, sagittal, DAPI; paraplast section

E Detailed view of ovary with distinct ovarioles and oocytes; upper right, a ready-to-lay-egg after vitellogenesis can be seen, sagittal, DAPI, paraplast section

2.3.2 Hemiptera

2.3.2.1 Biology of *Pyrrhocoris apterus*

Systematic position:

Arthropoda
Insecta
Hemiptera: Heteroptera
Pyrrhocoridae
Pyrrhocoris

The family Pyrrhocoridae comprises about 340 species in 33 genera, and is distributed mainly in paleotropical or subtropical-palearctic areas. Only a few species, including *Pyrrhocoris apterus* have a more northern distribution in temperate parts of the palaearctic region (Stichel 1959, Kulik 1973, Puchkov 1974, Socha 1993). *Pyrrhocoris apterus* also occurs in the USA, Central America and India (Barber 1911).
This species feed mainly on seeds of *Tilia cordata* or other Malvaceae (Tischler 1959, Polohencev and Polohencev-Korovina 1961). But in contrast to other species of the Pyrrhocoridae, it is more polyphagous and can easily adapt to other food sources (Socha 1993).

Adults of *P. apterus* undergo a diapause, which is controlled by photoperiod. In Central Europe the diapause lasts from August to December. From December to spring the adults adopt a post-diapause state that is triggered by starvation (Socha 1993). They spend this time under litter, mostly at the base of lime trees. In Germany, the adults start to mate in early April and then oviposit (Tischler 1959), and they reproduce via normal diploid bisexual reproduction. Mating takes from a few minutes to several hours (Zdarek 1967 in Socha 1993). Females lay egg-batches of up to 50 individual eggs loosely on the ground (Tischler 1959). Newly laid eggs have an ovoid shape and are white to off-white in color, turning to yellow-red during embryonic development (Rizki and Slama 1968, Smith and Forrest 1969). Embryonic development takes 7.5 days at 25 °C (Matolin 1973a), but the exact duration depends on temperature (Socha 1993). There are 5 nymphal instars. Instars 1 to 4 lasts about 10 to 14 days, whereas the last instar takes about 7 to 10 days (Rizki and Slama 1968, Socha 1993). Immature stages tend to aggregate, and this is triggered by visual and chemical stimuli (Staddon and Daroogheh 1982, Schmuck 1987).
Newly moulted adults are able to mate by the end of summer, but they overwinter and normally copulate in the subsequent spring. Therefore, this species is univoltine in our temperate zone (Tischler 1959).

2.3.2.2 Morphology of *Pyrrhocoris apterus*

External male genitalia

The outer male genitalia comprise a phallobase, a phallotheca, an endotheca and an aedeagus (Fig. 6B, C). From the basal sclerotized phallobase, the phallotheca arises (Fig. 6B). It is characterized by a weakly sclerotized region with several cuticular ridges on the ventral, but not dorsal, side (Fig. 6B). A membranous endotheca is invaginated within the phallobase and can be extended during copulation. The endotheca is tubular with 3 different lobes (Fig. 6B,C); medially, a basal lobe (0.6 mm in length) and a parabasal lobe (0.4 mm in length) (termed after Gapon 2007) are present. A dorsal lobe arises on the tip of the endotheca dorsally, whereas the aedeagus is situated ventrally (Fig. 6D). The aedeagus forms an intense sclerotized hook (0.7 mm in length) with the phallotrema on its tip (Fig. 6B, D).

Additionally, the 9^{th} segment bears two lateral hook-shaped styli (harpagones), which are periphallic organs with individually provided muscles (Fig. 6A).

Internal male genitalia

The genitalia consist of a pair of testes, two seminal vesicle, two vasa deferentia, two accessory glands, an ejaculatory bulb and an ejaculatory duct (Fig. 6E, F, G).

Each testis comprises 7 testicular tubules, each of which contains several cysts. Production of sperm and maturation occur during migration from the anterior to the posterior end (Fig. 6E). The mature sperm are 623.4 ± 111.2 µm (n = 100) long and filamentous in shape without any visible head region. They are stored within the adjacent tubular seminal vesicle (about 290 µm x 65 µm) posterior to each testis (Fig. 6E, F). The large seminal vesicle leads to the vasa deferentia, which are surrounded by the spongy accessory gland (Fig. 6F). Both vasa deferentia connect into the ejaculatory bulb, which in turn connects with the ejaculatory duct and the outer genitalia (Fig. 6F, G). The ejaculatory bulb is characterized by a dorsad muscle- and ventrad gland layer of three different epithelial coats (Fig. 6G).

External female genitalia

The genital segments 8 and 9 are a dark blackish in color, which distinguishes them from the other segments with red-colored marks. Sternites VIII and IX are short and plate-shaped (Fig. 7A). The female genital opening is covered by the VIII sternite (Fig. 7A). In this plate-shaped ovipositor type, the whole typical ovipositor apparatus is dorso-ventrally flattened, with the paratergites and gonocoxites (sternite VIII, IX) providing protection to the genital aperture (Tuxen 1970).

Internal female genitalia

The internal genitalia are formed by paired ovaries, two lateral oviducts, a common oviduct and a vagina with an attached spermatheca. Several glands support the functionality of the genitalia. Each ovary consists of 7 meroistic, telotrophic ovarioles (Fig. 7B, C). They are connected via a short pedicel with the lateral oviducts, which lead to the common oviduct (Fig. 7I). These structures are surrounded by a muscle layer. The common oviduct is attached to the ventral bursa copulatrix, the sac-like vagina with a thin sclerotic intima (Fig. 7H). On the

opposite side, the spermathecal duct arises from a spermathecal bulb, an extension of the vagina (Fig. 7H); this duct leads to the mushroom-shaped spermatheca (Fig. 7D, E). Both structures are lined with chitin (Fig. 7E, G). The spermatheca is build of a short stalk, surrounded by longitudinally orientated muscles, and a capsule-shaped head region (Fig. 7D, E). In the middle of the stalk a cuticular valve is integrated to control sperm flow. The head region is enclosed by glandular cells class 3 (according to Noirot and Quennedey 1974), which are characterized by different cell types of secretory- and conducting cells with extracellular cavities and conducting canals (Fig. 7F, G). After mating the stored spermatozoa are arranged evenly in a bundle.
Females of *P. apterus* possess two more additional glands. The orange-gland is located within the common oviduct (Fig. 7I), but the shape is dependent on mating activity and egg development. The gland consists of elongate columnar cells, basal nuclei and a microvilli-bordered secretion area (Fig. 7I). Basally, it is bordered by a basal lamina and an adjacent muscle layer; apically it is framed by the cuticular intima. The second gland, the parietovaginal gland, is located within the bursa copulatrix near the spermathecal bulb. It comprises a row of cells with basal nuclei and an apical secretion zone (Fig. 7H).
A third gland, the oil-gland (uradenies), belongs to the paragenital glands and is therefore not described here.

Describing the external male organs has proved to be difficult, because various authors (see Tab. 3) used different names and definitions. In order to improve comparability, structural term used here follow Snodgrass (1935) (Tab. 1, 2).
One example of the problems encountered is as follows. Merle (1969) referred to the styli (harpagones) as parameres, which should be connected to the phallotheca according to the definitions of Snodgrass (1935). But the styli are attached via a membran to the periphallic organs of the 9^{th} segment. Additionally in several studies (Ludwig 1926, Merle 1969, Gapon 2007) phallotheca and endotheca are not divided into two structural processes, but our SEM studies support recognition of two independently sclerotized structures (Fig. 6).

Table 3 Comparison of used terms in this study with other studies of morphology of external male organs in *Pyrrhocoris apterus*

Male *Pyrrhocoris apterus*	Name in this study	Synonyms	Reference
	phallotheca	theca	Gapon 2007
		phallosoma	Merle 1969
		basale Penisblase	Ludwig 1926
	endotheca	conjunctiva	Merle 1969
		Penisblase	Ludwig 1926
	lobi (basal,	processus conjunctivae	Merle 1969
	parabasal)	Flügelplatten	Ludwig 1926
	aedeagus	penis	Merle 1969
		vesica	Gapon 2007
		Hakenrohr	Ludwig 1926
	mushroom bodies	processus capitati	Merle 1969
		muschelförmige Platte	Ludwig 1926
	styli	genital styles	Yang 2005
		parameres	Merle 1969
		Genitalhaken	Ludwig 1926
Female *Pyrrhocoris apterus*			
	bursa copulatrix	vagina	Merle 1969
		gynatrium	Stys 1960
		ovipositor posterior	Ludwig 1926
	spermathecal bulb	gynatrial sack	Stys 1960
		diverticulum duct	Pluot 1970

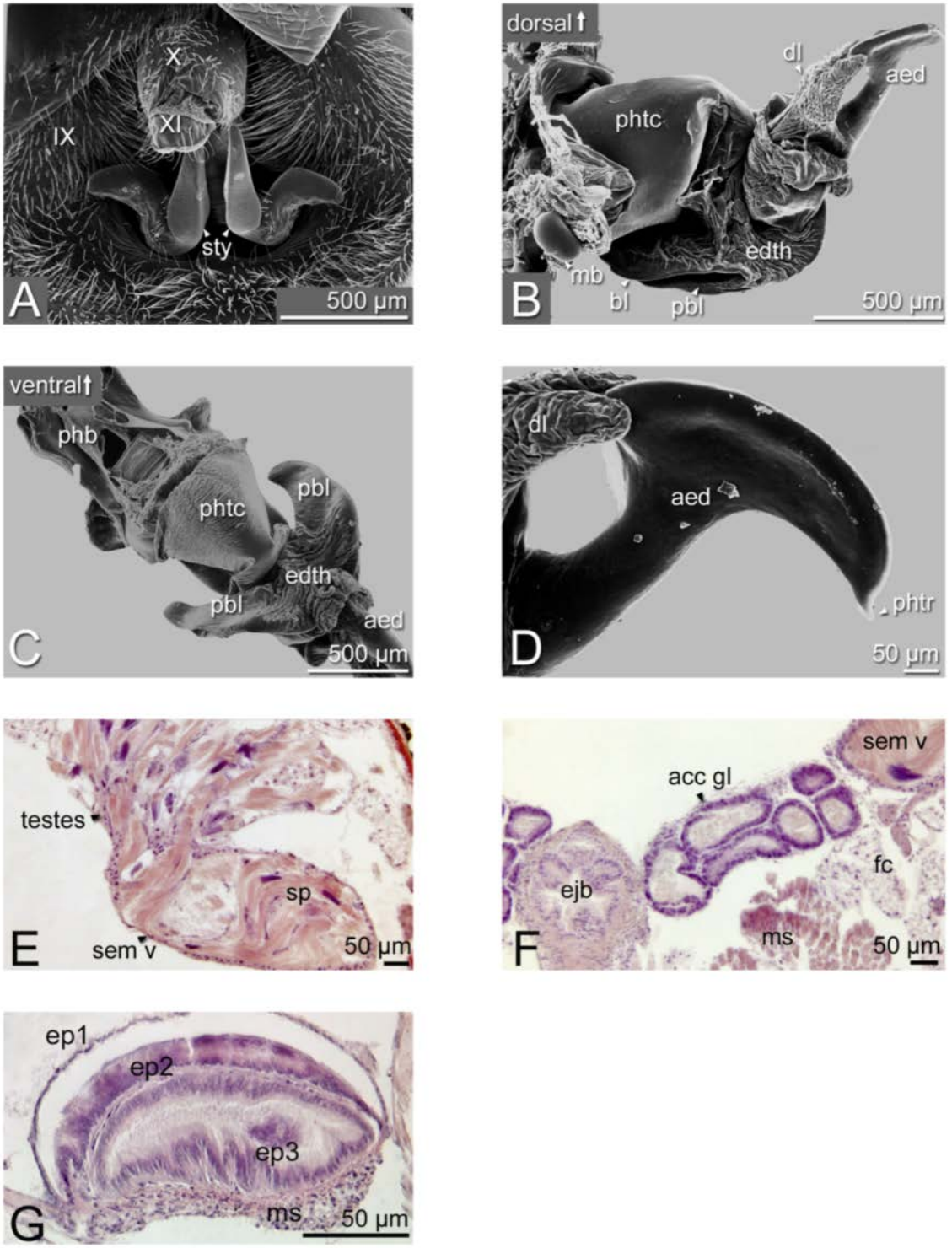


Fig. 6 External and internal male reproductive organs of *Pyrrhocoris apterus*

A Genital segment 9 with evaginated styli and segment 10 and 11, SEM

B Dissected and partly evaginated phallus with phallotheca, endotheca and aedeagus, dorsad-lateral, SEM

C Dissected and evaginated phallus with phallobase, sclerotized phallotheca, endotheca and aedeagus, ventral, SEM

D Detail of sclerotized aedeagus and dorsal lobe of the endotheca, lateral, SEM

E Transition of testes tubes with mature spermatozoa and seminal vesicle, frontal, HE, paraplast section

F Transition of seminal vesicle, vas deferens surrounded by the accessory gland and the muscular ejaculatory bulb, frontal, HE, paraplast section

G Ejaculatory bulb with three epithelial layers and a muscle layer, sagittal, HE, paraplast section

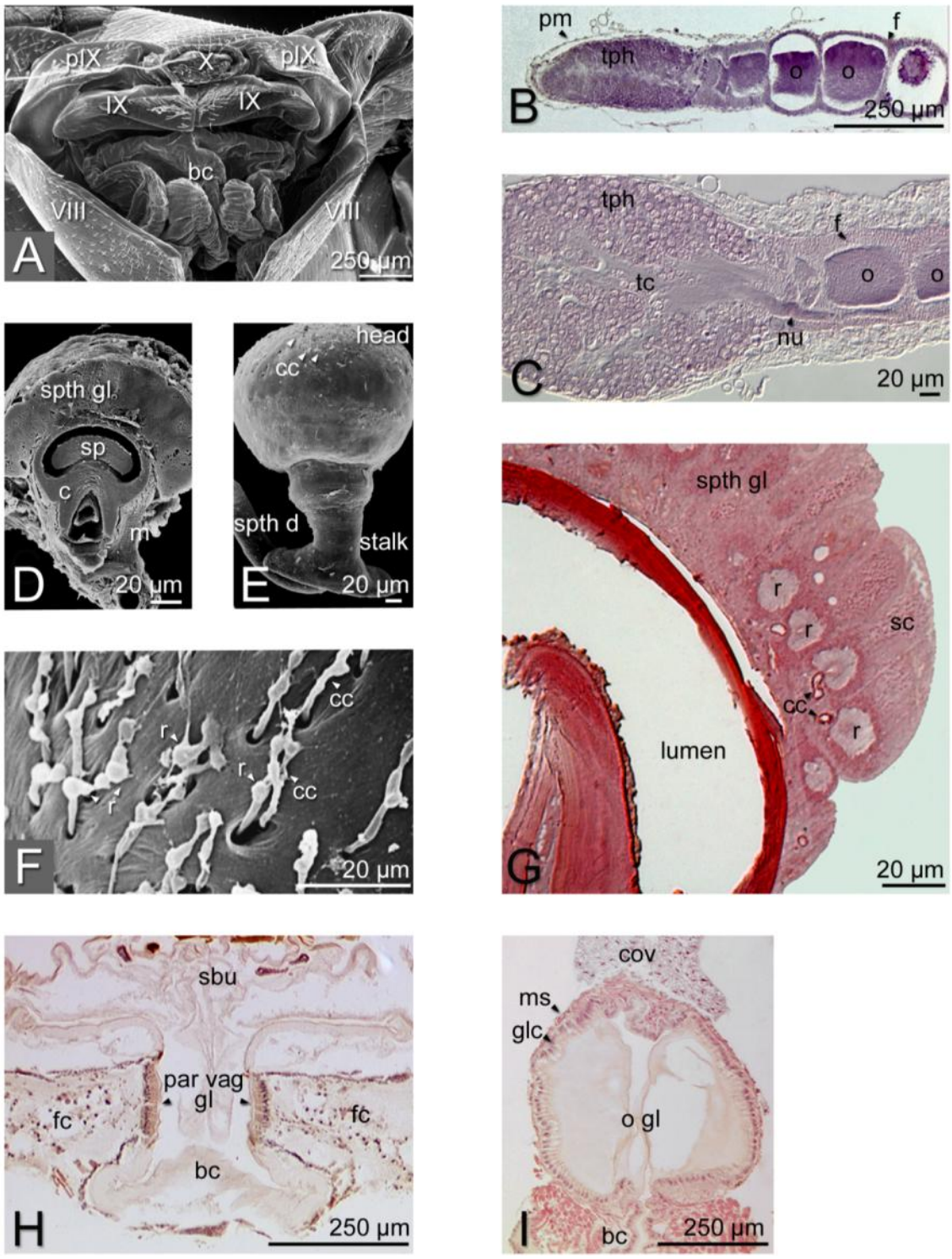


Fig. 7 External and internal reproductive organs of female *Pyrrhocoris apterus*

Fig. 7 External and internal reproductive organs of female *Pyrrhocoris apterus*
A Caudad view of external female genitalia with reduced ovipositor, SEM
B Meroistic telotrophic ovariole, paraplast section, HE
C Detailed view of ovariole with nutrition cord, paraplast section, HE
D Half-cutted spermatheca of mated female with sperm inside the head region, note the strong muscles attached to the stalk, SEM
E KOH-treated spermatheca without cellular material, note the conducting canal and reservoirs on the top of the head region, SEM
F Detailed view of head region of spermatheca with conducting canals and reservoirs
G Spermatheca with glandular area and lumen, note the complex gland cells with secretory- , and conducting unit, lateral, semi-thin section, stained with safranin
H Parieto-vaginal gland in transition of bursa copulatrix and spermathecal bulb, frontal, paraplast section, HE
I Orange gland within the common oviduct with large gland cells and releasing area, note the surrounding muscular sheath, frontal, paraplast section, HE

2.3.2.3 Biology of *Campyloneura virgula*

Systematic position:

Arthropoda
Insecta
Hemiptera: Heteroptera
Miroidea
Miridae
Campyloneura

The Miridae is the largest family of true bugs with about 1,200 genera and 10,000 species (Schuh and Slater 1995), but the genus *Campyloneura* includes just this one valid species (Wachmann et al. 2004). This species is about 3.9-4.7 mm in length, and is common from southern Scandinavia, to North Africa and in the southeast through Ukraine to the Caucasus. *C. virgula* lives on several deciduous trees *Tilia, Coryleus, Alnus, Crataegus* and *Carpinus,* but especially on *Fraxinus excelsior.* Nymphs and adults feed on small arthropods and honeydew (Wachmann et al. 2004). This is the only heteropteran species with a thelytokous reproduction mode in Europe (Carayon 1989), where only females are found, despite males being quite common in North Africa (male sex ratio 0.5, Wagner 1968, Carapezzo 1997). The mediterranean region seems to be a transition zone, with only a single crippled male found (Wagner 1958).
Overwintering occurs as an egg, with larval hatching from May to June, and adults found from June to September (Wachmann et al. 2004). Presumably eggs were deposited in plant material, such as twigs. Other details about this species are unknown.

2.3.2.4 Morphology of *Campyloneura virgula*

External female genitalia

Females possess a laciniate ovipositor (Fig. 8A) that is composed of the first and second valvulae (derived respectively from the 8th and 9th segments), fused with the 1st and 2nd valvifers (Fig. 8A, B). The 2nd valvifers form elongate plates and ensheath the ovipositor on both sides when this is not extended (Fig. 8B). The first valvulae bear several campaniform sensilla on their dorsal side, tooth-shaped edges and a distinct ridge, but the second valvulae lack characteristic features (Fig. 8E, F) - only small cuticular markings can be seen near the tip (Fig. 8D). The third valvulae are fused to the ventro-posterior margins of the 2nd valvifers and form a small part of the ovipositor sheath posterior to the abdomen.

Internal female genitalia

There are two ovaries with corresponding lateral oviducts, a short common oviduct, a large genital chamber with several compartments, and an accessory gland (Fig. 8H).

The paired ovaries each comprise 7 meroistic telotrophic ovarioles (Fig. 8E), and each ovary is connected to the gynatrium via a lateral oviduct (Fig. 8G, H). The lateral oviducts have a layer of inner epithelial cells and an outer muscle sheath (Fig. 8G). The following gynatrium (gyn = female, atrium = hall) is a complex ectodermal structure of 3 compartments (Fig. 8H). Several terms for each compartment have been used in the literature (see Tab. 4), but each is defined by its role during copulation (see Snodgrass 1935). Because of the thelytoky of this species, the functions of each compartment could not be investigated. Therefore, each compartment is designated by a number to avoid any inference of a possible function.

The lateral oviducts open directly into the anterior compartment 1 (Fig. 8G, H). This compartment is characterized by a strong cuticular intima and cuboid epithelial cells. As well as the connection to the lateral oviducts, a tube-like accessory gland (accessory gland to compartment 1) is attached (Fig. 8H). Compartments 2 and 3 are posterior to compartment 1. The pouch-like compartment 3 is characterized by two laterally located gland structures within the wall of the compartment; these have elongate columnar cells with basal nuclei (Fig. 8H).

The genital opening is formed by a short and slender vagina, which connects the 2nd valvifers and compartment 1.

Table 4 External female genitalia of *Campyloneura virgula* - comparison of terms used in this study with those used by other authors

Female *Campyloneura virgula*	Name in this study	Synonyms	Reference
	compartment 3	seminal depository	Davis 1955
		gynatrial sack	Masner 1966
		Vorderer Sack	Kullenberg 1947
		spermatheca	Groot & Smid 2000
	compartment 2	gynatrial bulb	Masner 1966
		common oviduct	Davis 1955
		Dorsalsack	Kullenberg 1947
	compartment 1	gynatrial chamber	Masner 1966
	accessory gland of compartment 1	spermathecal gland	Masner 1966
	accessory gland of compartment 3	gynatrial gland	Masner 1966
		vestibulum	Davis 1955
		intervalvular space	Bonhag& Wick 1953

Fig. 8 External and internal female reproductive organs of *Campyloneura virgula*

A Genital segments with the ovipositor shaft comprising the 1st and 2nd valvulae, and the base comprising the 1st and 2nd valvifers, lateral, SEM

B Base of the ovipositor during resting stage with the ensheathing 2nd valvifers, ventral, SEM

C Detail of the ovipositor showing several sensilla on 1st valvulae, lateral, SEM

D Detail of anterior ovipositor showing several sensilla, lateral, SEM

E Detail of meroistic telotrophic ovariole with tropharium and developing oocyte, frontal, HE, paraplast section

F Detail of the anterior ovipositor with markings on the 2nd valvulae and with downward-directed tooth on the 1st valvulae, dorsal, SEM

G Detail of the transition of lateral oviduct, sigmoid process and compartment 1, with sclerotized intima and cuboidal cell, sagittal, HE, paraplast section

H Overview of the internal genital organs with lateral oviduct, all 3 compartments of the gynatrium, both accessory glands and vagina, sagittal, HE, paraplast section

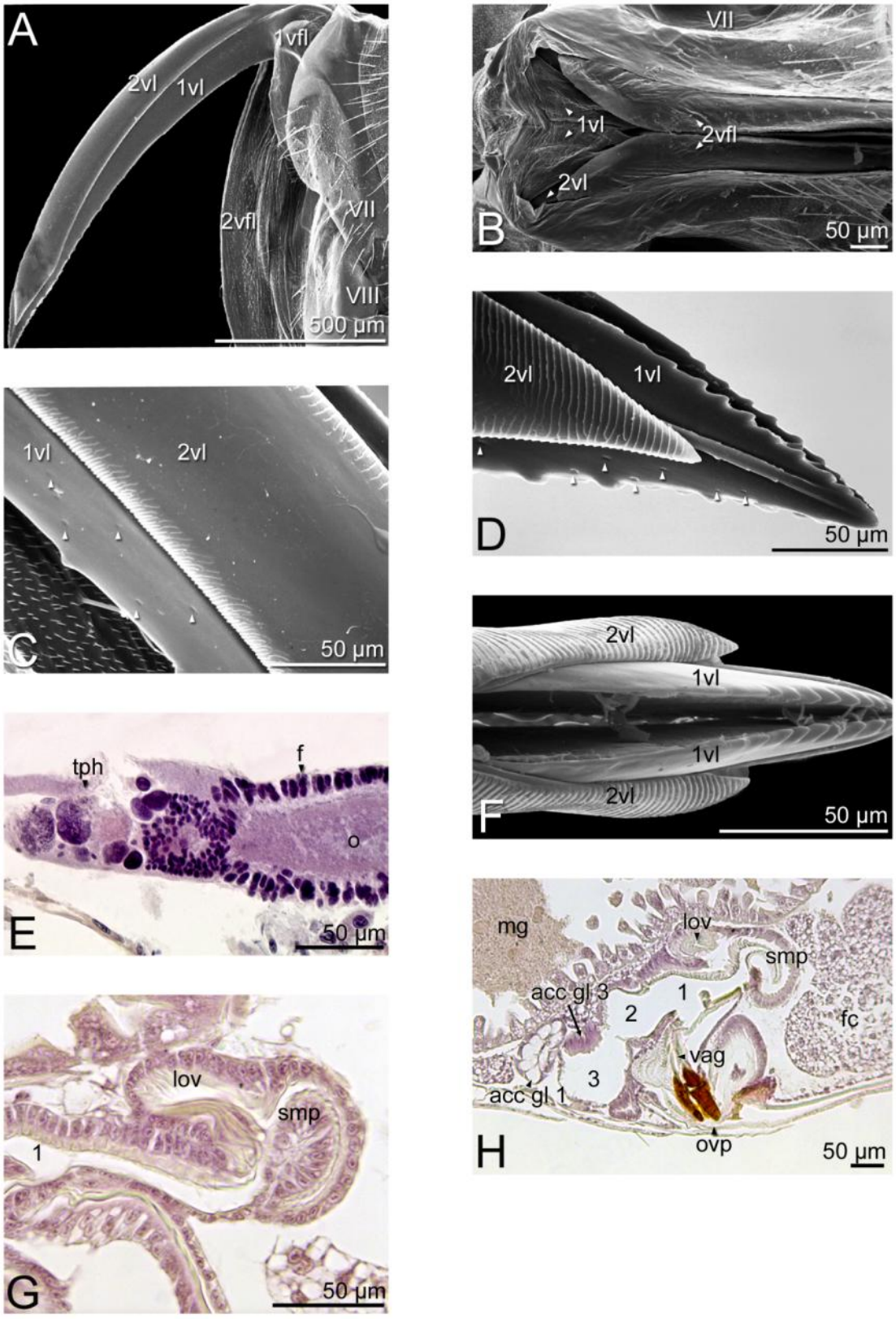


Fig. 8 External and internal female reproductive organs of *Campyloneura virgula*

2.3.2.5 Biology of *Arboridia ribauti*

Systematic position:

Arthropoda
Insecta
Hemiptera: Auchenorrhyncha
Membracoidea
Cicadellidae
Arboridia

The Cicadellidae comprises 20,000 to 25,000 species in about 50 subfamilies (Strümpel 2006), and the genus *Arboridia* includes about 60 species from the Palaearctic and Oriental Regions (Dmitriev and Dietrich 2003, Dietrich and Dmitriev 2006). Adults of *Arboridia ribauti* are about 2.9-3.4 mm in size, and are present from mid- July until the beginning of June, because they overwinter as adults, they produce one generation per year (Nickel 2003). *A. ribauti* feed on the leaf parenchyma of their host plant. However, nothing is known about their oviposition sites, or their embryonic and larval development. Given their well sclerotized ovipositor, it is likely that oviposition occurs on twigs or other lignified plant material.
In Germany this species is recorded only from the edge of the Alps to Düsseldorf, Göttingen, Halle and Dresden. In our local population in Halle only females are present (n = 399), whereas in Göttingen both sexes are known (Witsack, personal communication).
Species of *Arboridia* are usually identified by their external male genitalia. Because the Halle population is thelytokous, the species was identified via ITS-RFLP (data see Supplementary Material S2).
Parthenogenesis among Auchenorrhyncha is known for species of *Delphacodes*, and for *Agallia quadripunctata* (Cicadellidae). Some species of *Delphacidae* in Central Europe have developed pseudogamy, in which penetration of an egg by a spermatozoon is needed to initiate development, but no fusion of their nuclei occurs. Some *Muellerieanella* species exhibit another unusual system, with triploid females which produce in turn only female offspring (reviewed in Strümpel 2010).

2.3.2.6 Morphology of *Arboridia ribauti*

External female genitalia
The 9th segment is modified to form the pygofer and provides a protective cavity for the ovipositor (Fig. 9A, B). The ovipositor consists of three pairs of valvulae and their corresponding valvifers. The first valvulae split distally and lie outside the paired second valvulae. The first valvulae are slightly curved without any tooth-like structures (Fig. 9A, B), but lined with several campaniform sensilla (Fig. 9C, D). The second valvulae lie laterally in a groove of the first valvulae (Fig. 9A, B), and are long and appear like a curved saw-blade with dorsally-orientated teeth (Fig. 9C, D).
The third valvulae enclose both 1st and 2nd valvulae and have a smooth surface with several bristles (Fig. 9A, B, D).

Internal female genitalia

These consist of a paired ovary, a pair of lateral oviducts, a short common oviduct, a vagina, a bursa copulatrix and an accessory gland (Fig. 9G).

Each ovary comprise only 3 meroistic telotroph ovarioles per side (Fig. 9E, F). They are connected via a long pedicel to the lateral oviducts in the 7th segment (Fig. 9E), and these unite to the common oviduct in the 8th segment. The common oviduct in turn opens caudad into the tube-shaped vagina (Fig. 9G). The vagina opens to the ovipositor at sternite VIII. Two additional structures join the vagina: on the dorsal side a pouch-like bursa copulatrix (Fig. 9G), and on the ventral side a tubular-shaped accessory gland (Fig. 9G, H). This gland entwines the vaginal complex from the left to the right side of the vagina (Fig. 9H).
The vagina creates a connection to the genital opening at the ovipositor (Fig. 9G).

In the leafhoppers literature the term bursa copulatrix and spermatheca, unfortunately, have been used inaccurately and synonymously (Hummel et al. 2006, Strümpel 2010). During copulation, spermatophore, sperm or spermatodesm are deposited within the bursa copulatrix. The storing process of spermatozoa is occurred within vaginal crypts or convexities of the oviducts (Strümpel 2010).

The genital tract is typical of the family Membracidae; also having three ovarioles per ovary is not uncommon within the Auchenorrhyncha (Strümpel 2010), although some Cicadidae up to 80 ovarioles can be found (Strümpel 2010). Within the Membracoidae several accessory glands (tube-shaped gland, accessory gland in stricter sense, dorsovaginal gland) can be observed. However, in our specimen only one tube-shaped gland is connected with the vagina.

Fig. 9 External and internal female reproductive organs of *Arboridia ribauti*
A Segment 7 to 9 with everted ovipositor and sternite VII (S VII) modified to form a subgenital plate, SEM
B Ventral view of sternite VII (S VII) and segment 9 with 2nd and 3rd valvulae, SEM
C Ovipositor with several sensilla on 1st valvula, sagittal, HE, paraplast section
D Detail of anterior ovipositor with sensilla, ensheathed by the 3rd valvulae, sagittal, HE, paraplast section
E Paired meroistic telothrophic ovary connected via terminal filament at the mesothoracic diaphragm, frontal, HE, paraplast section
F Detail of an ovariole with germarium, tropharium, nutrition cord and oocyte, frontal, HE, paraplast section
G Overview of genital organs with sac-like bursa copulatrix, vagina, lateral oviduct and accessory gland, sagittal, HE, paraplast section
H Location of vagina, bursa copulatrix and accessory gland surrounded by the 1st and 2nd valvulae, frontal, HE, paraplast section

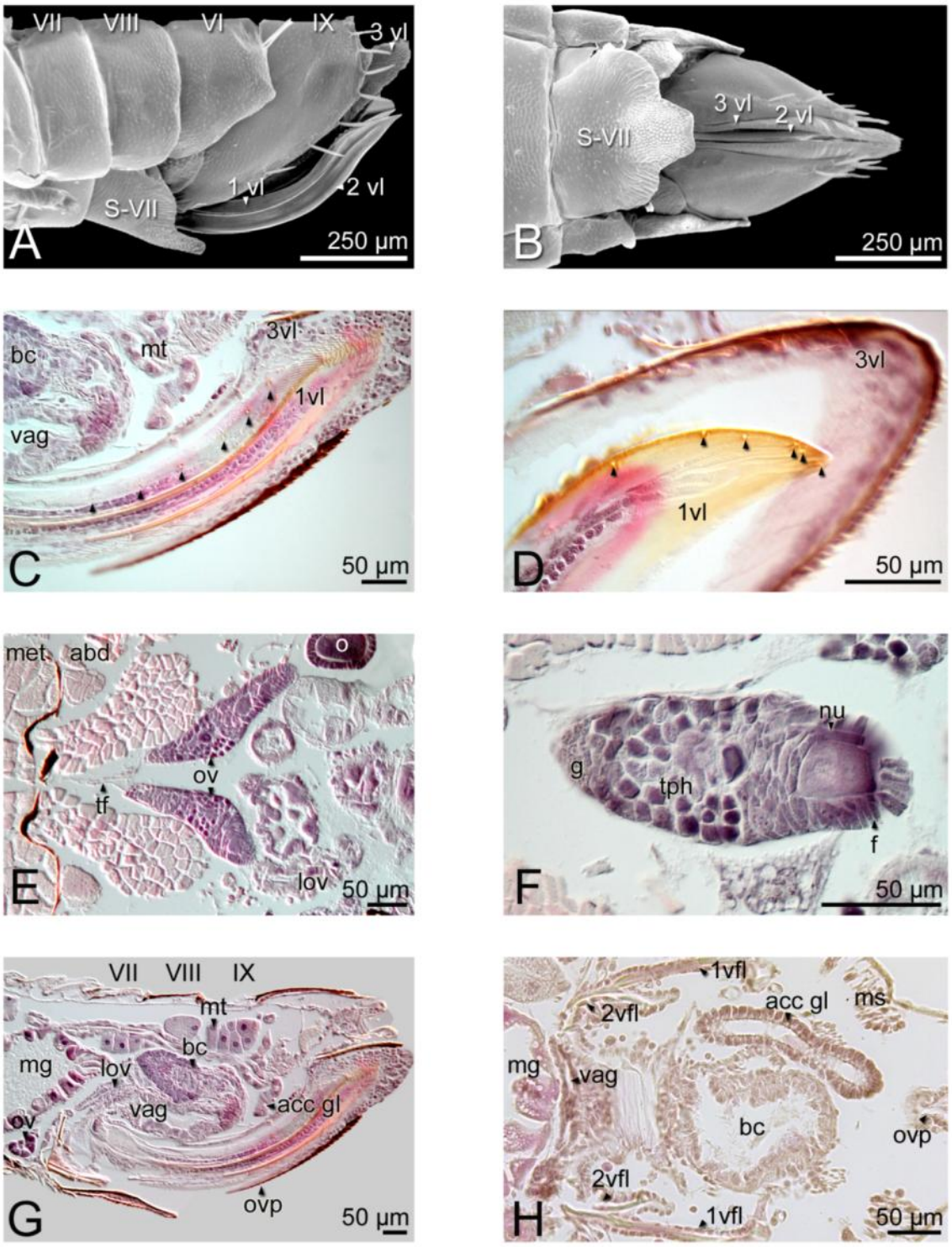


Fig. 9 External and internal female reproductive organs of *Arboridia ribauti*

2.4 Discussion

External male genitalia

The general structure of the male genitalia observed in *E. americanus* agrees with the description of other terebrantian species (Heming 1970b, Moritz 1997, Dallai 1997, Kumm 2002). Similarly, the phallus of *Pyrrhocoris apterus* is typical of that found among the Heteroptera (Tuxen 1970). However, within Heteroptera the phallus is more dimorphic within several groups (Snodgrass 1935), presumably in accordance with the dimorphic structure of the female genital tract (Snodgrass 1935, Pendergrast 1957, Scudder 1959) and lock and key system between these structures.

Nevertheless, both Heteroptera species examined share a similar phallus type, consisting of a phallobase, phallotheca and endotheca. Only the commonly used terms are different as is usual between different insect groups (Tuxen 1970). Definitions of Snodgrass (1935) were used to enable comparison between species, because they depend more on functionality than on homology.

Whereas the aedeagus in *Pyrrhocoris apterus* is reduced to a hook-like sclerotized part, it seems to be completely reduced to a plate like process in *E. americanus*, although another representative of the same family Thripidae (*Frankliniella occidentalis*) possesses a small tongue shaped aedeagus (Joel 2011).

Internal male genitalia

E. americanus possess monocystic testes with only one sperm tube, and adults have mature spermatozoa apparently a common feature among Terebrantia (Heming 1970b, Moritz 1997, Dallai 1997, Kumm 2002). Species in the suborder Tubulifera species have polycystic testes with fully differentiated spermatozoa (*Haplothrips verbasci, Bactrothrips brevitubus,* Heming 1970b, Kumm 2002) but also with different stages of development are known (*Suocerathrips linguis*, *Gynaikothrips ficorum*, Kumm 2002, Moritz 2006). Reasons and resulting consequences of these different mechanisms in spermiogenesis are unknown and discussed further in Chapter 3 and 7. The measured spermatozoa length of *E. americanus* corresponds to that known for other species of Thysanoptera. Size in other terebrantian species range from 20-200 µm, and in tubuliferan species from 70-130 µm, with some exception as e.g. *Megathrips inermis* with 1,200 µm (Heming 1995, De Marzo 2005, Paccagnini et al. 2009).

In contrast, *P. apterus* has seven sperm tubes per side, each with several cysts and different developmental stages of spermatozoa. Within Heteroptera, this type of testis morphology is frequent. Normally, the number of testicular tubes is less than the number of ovarioles in females (Snodgrass 1935), but in *P. apterus* the number of testicular tubes and ovarioles is congruent.

External female genitalia

Females of *E. americanus* have a lacinia-type ovipositor, as is typical for terebrantian species, except members of the family Merothripidae, which have a reduced ovipositor (Heming 1970a, Mound and O`Neill 1974, Bode 1975, Mound et al. 1980, Moritz 1982, 1989). Within the

Tubulifera, the ovipositor is in the form of a simple chute, without any muscle insertion; presumably movement is more passive than active (Heming 1970a).

Among the other species examined, all females, except *P. apterus,* possess a similar laciniate ovipositor. But they differ greatly in the structure of the valvifers and 3rd valvulae.
The greatly enlarged 3rd valvulae in *Arboridia ribauti* form a sheath for the ovipositor. Within the heteropteran species *Campyloneura virgula* the valvulae are fused to the ventro-posterior margins of the 2nd valvifers and form only a small part of the ovipositor sheath posterior of the abdomen. Among Heteroptera, the 3rd valvulae normally form an ovipositor sheath, but this is frequently absent in groups such as Nepomorpha, Pentatomorpha (including *P. apterus*), and a few other taxa (Schuh and Slater 1995). As we show here, in Miridae it is reduced (Davis 1955), and in Thysanoptera it is completely absent (Heming 1970a, Kumm 2002).

Internal female genitalia
There are conspicuous differences in the shape of the spermatheca among the various species. Female firebugs possess a well-developed spermatheca with complex glandular units and a distinct release mechanism (Pendergrast 1957, Merle 1969, Pluot 1970). Presumably, as an adaption to copulation in the late summer months with subsequent hibernation and oviposition in spring (Tischler 1959), a greater capacity for spermatozoa storage is needed to ensure successful fertilization in the following Spring. *Echinothrips americanus* has a more simple spermatheca, typical of Terebrantia (Bode 1975, Dallai 1996, Kumm 2002). A vesicular shaped structure, enclosed by a unicellular epithelial layer and interspersed gland cells, is situated near the vagina (Kumm 2002, Dallai 1996). Presumably, the much shorter lifespan of terebrantian species requires less effort for maintaining spermatozoa.
Because of the thelytokous strains of *Campyloneura virgula* and *Arboridia ribauti* used in this study, a precise description of sperm storage organs was not possible. Within Heteroptera, the sperm storage organ shows distinct structural variations (Pendergrast 1957). In Miridae the spermatheca is reduced to a sac-like organ without special structures (Schuh and Slater 1995). Similarly, Cicadomorpha lack a spermatheca in the narrow sense; they store spermatozoa within an expansion of the common oviduct, whereas Fulgoromorpha possess fully developed sperm storage organs (Strümpel 2010).

The two species of Heteroptera demonstrate two different forms of ectodermal reproductive organs that Stys (1960) named the coreid and the lygeoid type. The coreid type is characterized by a narrow and slender gynatrium/vagina. Mostly it is associated with a well-developed ovipositor. It can be found within the Lygaeidae, Largidae, Stenocephalidae, Miridae and probably in Piesmatidae.
In the coreid type the vagina is not developed, but the presence of a sac-like organ is common, mostly this type is associated with a reduced ovipositor. It occurs in the Coreidae-Pseudophloeinae, Alydidae- Micrelytrinae, Rhopalidae and Pyrrhocoridae.
Nevertheless, *Campyloneura virgula* exhibits several morphological specialisations. The common oviduct is extremely reduced; mature eggs are transferred directly from the lateral oviduct through compartment 1 to the ovipositor (see Davis 1955). A sperm storage organ in

the stricter sense is not present. In other mirid species, without thelytokous reproduction, the pouch-like bursa copulatrix/compartment 3 is used as storage organ (Schuh and Slater 1995).

2.5 References

Andjus L, Jovic M, Trdan S, 2009. First record of *Echinothrips americanus* in Serbia. HPPJ 2, 71-73.

Barber HB, 1911. *Pyrrhocoris apterus* Linn. in the United States. J NY Entomol Soc 19, 111-112.

Bode W, 1975. Der Ovipositor und die weiblichen Geschlechtswege der Thripiden (Thysanoptera, Terebrantia). Z Morphol Ökol Tiere 81, 1-53.

Bonhag PF, Wick JR, 1953. The functional anatomy of the male and female reproductive systems of the milkweed bug *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). J Morphol 93, 177-283.

Carapezzo A, 1997. Heteroptera of Tunisia. Nat sicil 21, 1-331.

Carayon J, 1989. Constant Parthenogenesis proved in 2 Heteroptera: the Mirid *Campyloneura virgula* and the Anthocorid *Calliodis maculipennis*. Ann Soc Entomol Fr 25, 387-391.

Chapmann RF, 2013. The Insects- Structure and Function. Cambridge University Press, Cambridge, UK.

Collins DW, 1998. Recent interceptions of *Echinothrips americanus* Morgan (Thysanoptera, Thripidae) imported into England. Entomol mon mag 133, 1-3.

Dallai R, Del Bene G, Lupetti P, 1996. Fine structure of spermatheca and accessory gland of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Int J Insect Morphol Embryol 25, 317-330.

Dallai R, Del Bene G, Lupetti P, 1997. Fine structure of the male reproductive organs of the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Int J Insect Morphol Embryol 26, 97-112.

Davis NT, 1955. Morphology of the female organs of reproduction in the miridae (Hemiptera). Ann Entomol Soc Am 48, 132-150.

De Marzo L, 2005. Lunghezza degli spermatozoi in *Megathrips inermis* Priesner (Thysanoptera. Phlaeothripidae). Boll Zool Agrar Bachic Ser II 37, 179-183.

Dietrich CH, Dmitriev DA, 2006. Review of the New World genera of the leafhopper tribe Erythroneurini (Hemiptera: Cicadellidae: Typhlocybinae). Bull Illinois Nat Hist Survey 37, 119–190.

Dmitriev DA, Dietrich CH, 2003. An interactive key to genera of Erythroneurini. available online: http://ctap.inhs.uiuc.edu/dmitriev/key.asp?key=Erythroneura&lng=En&i=l&keyN=1

Ferguson G, Shipp L, 2002. New pests in Ontario greenhouse vegetables. Bull. OILB 25, 69-72.

Gapon DA, 2007. Structure, Function, and Morphological Conformity of the Male and Female Genitalia in the True Bugs Genus *Pyrrhocoris* Fall. (Hymenoptera*, Pyrrhocoridae). Entomol Rev 87, 1099-1108.

Grimaldi D, Engel MS, 2005. Evolution of the Insects. Cambridge University Press, New York, USA.

Groot AT, Smid HM, 2000. Polyandry in the mind bug *Lygocoris pabulinus* (L.)- effects on sexual communication and fecundity. Invertebr Reprod Dev 38, 143-155.

Gullan PJ, Cranston PS, 2010. The Insects- an Outline of Entomology. Wiley-Blackwell, Oxford, UK.

Heming B, 1970a. Postembryonic development of the female reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc Publ Entomol Soc Am 7, 199-234.

Heming B, 1970b. Postembryonic development of the male reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc Publ Entomol Soc Am 7, 237-272.

* this order was originally mentioned in the title, Hemiptera would be correct

Heming BS, 1995. History of the Germ Line in Male and Female Thrips. In: Thrips Biology and Management. Ed. by Parker BL, Skinner M, Lewis T, Plenum Press, New York, USA, 505-535.

Hummel N, Zalom FG, Peng CYS, 2006. Anatomy and Histology of Reproductive Organs of Female *Homalodisca coagulate* (Hemiptera: Cicadellidae, Proconiini), with Special Emphasis on Categorization of the Vitellogenic Oocytes. Ann Entomol Soc Am 99, 920-932.

Itoh K, Oguri A, Suzuki A, 2003. Occurrence and control of insect pests in perilla on Aichi Prefecture. Proc Kansai Pl Protect Soc 45, 71-72.

Joel JC, 2011. Inner and outer anatomy of the male reproductive system in Thysanoptera (Insecta). Diploma-thesis, Martin-Luther University Halle-Wittenberg, Halle, Germany.

Kulik SA, 1973. The Coreidae and the Pyrrhocoridae (Heteroptera) of the East Siberia and the Far East. In: Insect Fauna and Ecology of the East Siberia and Far East. Ed. by Kulik SA, University Publishing, Irkutsk, Russia, 32-43.

Kullenberg B, 1947. Der Kopulationsapparat der Insekten aus phylogenetischen Gesichtspunkt. Zool Bids Uppsala 25, 79-99.

Kumm S, 2002. Reproduction, progenesis and embryogenesis of thrips (Thysanoptera, Insecta). PhD-thesis, Martin-Luther University Halle-Wittenberg, Halle, Germany.

Li X-W, Zhang X-C, Jiang H-X, Feng J-N, 2012. Comparisons of developmental and reproductive biology between parthenogenetic and sexual *Echinothrips americanus* (Thysanoptera: Thripidae). Environ Entomol 41, 706-713.

Ludwig W, 1926. Untersuchungen über den Copulationsapparat der Baumwanzen. Z Morphol Oekol Tiere 5, 291-380.

Matolin S, 1973. The embryonic development of *Pyrrhocoris apterus* (L.) (Heteroptera, Pyrrhocoridae). Acta Entomol Bohemslov 70, 150-156.

Masner P, 1966. The structure, function and imaginal development of the female inner reproductive organs of *Adelphocoris lineolatus* (Goeze) (Heteroptera, Miridae).Acta ent bohemslov 63, 177-199.

Merle J, 1969. Anatomie des appareils genitaux male et femelle et fonctionnement ovarien de *Pyrrhocoris apterus* (L.)(Hemiptera, Pyrrhocoridae). Acta ent bohemslov 66, 275-288.

Mirab-Balou M, Lu H, Xue-Xin C, 2010. First record of *Echinothrips americanus* Morgan (Thysanoptera, Thripidae) in mainland China, with notes on distribution and host plants. Acta Zoo Sinica 35,674-679.

Moritz G, 1982. Zur Morphologie und Anatomie des Fransenflüglers *Aeolothrips intermedius* Bagnall. 3. Mitteilung: Das Abdomen. Zool Jb Anat 108, 293-340.

Moritz G, 1989. Die Ontogenese des Thysanoptera (Insecta) unter besonderer Berücksichtigung des Fransenflüglers *Hercinothrips femoralis* (O.M. Reuter 1891) (Thysanoptera, Thripidae, Panchaetothripinae). V. Mitteilung: Imago, Abdomen. Zool Jb Anat 119, 157-217.

Moritz G, 1997. Structure, growth and development. In: Thrips as crop pests. Ed. by Lewis T, CAB International, Oxon, UK, 15-63.

Moritz G, 2006. Thripse - Fransenflügler, Thysanoptera. Westarp-Verlag, Hohenwarsleben, Germany.

Mound LA, 2000. The aquatic thrips *Organothrips indicus* Bhatti (Thysanoptera: Thripidae) in Queensland, and a new species, *O. wrighti*, from tropical Australia. Aust J Entomol 39, 10-14.

Mound LA, O´Neill K, 1974. Taxonomy of the Merothripidae, with ecological and phylogenetic considerations (Thysanoptera). J nat Hist 8, 481-509.

Mound LA, Heming BS, Palmer JM, 1980. Phylogenetic relationship between the families of recent Thysanoptera (Insecta). Zool J Linn Soc 69, 111-141.

Mound LA, Marullo R, 1996. The thrips of Central and South America: An introduction (Insecta: Thysanoptera). Associated Publishers, Gainesville, USA.

Mound LA, Ng YF, 2009. An illustrated key to the genera of Thripinae (Thysanoptera) from South East Asia. Zootaxa 2265, 27-47.

Mound LA, Tree DC, Sartiami D, 2013. The greenhouse pest, *Echinothrips americanus* Morgan, recorded for the first time from Australia and Java. Myrmecia 49, 54-57.

Nickel H, 2003. The leafhoppers and planthoppers of Germany (Hemiptera, Auchenorrhyncha): Patterns and strategies in a highly diverse group of phytophagous insects. Pensoft Publishers, Sofia, Bulgaria.

Noirot C, Quennedey A, 1974. Fine structure of insect epidermal glands. Ann Rev Entomol 19, 61-80.

Oetting RD, Beshear RJ, 1993. Biology of the greenhouse pest *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Int J Pure Appl Zool 4, 307-315.

Oetting RD, Beshear RJ, Liu TX, Braman SK, Baker JR, 1993. Biology and identification of thrips on greenhouse ornamentals. Geo Agr Exp St Bull 414, 20.

Paccagnini E, Lupetti P, Afzelius BA, Dallai R, 2009. New findings on sperm Ultrastructure in thrips (Thysanoptera, Insecta). Arth Struct & Dev 38, 70-83.

Pendergrast JG, 1957. Studies on the reproductive organs of the Heteroptera with consideration of their bearing on classification. Proc R Entomol Soc Lond 109, 1-63.

Plant Health Australia, 2010. Poinsettia thrips contingency plan. available online: http://www.planthealthaustralia.com.au/pests/poinsettia-thrips/.

Pluot D, 1970. La spermathèque et les voies genitals femelles des Pyrrhocoridès (Hemiptera). Ann Soc ent Fr 6, 777-807.

Polozhencev PA, Polozhenceva-Korovina NJ, 1961. Studies on the biology of the red firebug. Les Zh 1961, 10-14.

Puchkov VG, 1974. Berytidae, Pyrrhocoridae, Piesmatidae, Aradidae, Tingidae. In: The Fauna of Ukraine 21 (4) Ed. by Puchkov VG, Akademija Nauk Ukrainskoi, Kiev, Ukraine.

Rizki T, Slama K, 1968. An autosomal recessive gene in *Pyrrhocoris*. J Hered 59, 327-328.

Schmuck R, 1987. Aggregation behaviour of the fire bug, *Pyrrhocoris apterus,* with special reference to its assembling scent (Heteroptera, Pyrrhocoridae). Entomol Gener 12, 155-169.

Schuh RT, Slater JA, 1995. True bugs of the world (Hemiptera: Heteroptera) - Classification and natural history. Cornell University Press, Ithaca, USA.

Scudder GGE, 1959. The female genitalia of the Heteroptera: Morphology and bearing on classification. Trans R Ent Soc Lond 111, 405-467.

Scudder GGE, 1971. Comparative Morphology of Insect Genitalia. Ann Rev Entomol 16, 379-406.

Seifert G, 1970. Entomologisches Praktikum. Thieme Verlag, Stuttgart, Germany.

Shipp JL, Gillespie DR, Fry KM, Ferguson GM, 2001. *Echinothrips americanus* (Morgan), *Frankliniella occidentalis* (Pergande), western flower thrips, and *Thrips tabaci* Lindemann, onion thrips (Thysanoptera: Thripidae). In: Biological control programs in Canada. Ed. by Mason PG, Huber JT, CABI Publishing, Wallington, 115-119.

Simmons LW, 2001. Sperm competition and its Evolutionary Consequences in the Insects. Princeton University Press, Princeton, USA.

Smith RL, Forrest HS, 1969. The pattern of pteridine accumulation in eggs of *Pyrrhocoris apterus*. J Insect Physiol 15, 953-957.

Snodgrass RE, 1935. Principles of Insect Morphology. McGraw-Hill Book Company, New York, USA.

Socha R, 1993. *Pyrrhocoris apterus* (Heteroptera) - an experimental model species: A review. Eur J Entomol 90, 241-286.

Staddon BW, Daroogheh H, 1982. Allometry of scent oil accumulation in Heteroptera. Comp Biochem Physiol 71, 149-154.

Stannard L, 1968. The thrips, or Thysanoptera, of Illinois. Bull Ill Nat Hist Surv 29, 215-552.

Stichel W, 1959. Illustrierte Bestimmungstabellen der Wanzen. II. Europa. Stichel, Berlin, Germany.

Strümpel H, 2010. Die Zikaden, Auchenorrhyncha. Westarp-Verlag, Hohenwarsleben, Germany.

Stys P, 1960. Morphology of the abdomen and female ectodermal genitalia of the Trichophorous Heteroptera and bearing on their classification. Proceedings of XI International Congress of Entomology Vienna, 37-43.

Tischler W, 1959. Zur Biologie der Feuerwanze (*Pyrrhocoris apterus* L.). Zool Anz 163, 392-396.

Trdan S, Milevoj L, Raspudic E, Zezlina I, 2003. The First Record of *Echinothrips americanus* Morgan in Slovenia. Acta Phytopathol Hun 38, 157-166.

Tuxen SL, 1970. Taxonomist`s glossary of genitalia in insects. Munksgaard, Copenhagen, Denmark.

Varga L, Fedor PJ, 2008. First interception of the greenhouse pest *Echinothrips americanus* Morgan, 1913 (Thysanoptera: Thripidae) in Slovak Republic. Plant Protect Sci 44*,* 155-158

Vierbergen G, 1998. *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). Proc Exp Appl Entomol 9, 155–160.

Vierbergen G, Cean M, Hatalànè Szellèr I, Jenser G, Masten T, Simala M, 2006. Spread of two thrips pests in Europe: *Echinothrips americanus* and *Microcephalothrips abdominalis* (Thysanoptera: Thripidae). Acta Phytopathol Hun 41, 287-296.

Wachmann E, Melber A, Deckert J, 2004. Wanzen Bd 2. Goecke and Evers, Keltern, Germany.

Wagner E, 1958. Ein Männchen von *Campyloneura virgula* H.S. 1839. Nachri bl Bayer Entomol 9, 81-83.

Wagner E, 1968. Über *Campyloneura virgula* Herrich Schäffer (Hem. Het. Miridae). Mitt Dtsch Entomol Ges 27, 46-47.

Weber H, 1974. Grundriss der Insektenkunde. Ed. by Weidner H, 5th edition, Fischer Verlag, Stuttgart, Germany.

Yang C-T, 2005. External Male Genitalia of the Pyrrhocoroidea, Coreoidea, and Lygaeoidea (Hemiptera: Heteroptera). NMNS Spec. Publ. 11, 1-158.

CHAPTER 3

REPRODUCTIVE BEHAVIOR OF *ECHINOTHRIPS AMERICANUS* (THYSANOPTERA: THRIPIDAE)

Stephanie Krueger, Marcus Jilge, Gerald B. Moritz

Martin Luther University Halle- Wittenberg, Institute of Biology, Faculty of Natural Science I, Heinrich-Damerow Str. 4, 06120 Halle, Germany

3.1 Summary

Most Thysanoptera possess a haplo-diploid reproduction mode and reproduce via arrhenotoky. While females can mature eggs successively throughout almost their entire life, in most Terebrantia spermiogenesis is complete by adult male eclosion. In parasitoid wasps this phenomenon of only pre-adult spermiogenesis is described as prospermatogeny. It is unclear if prospermatogeny in terebrantian Thysanoptera will result in sperm limitation and thus have implications for their mating strategy and fitness. In this study we give a detailed description of mating behavior of the terebrantian species *Echinothrips americanus*, which largely corresponds with the only available data of a terebrantian species, *Frankliniella occidentalis* (Thysanoptera: Thripidae). With the help of light microscopic investigations, we describe for the first time the chronological sequence of internal processes during copulation. The sequential release of male accessory gland material and afterwards spermatozoa indicates production of a female-determined type 1-spermatophore. Despite prospermatogeny, males are able to inseminate 10 females with an equal amount of spermatozoa. Only quantity of glandular material decreases with the number of previous copulations. Based on these new findings, the reproductive strategy of this species is discussed.

Keywords:
sperm transfer, copulation, ejaculate volume, prospermatogeny

Own contribution: 85 %

Abbreviations:
cov--common oviduct, edth-endotheca, hg-hindgut, o-oocyte, ovp-ovipositor, p-paramere, paed-primitive aedeagus, phtc-phallotheca, pph-paraphyses, sb-sperm ball, spth-spermatheca, spth d-spermathecal duct, vag-vagina

3.2 Introduction

Detailed knowledge of reproduction is critical to managing those species that compete with us in the field, forest and greenhouse (Heming 1995). Each year, Thysanoptera cause millions of $US of crop loss (Lewis 1997). Mainly species from the suborder Terebrantia produce significant damage by piercing and sucking out leaves, flowers and fruits. Additionally some of the 1 to 2 mm small species are able to transmit plant viruses (Machlomovirus, Ilarvirus, Carmovirus, and Sobemovirus), bacteria and fungi (Ullman et al. 1997, Gitaitis et al. 2003, Jones 2005). However, our knowledge of their reproduction is still anecdotal (Moritz 2006).

Most Thysanoptera possess a haplo-diploid reproduction mode and reproduce via arrhenotoky. Haploid males originate from unfertilized eggs, whereas fertilized eggs develop into diploid females. While females can mature eggs successively throughout almost their entire life, in most terebrantian males spermiogenesis is completed at eclosion of the adult (Bournier 1956, Heming 1970, Bode 1983). In parasitoid wasps this phenomenon of spermiogenesis completed prior to emergence is described as prospermatogeny (Boivine et al. 2005). In contrast synspermatogenetic species are able to produce spermatozoa throughout their lifetime.

Whether prospermatogeny in Thysanoptera will result in sperm limitation and consequent implication for their mating strategy and fitness, is unclear. Male fitness is defined as the quantity of daughters that their mates produce. Males are expected to optimize the use of their sperm (Pitnick and Markow 1994).

Detailed description of mating behavior within the Terebrantia is available only for *Frankliniella occidentalis* (Terry and Schneider 1993), *Frankliniella schultzei* (Milne et al. 2007) and *Scirtothrips aurantii* (Rafter and Walter 2013). In *Frankliniella occidentalis* males attempt to mate with as many virgin females as possible. In contrast, females reject mating attempts after an initial copulation for many days. Strong competition among males to find virgin females occurs (Terry and Schneider 1993). After a very brief precopulatory period, the male mounts a female, clasps her pterothrorax with his legs and twists the tip of his abdomen underneath hers. Insertion of the aedeagus follows. For successful copulation, antennal contact between individuals, as well as stroking the females´ back with the mesothoracic legs of the male is necessary. Later, several calm periods are characteristic. Copulation lasts on average after 238.6 seconds. The other species reported *F. schultzei* and *S. aurantii,* show similar behavioral patterns (Milne et al. 2007, Rafter and Walter 2013) and similar mating position, as is also known for several other Terebrantia (Franssen and Mantel 1964, Heming 1995), where knowledge of behavior is lacking.

In this study of *Echinothrips americanus,* we give a precise description of mating behavior, mating frequency, duration, chronological sequence and quantity of spermatozoa transfer.

E. americanus is native in the eastern United States (Stannard 1968). As a greenhouse pest it has spread over Canada, Europe, Asia and Northern Australia within the last 30 years (see Krueger et al. 2015). The so-called Poinsettia thrips has a broad host range and causes damage by its feeding (Vierbergen 1998, Oetting and Beshear 1993, Oetting et al. 1993, Trdan et al.

2003). As a result, it has the potential to become a major pest insect. But as in most species of Thysanoptera, little is known about the biology, and such an understanding is essential for efficient control.

3.3 Material and Methods

Animal breeding and keeping

E. americanus laboratory culture was maintained at the University of Halle, Germany. In acrylic cages (50 x 50 cm), they were reared on *Phaseolus vulgaris* and *Gossypium sp.* as host plants. The culture was kept at 23 ± 1 °C, 60 ± 10 % RH, and photoperiod of 16:8 h L:D at 5000 Lux during the photophase.

To get virgin and naive males and females, females were allowed to lay eggs in wells of 12-well Greiner Plates (Sigma- Aldrich, Munich, Germany). Each well (Ø 2.2 cm) was filled with 1.5 ml of 1.4 % (w/v) agar and topped with a leaf discs (Ø 1.6 cm) of *P. vulgaris.* Hatched larvae were reared individually, raised up until the adult moult and then sexed. The plates were kept in a climate chamber under the same rearing conditions as lab culture.

1) *Mating behavior and chronological sequence*

Mating behavior was observed on an agar and leaf disc well of a 12-well Greiner plate (observation arena). Three females and one male were recorded within 1h with a HD Webcam C525 (Logitech, Switzerland). Males were classified in two age groups. Males of *E. americanus*have an average life span of 10 to 14 days (Krueger et al. 2015). One to three day old males (post emergence) were categorized as “young males”; 10 to 12 day old males were categorized as “old males”. Virgin females had an age of 2 to 5 days post-emergence at the time of test.

Video playbacks have been used for investigation and analysis of duration and number of behaviors. Due to the small size of the insects, we were unable to analyse all behaviors via video playbacks (antennation, stroking), therefore data was supplemented by direct observations. Direct observations were conducted in similar observation arenas under a stereomicroscope (Leica S8 APO, Wetzlar, Germany). To achieve greater comparability with the available data for mating behavior within Terebrantia, the behaviors observed were chosen analogous to the study of Terry and Schneider (1993) (Tab. 1).

Detailed chronological sequences of copulation were analysed using histological methods. Couples were frozen in supercooled carnoy´s fluid (-80 °C) at different time intervals after insertion of the phallus (1 min, 3 min, 5 min, 7 min, control: recently finished copulation). Afterwards specimens were prepared for light microscopy and stained with haematoxylin-eosin (Supplementary Material S2). Slides were observed with a Leitz DMBRE (Leica, Wetzlar, Germany) fitted with a Leica DFC 450C. Images were processed with Photoshop CS5 (Adobe, Ireland).

Table 1 Observed mating behaviors with its description in *Echinothrips americanus*

Behavior observed	Description
test start to male mounting	time between start of video recording and male mounting
number of prior matings of male	number of previous matings of male before recorded copulation
female rejects male prior mating	number of rejections of female prior copulation (same individuals)
initial contact to male mounting	time between initial contact (antennal contact) and male mounting
contact position	position of male and female during initial contact (head to head, head to abdomen, undetermined)
antennation	antennal contact of male and female
stroking	male palpate females back with his mesothoracic legs
duration of mating	time between insertion of aedeagus and separating
calm period during mating	period during copulation without any observable movement of both sexes
female walking during mating	period of female walking with male on her back during copulation

2) Frequency

Re-mating frequency was observed for copulations with the same male, as well as for copulations with new virgin males. Couples were placed in an observation arena for 24h. After initial mating, couples were separated. Every 24h either the same or another male was presented to each female. Within 30 min mating attempts and mating, each within 30 min, were counted visually under a stereo microscope (Leica S8 APO, Wetzlar, Germany). After 30 min the individuals were separated and kept in plates as described before (animal breeding). The test stopped with female death (re-mating trial with different male) or with male death (re-mating trial with the same male).

3) Quantification of sperm transfer

In order to measure transfer of spermatozoa into the female and sperm limitation in the males, one male and two females were placed in a well of a 12- well Greiner-plate, prepared as described above, and kept in a climate chamber. Every 2 days, the male was transferred to two new virgin females. Previously mated females were removed and processed for histological analysis as illustrated below. The procedure was stopped after 0, 5 or 10 consecutive mating. To check for age-dependent effects, virgin males were treated similarly but without females. Afterwards males were prepared for histological observation (Supplementary Material S2). DAPI- staining was applied, as this intercalates with DNA. The brightness of the coloration indicates the amount of DNA present and thus the quantity of spermatozoa. The detailed

staining procedure is explained in the Supplementary Material S 5.1 DAPI-staining. Slides were examined with a light microscope Leitz DMBRE (Leica, Wetzlar, Germany), fitted with a Leica DFC 450C and processed with ImageJ (version 2.0.0; Wayne Rasband, http://imagej.nih.gov/ij/) software. The area and grey values of the spermball within the spermatheca and of the spermatozoa remaining within the testes were measured. To reduce any artefacts, all slides were stained at the same time, with the same chemicals, and with the same interval between staining and observation. Additionally, the room was darkened and the microscope settings were retained during all measurement.

The index of the grey values of area of interest (testes or sperm ball) was calculated as follows:

$$\frac{(mean\ grey\ value\ area\ of\ interest - mean\ grey\ value\ backround)}{(mean\ grey\ value\ nucleus - mean\ grey\ value\ background)}$$

Therefore grey value was taken in relation to background and the maximum coloration produced (nucleus of somatic tissue).

Statistical analysis

Data analysis was performed with WinStat® for Excel (Fitch Software, Bad Krozingen, Germany) and SPSS Statistics 22 (IBM Corp., Armonk, New York, USA). Prior to analysis, data were checked for normal distribution using Shapiro-Wilk test ($p > 0.05$). Data on mating behavior was not normally distributed ($p < 0.05$), therefore nonparametric tests were conducted. Data on sperm quantification were normally distributed ($p > 0.05$), consequently parametric tests were used.

1) Mating behavior

Times of test start to mounting, initial contact to male mounting, duration of mating, calm period and walking of female during copulation, were analysed with Mann-Whitney U-test to distinguish between young and older males. Frequency of contact position was analysed with 2 x 3 Fishers-Exact-test, $p < 0.05$.

2) Frequency

Frequency of mating attempts and mating in both remating trials were analysed with 2 x 2 Fishers-Exact-test, $p < 0.05$.

3) Sperm quantification

We tested for differences between both testes of one individual using a paired t-test. No difference was observed ($p > 0.05$), therefore data were pooled. Testis size, index of testis grey value, size of sperm ball and index of grey value of sperm ball were analysed with ANOVA, means were separated by LSD posthoc test ($p < 0.05$).

3.4 Results

1) Mating behavior and chronological sequence

Copulation involves three phases: precopulation, copulation and postcopulation. The precopulatory phase starts with an initial contact of both sexes (Tab. 2). The male contacts the female with his antenna either in a head-to-head position (50 %) or head to female abdomen/thorax (27.7 %). Then, a receptive female lowers her abdomen and thorax, while the male mount onto the female´s back. Unreceptive females threw off males by raising their abdomen. Males repeatedly attempt to mate with a female, even when not receptive. In the following copulation period, the male twisted his abdomen underneath that of the female and tried to insert his aedeagus into the female genitalia. Meanwhile he palpates her antennae with his antennae, as well as stroking her back with his mesothoracic legs. Successful insertion is followed by a calm period, which can greatly vary in duration (Tab. 2). This phase is characterized by no movement of any part of the body. Prior to separation of the couple, the female sometimes starts to walk around with the male on her back (Tab. 2).

Mating behavior was analysed with to compare male age at mating. Younger males had a significant shorter duration of mating (U-test, Z = -2.711, p = 0.006), than older males. All other recorded behaviors did not differ significantly.

Table 2 Mating behaviors of *Echinothrips americanus* analyzed from video observations, letters indicate for significant differences

Behavior	n	Duration in s ± SD	Frequency in %	Range in s
Precopulation				
Test start to male mounting				
Young males	10	633 ± 533.8	a	165-1641
Old Males	9	1113.2 ± 860.6	a	105-2304
Initial contact to male mounting				
Young males	10	8 ± 2.8	a	5-13
Old males	9	9.7 ± 5.1	a	4-20
Contact position				
head to head	18		50	
head to female abdomen	18		27.7	
undetermined	18		27.7	
Copulation				
Duration				
Young males	10	403.2 ± 31.28	a	342-444
Old males	8	610.66 ± 202.2	b	389-995
Calm period				
Young males	10	297.3 ± 128.2	a	91-830
Old males	8	373.22 ± 271.1	a	88-423
Female walking				
Young males	10	11.1 ± 35.1	a	0-131
Old males	8	82.8 ± 145.4	a	0-111

During copulation, both parameres and the primitive aedeagus were completely inserted within the females´ vagina (Fig. 1 A-E). Endotheca and phallotheca were evaginated from the beginning of the copulation (Fig. 1D), and the paraphyses were located near the opening of spermathecal duct (Fig. 1 D,E). One minute after insertion, the endotheca seems to be filled mainly with accessory gland secretion (magenta-stained) and only few spermatozoa (Fig 1D). After 3 min, more spermatozoa are visible within the endotheca (Fig. 1E). With increased time after the start of copulation, the number of visible spermatozoa within the endotheca increased. But spermatozoa were first observed in the spermatheca at 7 min after start of copulation (Fig. 1F). After successful copulation, a sperm ball of twisted spermatozoa was located within the spermatheca (Fig. 1F).

2) Frequency

In both conditions only one female mated again with the same male 3 days after the initial copulation. But no significant difference in mating attempts or frequency of mating was observed between both re-mating trials (mating attempts, Fishers-Exact-test, $p = 0.766$; matings, Fishers-Exact-test, $p = 1$) (Tab. 3).

Table 3 Frequency of mating attempts and matings in re-mating trials with different male and with the same male after initial copulation

	Re-mating trial with different male	Re-mating trial with the same male
n	18	27
Mating attempts	9	11
Matings	0	1

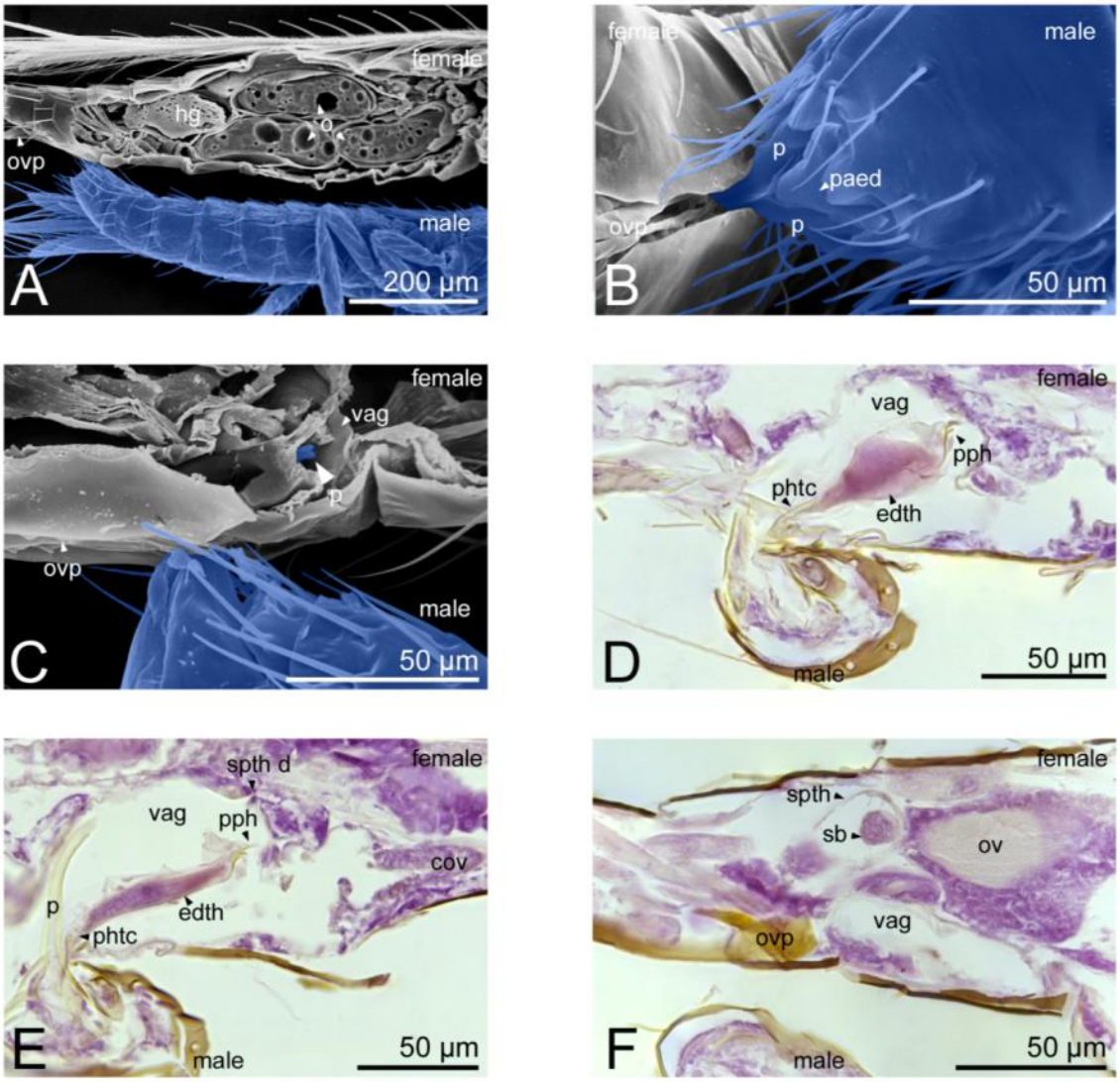


Fig. 1 Copulation of *Echinothrips americanus*,
A-C Male in false color, SEM, D-F light microscope, hemalum-eosin stained

A Sagittal view of male and female in copula, note in life male is on the back of the female, clasps her pterothorax with his legs and twists the tip of his abdomen underneath that of the female

B Male with genitalia fully inserted into female, cranio-dorsad view of couple

C Detailed view of inserted male genitalia, note the parameres within the females vagina

D Couple fixed 1 min after insertion of aedeagus, note the magenta colored secretion of males accessory gland within endotheca

E Couple fixed 3min after insertion of aedeagus, note the magenta colored gland secretion and few sperm (lilac) within edotheca

F Couple fixed 7 min after insertion of aedeagus, note the filled spermatheca of the female

3) *Quantification of sperm transfer*

The area of sperm content within the testes decreased with the number of repeated copulations (ANOVA, F = 23.673, p < 0.001). Additionally, we also observed a decrease in sperm content over time, regardless of the number of previous matings. The area of sperm within the testes of 10 day old virgin males was significantly smaller than in 5 day old virgin males or in freshly emerged males (0d virgin) (10 d virgin vs. 5 d virgin, Posthoc LSD, p = 0.001; 10 d virgin vs. 0 d virgin, Posthoc LSD, p < 0.001) (Fig. 2).

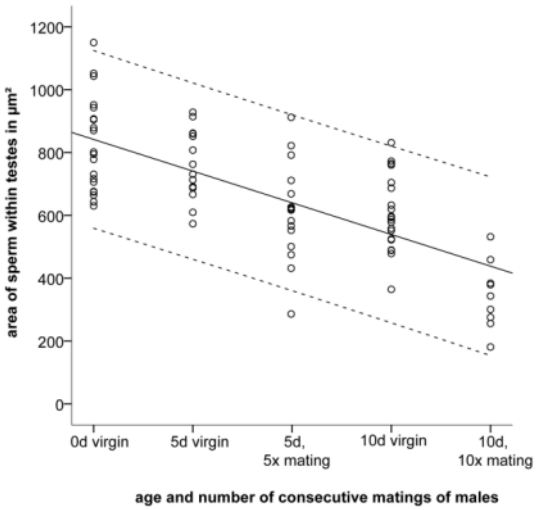


Fig. 2 Area of sperm within testes of male *E. americanus* depending on age and number of previous matings

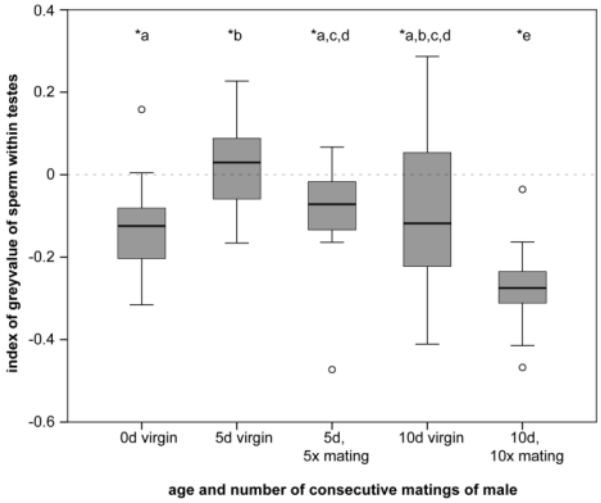


Fig. 3 Index of grey value of sperm within testes of male *E. americanus* depending on age and number of previous matings, letters indicate for significant differences

The index of grey value of sperm within the testes differs significantly between the conditions (ANOVA, F = 6.211, p < 0.001). Freshly hatched adult males (0d virgin) have a low index compared to 5 d virgin males (Posthoc LSD, p = 0.003). Afterwards, the index decreased subsequent to consecutive matings (Fig. 3).

The sperm ball size within the spermatheca decreased after copulation with males with increased number of previous matings (ANOVA, F = 5.504, p = 0.001) (Fig.4). Interestingly, the index of grey value and therefore brightness of sperm ball did not differ with consecutive matings of the male (ANOVA, F = 1.239, p = 0.306). Detailed data of measurement of spermatheca is shown in Supplementary Material S3.

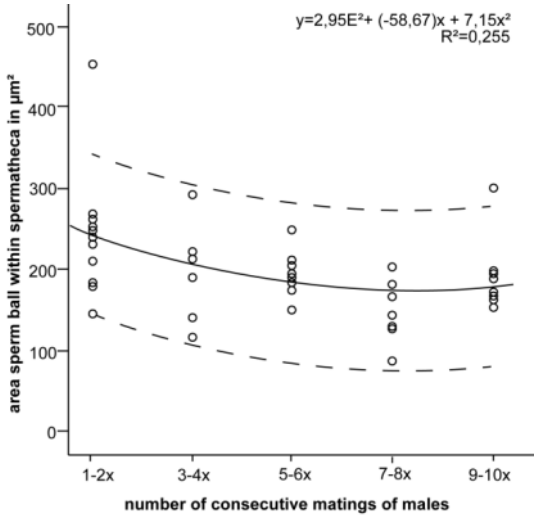


Fig. 4 Area of sperm ball within spermatheca after different numbers of consecutive copulations of male in *E. americanus*

3.5 Discussion

Mating behavior

Mating behavior was analysed with video playbacks, as well as direct observations. Behavior in *E. americanus* corresponded largely to that of *F. occidentalis* described by Terry and Schneider (1993), *F. schultzei* (Milne et al. 2007) and *S. aurantii* (Rafter and Walter 2013). After the phase of initial contact, which was much shorter in *E. americanus* (about 8.61 s in *E. americanus,* 19 s in *F. occidentalis*), mounting occurred. The male twisted his abdomen underneath that of the female and tried to insert his aedeagus into the female genitalia. The duration of copulation varied with male age. Older males copulated significantly longer than younger males, a phenomenon which is known also in *Tenebrio molitor* (Carazo et al. 2011). Presumably longer copulation is necessary to ensure transfer of enough sperm to the female, because we demonstrated a decrease in spermatozoa quantity within the testes (see section below: sperm quantification), or older males are less effective in sperm transfer (Carazo et al. 2011).

However, copulation duration in *E. americanus* was much longer than in *F. occidentalis* (Terry and Schneider 1993), but seems typical for terebrantian species (*Caliothrips fasciatus* 1-10 min Lewis 1973; *Thrips major* and *Thrips fuscipennis* 6 min Kirk 1985, *Frankliniella Schultzei* 9 min Milne et al. 2007, 13 min *Scirtothrips aurantii* Rafter and Walter 2013).

Chronological sequence

Copulation starts with insertion of the phallus into the vagina. Phallotheca and endotheca are everted, presumably by hydrostatic pressure (Heming 1970, Pitkin 1972). The vagina is heavily stretched, compared to virgin females (see Chapter 2). The placement of the gonopore near the mouth of the spermathecal duct has also been described in *Frankliniella fusca* (Heming 1970). Sensilla on the outside of the parameres presumably assist in positioning the phallus within the female (Chapter 2).

First, accessory gland material is transferred into the female, whereas spermatozoa transfer starts 3 min after phallus insertion. Since no glandular material or spermatozoa were found

inside the vagina, and the paraphyses were located close to the spermathecal duct, it can be assumed that the ejaculate migrates directly to the duct and to the spermatheca. However, this process seems to take some time to complete, as filling of the spermatheca could be observed only after 7 minutes. After copulation, a sperm ball of twisted spermatozoa is observable within the spermatheca. This sperm ball consists of a spherical capsule of densely-packed secretion outside and less- electron- dense material and spermatozoa inside (Teuber 2011). The encapsuled sperm ball is similar to that of other species within the Thripidae (Heming 1995).

Bournier (1956) supposed this type of sperm ball to be a spermatophore. He uses the term spermatophore in a stricter sense as an ampulla or capsule created by males and transferred to the female. Teuber (2011) argued that the unequal distribution of secretion within the sperm ball suggested a spermatophore rather than a coagulation within the female reproductive tract. But the gradual release of glandular secretion, and later the spermatozoa shown in this study, are reasons for these different distribution patterns of spermatozoa and glandular material. Therefore the time-delayed transfer of secretion and spermatozoa, as well as the delivery near the spermathecal duct shown here, support Bode`s (1975) assumption of coagulation of the secretion within the spermatheca.

Therefore the sperm ball seems to be a special form of “female-determined type 1 spermatophore” according to Gerber (1970), which is characterized by an ejection of secretion in a definite sequence before or after the spermatozoa and a form of coagulated material, determined by the female genital tract. This was already suspected by Heming (1995). A similar “spermathecal spermatophore formation” is known only in the psocids *Leptinotus patruelis* (Finlayson 1949) and *Trogium pulsatorium* (Klier 1956). Within the tubuliferan species only *Tiarothrips subramanii* is known to produce spermatophore-like objects (Ananthakrishnan 1990).

Frequency

Remating frequency was extremely low in *E. americanus.* While males mate multiple times, females reject mating after initial copulation, also noted by Li et al. (2014). Females of *F. occidentalis* refuse matings over a period of 15 days (Terry and Schneider 1993). Whereas *Thrips tabaci* is the only known species within the Terebrantia with repeated matings (Li et al. 2015). Frequent matings are also typical within the Tubulifera (Crespi 1986 a,b, 1988 a,b).

In *E. americanus* a single copulation is enough to provide sufficient sperm to fertilise eggs throughout a females life time (Krueger et. al. 2015, Chapter 4). But monandry (female mate with one male during lifetime) is rare in insects (Arnqvist and Nilsson 2000, Hosken et al. 2009).

Quantification of sperm transfer

As expected, males show a significant decrease in area of spermatozoa within the testes in relation to the number of consecutive copulations. Additionally, the lifespan of males do also has an impact on spermatozoa area quantity. Possibly muscle contraction in the testis wall, suppression by other internal organs, or consumption of possible secretions within the testes are reasons for this observation. Brightness of sperm within testes, and therefore the amount

of contained sperm, decreases with the number of matings. But after 10 copulations, the testes still contain 70 % of spermatozoa compared to 5 d old virgin males.
Freshly emerged virgin males had a lower index of grey value, than 5 or 10 day old virgin males. Possibly, maturation of sperm is not entirely completed at this point. Afterwards (5 or 10 day old males) age of virgin males had no impact on index of grey value of spermatozoa within testes, therefore no decrease in amount of spermatozoa with age is detected. However, we did not test fertility of aged sperm, which is known to decrease with progressive age of sperm cells (Reinhardt 2007).

In females, brightness of sperm ball within the spermatheca did not differ. In our study, females always receive the same amount of spermatozoa by copulation, even from a tenfold mated male. But area of sperm ball significantly decreased with number of copulations. Probably the amount of transferred male accessory gland material decreases over time. In histological sections flattened and emptied accessory glands were conspicuous in 10x mated males (Supplemental Material S3).

Nonetheless sperm limitation of males, prospermatogeny, is of minor importance. Males of *F. occidentalis* are able to successful inseminate up to 10 females. Males of *Trichogramma evanescens* transfer relatively constant numbers of sperm to the first 10 females, but subsequently fewer (Damiens and Boivin 2005). Transfer of accessory gland material might influence insemination success. Male *Drosophila melanogaster* run out of gland material after 4 to 5 consecutive matings and fail to transfer sperm (Lefevre and Jonsson 1962). A similar phenomenon is described in *Drosophila silvestris* after two matings (Schwartz 1991). Besides gland substances are linked to sperm transfer and sperm protection, these substances are also linked to the induction of refractoriness in females, reduction of attractiveness, sperm competition, regulation of egg development and induction of ovulation and oviposition (see Chen 1984, Gillot 1988, 2003).

The different mating strategies of polygynous males and monandrous females within this species lead to a high risk of sexual competition. It is not clear, if females control the opportunity to mate with their limited receptivity, or if the monandry is male determined. In addition to the resistance of the female to continuous male mating attempts, males are able to manipulate female receptivity either by their accessory gland products (see above) or by transferring of ant aphrodisiacs (Hosken et al. 2009). Such a pheromonal substance is detected for *E. americanus* (Chapter 6), but such substances have only a transient effect and serve as bridging until the female is able to produce her own pheromone (Simmons 2001). Further investigations are needed to distinguish the role of both sexes in this mating strategy.

Acknowledgement
We would like to thank Christin Hesse for technical support.

3.6 References

Ananthakrishnan TN, 1990. The reproductive biology of thrips. Indira Publishing House, Oak Park, Michigan, USA.

Arnqvist G, Nilsson T, 2000. The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60, 145-164.

Bode W, 1975. Der Ovipositor und die weiblichen Geschlechtswege der Thripiden (Thysanoptera, Terebrantia). Z Morphol Ökol Tiere 81, 1-53.

Bode W, 1983. Spermienstruktur und Spermiohistogenese bei *Thrips validus* Uzel (Insecta, Thysanoptera). Zool Jb (Anat) 109, 301-318.

Boivin G, Jacob S, Damiens D, 2005. Spermatogeny as a life-history index in parasitoid wasps. Oecologia 143, 198-202.

Bournier A, 1956. Contribution a L´ètude de la parthènogèse des Thysanopteres et de sa cytology. Arch. Zool exp gen 93, 219-318.

Chen PS, 1984. The functional morphology and biochemistry of insect male accessory glands and their secretions. Ann Rev Entomol 29, 233-255.

Carazo P, Molina-Vila P, Font E, 2011. Male reproductive senescence as a potential source of sexual conflict in a beetle. Behav Ecol 22, 192-198.

Crespi BJ, 1986a. Territoriality and fighting in a colonial thrips, *Hoplothrips pedicularis*, and sexual dimorphism in Thysanoptera. Ecol Entomol 11, 119-130.

Crespi BJ, 1986b. Size assessment and alternative fighting tactics in *Elaphrothrips tuberculatus* (Insecta: Thysanoptera). Anim Behav 34, 1324-1335.

Crespi BJ, 1988a. Alternative mating tactics in thrips: effects of sex ratio variation and body size. Am Midl Nat 122, 142-150.

Crespi BJ, 1988b. Risks and benefits from lethal male fighting in colonial *Hoplothrips karnyi* (Insecta: Thysanoptera). Behav Ecol Sociobiol 22, 293-301.

Damiens D, Boivin G, 2005. Male reproductive strategy in *Trichogramma evanescens*: sperm production and allocation to females. Physiol Entomol 30, 241-247.

Finlayson LH, 1949. The life-history and anatomy of *Leptinotarsus patruelis* Pearman (Psocoptera-Atropidae). Proc zool Soc Lond 119, 301-323.

Franssen CJH, Mantel WP, 1964. Notes on copulation of thrips. Tijdsch Entomol 107, 341-347.

Gerber GH, 1970. Evolution of methods of spermatophore formation in pterygote insects. Can Entomol 102, 358-362.

Gillot C, 1988. Accessory Sex Glands. In: Arthropoda-Insecta Reproductive Biology of Invertebrates, Vol 3. Ed. by Adiyodi KG, Adiyodi RD, Wiley, New York, USA, 356-471.

Gillot C, 2003. Male accessory gland secretions: Modulators of female reproductive physiology and behaviour. Ann Rev Entomol 48, 163-184.

Gitaitis RD, Walcott RR, Wells ML, Diaz Perez JC, Sanders FH, 2003. Transmission of *Pantoea ananatis*, causal agent of center rot of onion, by tobacco thrips, *Frankliniella fusca*. Plant Dis 87, 675-678.

Heming B, 1970. Postembryonic development of the male reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc Publs Entomol Soc Amer 7, 237-272.

Heming B, 1995. History of the Germ Line in Male and Female Thrips. In: Thrips Biology and Management. Ed. by Parker BL, Skinner M, Lewis T, Plenum Press, New York, USA, 505-535.

Hosken DJ, Stockley P, Tregenza T, Wedell N, 2009. Monogamy and the Battle of the Sexes. Ann Rev Entomol 54, 361-378.

Jones DR, 2005. Plant viruses transmitted by thrips. Eur J Plant Pathol 113, 119-157.

Kirk W, 1985. Aggregation and mating of thrips in flowers of *Calystegia sepium*. Ecol Entomol 10, 433-440.

Klier E, 1956. Zur Konstructionsmorphologie des männlichen Geschlechtsapparates der Psocopteren. Zool Jb Anal 75, 207-286.

Krueger S, Mound LA, Moritz GB, 2015. Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* MORGAN 1913 (Thysanoptera). J Appl Entomol, doi: 10.1111/jen.12280

Lefevre G, Jonsson UB, 1962. Sperm transfer, storage and displacement, and utilization in *Drosophila melanogaster*. Genetics 47, 1719-1736.

Lewis T, 1973. Thrips: Their Biology, Ecology and Economic Importance. Academic Press London, New York, USA.

Lewis T, 1997. Chemical control. In: Thrips as Crop Pests. Ed. by Lewis T, CAB International, Oxon and New York, USA 567-593.

Li X-W, Jiang H-X, Zhang X-C, Shelton AM, Feng J-N, 2014. Post-mating interactions and their effects on fitness of female and male *Echinothrips americanus* (Thysanoptera: Thripidae), a new insect pest in China. PLOS ONE 9, 1-7.

Li X-W, Fail J, Shelton AM, 2015. Female multiple matings and male harassment and their effects on fitness of arrhenotokous Thrips tabaci (Thysanoptera: Thripinae).Behav Ecol Sociobiol 69, 1585-1595.

Milne M, Walter GH, Milne JR, 2007. Mating Behavior and Species Status of Host-Associated Populations of the Polyphagous Thrips, *Frankliniella schultzei*. J Insect Behav 20, 331-346.

Moritz G, 2006. Thripse - Fransenflügler, Thysanoptera. Westarp-Verlag, Hohenwarsleben, Germany.

Oetting RD, Beshear RJ, 1993. Biology of the greenhouse pest *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Int J Pure Appl Zool 4, 307-315.

Oetting RD, Beshear RJ, Liu TX, Braman SK, Baker JR, 1993. Biology and identification of thrips on greenhouse ornamentals. Geo Agr Exp St Bull 414, 20.

Pitkin BR, 1972. On the so-called spermatophores of *Cirothrips manicatus* Haliday (Thysanoptera: Thripidae). J Entomol (A) 46, 149-151.

Pitnick S, Markow TA, 1994. Male gametic strategies: sperm size, testes size and the allocation of ejaculate among successive mates by the sperm limited fly *Drosophila pachea* and its relatives. Amer Nat 143, 785-819.

Rafter MA, Walter GH, 2013. Mate Recognition in the South African Citrus Thrips *Scirtothrips aurantii* (Faure) and Cross-Mating Tests with Populations from Australia and South Africa. J Insect Behav 26, 780-795.

Reinhardt K, 2007. Evolutionary consequences of sperm cell aging. Q Rev Biol 82, 375-393.

Schwartz JM, 1991. Effect of sexual experience on male mating success in *Drosophila silvestris*. Anim Behav 42, 1017-1019.

Simmons LW, 2001. Sperm competition and Its Evolutionary Consequences in the Insects. Princeton University Press, Princeton, USA.

Stannard LJ, 1968. The Thrips, or Thysanoptera, of Illinois. Bull Ill Nat Hist Surv 29, 214-552.

Terry I, Schneider M, 1993. Copulatory behaviour and mating frequency of the western flower thrips, *Frankliniella occidentalis* (Insecta, Thysanoptera). J Pure Appl Zool 4, 339-354.

Teuber J, 2011. Spermienultrastruktur von arrhenotoken und thelytoken Thysanoptera-Arten (Insecta). Diploma-thesis. Martin-Luther-University Halle-Wittenberg, Halle, Germany.

Trdan S, Milevoj L, Raspudic E, Zezlina I, 2003. The First Record of *Echinothrips americanus* Morgan in Slovenia. Acta Phytopathol Hun 38, 157-166.

Ullman D, Sherwood JL, German TL, 1997. Thrips as vectors of plant pathogens. In: Thrips as Crop Pests. Ed. by Lewis T, CAB International, Oxon and New York, 539-565.

Vierbergen G, 1998. *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). Proc Exp Appl Entomol 9, 155-160.

CHAPTER 4

OFFSPRING SEX RATIO AND DEVELOPMENT ARE DETERMINED BY COPULATION ACTIVITY IN *ECHINOTHRIPS AMERICANUS* MORGAN 1913 (THYSANOPTERA: THRIPIDAE)

Journal of Applied Entomology
doi: 10.1111/jen.12280

Stephanie Krueger[1], Laurence A. Mound[2], Gerald B. Moritz[1]

[1]*Faculty of Natural Sciences I, Institute of Biology, Martin Luther University Halle-Wittenberg, Germany*
[2]*Australian National Insect Collection CSIRO, Canberra, ACT, Australia*

4.1 Summary

The costs of parthenogenetic reproduction are more or less unknown in thrips. However, IPM strategies require an understanding of temporal and spatial life-history variations and sex ratios. Hence, different circumstances with regard to mating and sperm storage, and their effects on the life history of the adults and progeny, were tested in *Echinothrips americanus.* Different conditions were investigated: (1) one female and one male with permanent access to each other, (2) one female and one male with limited access to each other, (3) one virgin male only, (4) one virgin female only and (5) two virgin females permanently associated. Mating or not mating has a significant effect on the longevity of females and males, and on female fecundity. As a result of tested condition (2), limited access has a positive effect on longevity of a male. On the other hand, permanent access (1) or no access to a female (3) leads to shorter longevity of a male. For a female with permanent access to a male (1), longevity and oviposition rate are reduced. In addition, test conditions of parents have a significant effect on developmental time of the offspring and their sex ratio. Compared to a female under condition (1), a female with limited access to a male (2) delivers offspring with a more male-biased sex ratio, independent of the period of time after last male access. Concerning the time for development of the offspring, females under condition (2) and under condition (4) deliver an F1 generation with a shorter developmental time than those under condition (1). Therefore, *E. americanus* seems to have a feedback system that is offspring in low male-biased populations develop faster and generate an adapted sex ratio. Hence, the assumption of a lower infestation risk in arrhenotokous than in thelytokous parthenogenesis should be reconsidered.

Keywords: life history, mating, Poinsettia thrips, reproduction, sex allocation

Own contribution: 90 %

4.2 Introduction

Reproduction is costly. Partner search, courtship and mating are time-consuming, cost-intensive and associated with a high risk of predation (Burk 1982; Bailey et al. 1993; Hosken et al. 1994; Kotiaho et al. 1998a, b; Watson et al. 1998). Furthermore, investment in gamete production (van Voorhies 1992; but see Gems and Riddle 1996) or down regulation of immune functions influences reproduction behaviour (Siva-Jothy et al. 1998; Hosken 2001; McKean and Nunney 2001; Rolff and Siva-Jothy 2002). Several studies conclude that reproduction tends to shorten lifespan in most organisms (e.g. Reznick 1985; Bell and Koufopanou 1986; Barnes and Partridge 2003; Kenyon 2005; Flatt and Promislow 2007; Harshman and Zera 2007; Tatar 2010). These so-called costs of reproduction are a particular kind of trade-offs between life-history traits (Roff 1992; Stearns 1992). If reproductive success increases, another life-history trait, for instance, survivorship decreases. Furthermore, parental effects are known to adapt offspring life-history traits to prepare progeny for the expected environmental conditions. They could be an influence on offspring phenotype, diapause, fitness, developmental time, growth rate, resistance to infections and survival (reviewed in Mousseau and Dingle 1991).
Knowledge of reproduction strategies and of effects of reproduction on life history and on offspring is important for modern pest management systems, especially if insecticide control mechanisms fail. Thrips damage can cause a loss of yield in field crops from 10 to nearly 100% (see Lewis 1997). Due to their piercing–sucking mouth parts, thrips cause visible damage on leaves and/or fruits and can transfer fungi, bacteria and viruses (Tospoviruses, Machlomoviruses). Actually, for a few species, like *Frankliniella occidentalis*, in some regions no effective insecticide is available. A few species are known for their fast adaptability to different environments, rapid reproduction, enormous host range, worldwide distribution and extremely small body size (1–3 mm) compared with the majority of insects.

Most Thysanoptera have a haplo-diploid reproduction mode and reproduce via arrhenotoky. Diploid females arise from fertilized eggs, whereas unfertilized eggs produce haploid males. Only a few are diplodiploid (thelytokous parthenogenesis) and females develop from unfertilized eggs. However, at present our knowledge is low and anecdotal in terms of the real genetic background of these sex determination processes (Moritz 1997, 2006). In thrips and other insects with arrhenotokous parthenogenesis, females are able to control the sex of offspring. Evidence for adaptive sex ratio strategies was already described (Clausen 1939; Charnov 1982; King 1987; Antolin 1993; Wrensch and Ebbert 1993; Godfray 1994). Like in many insects, females store sperm in a spermatheca after mating and later release it from this organ to allow fertilization via the micropyle of each egg. Heming (1970), Moritz (1982, 1989) and Dallai et al. (1996) found muscle insertions on the spermatheca and spermathecal duct among Thysanoptera, suggesting these muscles control sperm flow. Most studies about sex allocation were focused on the influence of different environmental factors on reproduction, like host plants and temperature (e.g. Lublinkhof and Foster 1977; Nugaliyadde 1984; Anyango 1992; Lowry et al. 1992; Oetting and Beshear 1993; van Rijn et al. 1995; Hoddle et al. 2000; Murai 2000; Stacey and Fellows 2002; Premachandra et al. 2004; Chhagan and Stevens 2007; Zhang et al. 2007; Nielsen et al. 2010; Fathi et al. 2011), but never on reproduction strategies.

The aims of this study were to test differences and consequences of reproduction strategy by *Echinothrips americanus* (Poinsettia thrips) within different mating states. This species is an emerging pest thrips worldwide, and it may be a vector of several pathogens. Although potentially resistant strains are common in the USA, very little is known about this species (Plant Health Australia 2010).

Poinsettia thrips is native in eastern parts of the United States (Stannard 1968) and since 2002 has spread to the whole north-eastern USA and Canada (Shipp et al. 2001; Ferguson and Shipp 2002). In 1989, *E. americanus* was intercepted in Europe glasshouses (Collins 1998) and spread rapidly over 20 European countries (Vierbergen et al. 2006; Varga and Fedor 2008; Andjus et al. 2009). This greenhouse pest is also found in Thailand (Mound 2000), Japan (Itoh et al. 2003), Java of Indonesia (Mound and Ng 2009), Taiwan, China (Mirab-Balou et al. 2010) and Northern Australia (Mound et al. 2013).

Echinothrips americanus is phytophagous with hosts in about 24 plant families (Vierbergen 1998). In the USA, Poinsettia thrips is confirmed to reproduce on 40 cultivated and 59 wild-growing plant species (Oetting et al. 1993; Plant Health Australia 2010). Feeding activity causes small chlorotic areas and shallow feeding punctures, such that the plants appear to be shrunken (Oetting and Beshear 1993; Oetting et al.1993; Trdan et al. 2003). Additionally, many droplets of black faecal material are deposited on the leaf surface. Because of the damage, the rapid spread and wide host range this thrips has the potential to become a major thrips pest, needing further attention and control. In contrast to other thrips species (*F. occidentalis, Thrips tabaci), E. americanus* is known to be much less resistant and can, if identified, still be controlled easily with most common insecticides (Oetting and Beshear 1993; Oetting et al. 1993; Reynaud 1998; Vierbergen 1998).

4.3 Material and Methods

Rearing

Laboratory culture of *E. americanus* was reared under uniform conditions (23 ± 1°C, 60% humidity, light regime L:D 16:8, light on 6:00 am) on *Phaseolus vulgaris* and *Gossypium* sp.

Testing

All individuals and pairs were reared in wells (each 2 cm high, Ø 2.2 cm) of a 12-well Greiner-Plate (Sigma-Aldrich, Munich, Germany) filled with 1.5 ml of 1.4% (w/v) agar and topped with a leaf discs of *P. vulgaris* (Ø 1.6 cm). Plates were covered with a glass sheet and sealed with Parafilm M© (Pechiney Plastic Packaging, Chicago, IL). Plates were kept in a climatic chamber at 23 ± 1°C, 60% humidity, light regime L:D 16:8 (light on 6:00 am).

Influence of mating on parental individuals

Freshly emerged adults (0–24 h post-emergence) were placed into the plate chambers after the scheme in table 1. In every design, 15–17 individuals were tested. All designs (except female–female condition) were repeated three times. Every day, adults were checked for survival. Every 2–3 days, plates were changed to provide fresh food for adults. ‘Old’ plates

were stored in climatic chamber for 12 days. Hatched larvae were counted and separated daily. After 12 days, plates were stored at -18°C; frozen leaf discs were placed in ethanol to degrade chlorophyll in order to count non-hatched, prolarval egg stages. Observation stopped with female death. Previously deceased males were replaced with males from deceased females from the same repetition. If not possible, other mated males were added, but excluded from further analysis.

To enable similar conditions in designing female–female, dead females were replaced with individuals where partner also died or with an extra female of the same age and design conditions (added females were marked). To adapt number of laid eggs per female for this condition, number was halved. However, number of laid eggs per female and day were not investigated because of the design.

Influence of reproduction mode on progeny
All hatched larvae of experimental part 1) (except female–female condition) were raised up in identical culture plates. Developmental status was documented daily, and after adult hatching, sex was determined. Larval mortality was not analysed in this study, because an influence of handling could not be excluded. To determine male sex ratio of progeny over mothers' lifetime, mothers' individual adult lifetime was divided into 4 equal phases (early, first medial, second medial, late phase; phase = individual adult lifetime/ 4). Male sex ratio was calculated for each phase for each female.

Table 1 Experimental design and conditions.

design	condition
(1) 1♀, 1♂	1 male and 1 female permanent associated
(2) 1♀, 1♂, 24 h	1 male and 1 female 24 h associated, separation afterwards
(3) 1♂	1 virgin male
(4) 1♀	1 virgin female
(5) 2♀	2 virgin females permanent associated

Statistical analysis
Data analyses were performed with SPSS Statistics© 22 (IBM, Armonk, NY) and WinStat© for Excel (Fitch Software, Bad Krozingen, Germany). Prior to analysis, data were checked for normal distribution using nonparametric Shapiro–Wilk test ($P > 0.05$). All data were not normally distributed ($P < 0.05$), even after Box–Cox transformation, so nonparametric tests were used.

1 Influence of mating on parental individuals: Survival analyses were conducted with log-rank test. P-values were Bonferroni corrected (males: $P \leq 0.016$; females: $P \leq 0.00834$). Female number of laid eggs, fecundity and hatching rate were analysed with Kruskal–Wallis test for detecting differences between test conditions. Afterwards data were pairwise compared with

Mann–Whitney U-test. P-values were Bonferroni corrected (total fecundity $P \leq 0.00834$, oviposition rate $P \leq 0.0167$).

2 Influence of reproduction mode on progeny: Sex ratios of progeny were analysed with chi-square goodness-of-fit test ($P < 0.05$). Sex ratios of progeny over test period within the test condition were analysed with Friedman test ($P < 0.05$). Differences between test conditions within the different phases were analysed by Kruskal–Wallis test ($P < 0.05$). Development times of offspring were checked for differences with Kruskal–Wallis analysis. Afterwards differences were determined with Mann–Whitney U-test. P-values were Bonferroni corrected ($P < 0.0125$).

4.4 Results

Influence on parents

Effect of female presence on male survival probability/ longevity

Males with permanent or without female contact showed no difference in probability of survival (log-rank test, $\chi^2 = 1.46$, $P = 0.227$), (fig. 1). Therefore, the median survival differs (table 2). Males with just 24-h female contact show a significant higher probability of survival compared to permanent female-associated males (log-rank test, $\chi^2 = 9.025$, $P = 0.0027$) or virgin males (log-rank test, $\chi^2 = 13.4$, $P < 0.001$), (fig. 1).

Effect of male presence on survival probability/longevity of females

Females permanently associated with a male had a significantly lower probability of survival than mated female with 24-h male contact (log-rank test, $\chi^2 = 6.96$, $P = 0.008$). There was no significant difference between 24-h male-associated females and virgin females (log-rank test, $\chi^2 = 0.669$, $P = 0.41$). Females cohabited with a male show a trend to lower probability of survival compared with virgin females (log-rank test, $\chi^2 = 2.78$, $P = 0.095$) (fig. 2). To exclude a density effect as cause for the lower probability of survival of permanently associated males and females, two virgin females were kept together and checked for survival. Compared to single virgin females (log-rank test, $\chi^2 = 1.31$, $P = 0.252$) and single mated females (log-rank test, $\chi^2 = 0.23$, $P = 0.635$), no difference could be observed. In comparison with male-associated females, there is a slight difference in probability of survival (fig. 2), even though this is not significant after Bonferroni correction (log-rank test, $\chi^2 = 5.31$, $P = 0.021$). No difference between virgin males and virgin females probability of survival could be detected (log-rank test, $\chi^2 = 1.68$, $P = 0.195$), despite median survival differs (table 3, fig. 2).

Table 2 Longevity of male *Echinothrips americanus* depending on female presence, means followed by the same letter are not significantly different (Log-Rank-test).

design	sex	n	mean longevity in days ± SE	median survival in days
(1) 1♀, 1♂	male	47	13.57 ± 1.21 a	14
(2) 1♀, 1♂ 24 h	male	30	18.27 ± 2.81 b	20
(3) 1♂	male	42	09.62 ± 1.45 a	5,5

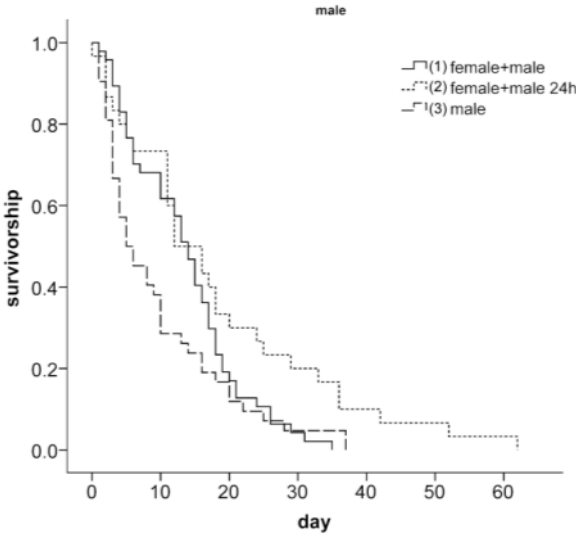


Fig. 1 Probability of survival of males depending on the presence of males

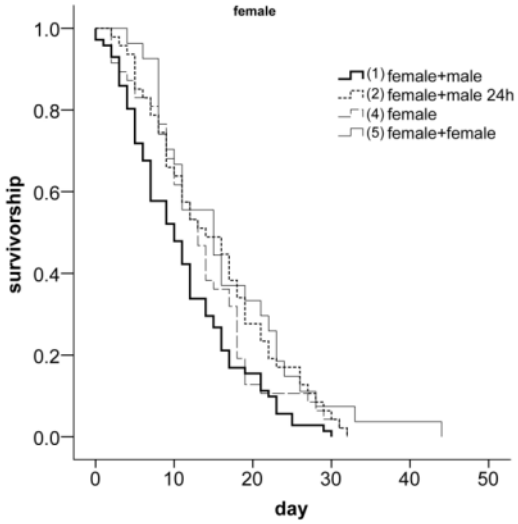


Fig. 2 Probability of survival of females depending on the presence of males or females

Effect on total fecundity

There was a significant difference in number of eggs laid per female depending on male presence (Kruskal–Wallis test, $P < 0.05$). Females permanently associated with males laid significantly fewer eggs than females 24-h male-associated (Mann–Whitney U-test, U = -3.17, P = 0.002) or virgin females (Mann–Whitney U-Test, U = -3.40, P = 0.001), (table 3). Number of laid eggs does not differ between the two-female condition and mated female condition (Mann–Whitney U-test, U = -0.832, P = 0.405) or virgin female condition (Mann–Whitney U-test, U = -0.842, P = 0.4). However, we could not find a significant difference in the number of laid eggs between the permanently associated female condition and the two-female condition after Bonferroni correction, which also may be caused by high standard error (Mann–Whitney U-test, U = -2.923, P = 0.03), (table 3).

Effect on oviposition rate

Number of eggs laid per female was analysed in dependence on longevity of female. Female oviposition rate per day differed significantly between conditions (Kruskal–Wallis test, $P < 0.05$) (table 3). Females permanently associated with males laid significantly fewer eggs per day, than individually reared mated females (Mann–Whitney U-test, U = -3.885, $P < 0.0167$), (fig. 3). A difference was also detected between females associated with a male and virgin females (Mann–Whitney U-test, U = -4.265,

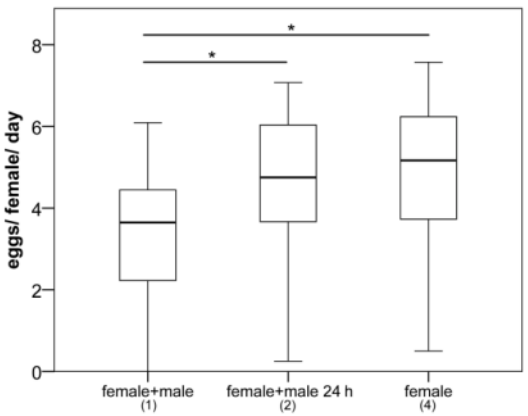


Fig. 3 Laid eggs per female and day depending on male presence in *Echinothrips americanus.*
* indicate significant differences

P < 0.0167). Mated females and virgin females do not differ in oviposition rate (Mann–Whitney U-Test, U = 0.351, P = 0.726), (fig. 3). It was not possible to record the individual oviposition rate per female due to the experimental design of the female–female condition (5).

Effect on hatching rate

Despite permanently associated females having a lower longevity and fecundity, there was no effect on hatching rate depending on male presence (Kruskal– Wallis test, P > 0.05) (table 3).

Table 3 Longevity, total fecundity, oviposition rate and mean number on non-hatched prolarval egg stages of female *Echinothrips americanus* depending on male presence, means followed by the same letter are not significantly different (longevity: Log-Rank-test; fecundity, oviposition rate, number of non hatched eggs: Kruskal-Wallis-test, Mann-Whitney-U-test).

design	sex	n	mean longevity in days ± SE	median survival in days	total fecundity (eggs per female ± SE)	oviposition rate (eggs per female/ day ± SE)	mean number of non hatched prolarval egg stages in % ± SE
(1) 1♀, 1♂	female	71	11.04 ± 0.86 a	10	41.13 ± 4.75 a	3.36 ± 0.19 a	6.13 ± 0.85 a
(2) 1♀, 1♂ 24 h	female	47	15.09 ± 1.22 b	14	76.07 ± 8.92 b	4.63 ± 0.27 b	4.11 ± 0.80 a
(4) 1♀	female	47	13.55 ± 1.12 ab	13	72.44 ± 7.60 b	4.81 ± 0.25 b	4.35 ± 0.61 a
(5) 2♀	female	27	16.37 ± 1.80 ab	15	86.54 ± 15.12 ab	-	-

Influence on progeny

Influence on sex ratio

There was a significant difference in sex ratio of progeny after adult moulting. Females with only 24-h male contact produced more sons, than females permanently associated with a male (chi-square goodness- of-fit test, $\chi^2 = 22.5$; d.f. = 1, P < 0.001). Virgin females produced only sons (table 4).

Table 4 Tertiary sex ratio of progeny (at adult moult) depending on design, sex ratio followed by the same letter are not significantly different (χ^2-godness-of-fit-test).

design	total number of progeny	female	male	sex ratio
(1) 1♀, 1♂	1236	966	270	0,22 a
(2) 1♀, 1♂ 24 h	1849	1303	546	0,30 b
(4) 1♀	1917	-	1917	1,00 c

Influence on sex ratio of progeny over test period

Lifespan of mothers was divided into four phases. Sex ratio of progeny was individually calculated for each female in each phase (fig. 4). Male sex ratio within the different phases did not significantly differ in both test conditions (permanent female–male condition: Friedman test, P > 0.05; mated female condition: Friedman test, P > 0.05). There was also no difference between the several phases compared in the two conditions (Kruskal–Wallis test; phase 1:

P = 0.335; phase 2: P = 0.996; phase 3: P = 0.654; phase 4: P = 0.814). Despite high variability within the data, in both conditions, no female produced exclusively male progeny in phase 4.

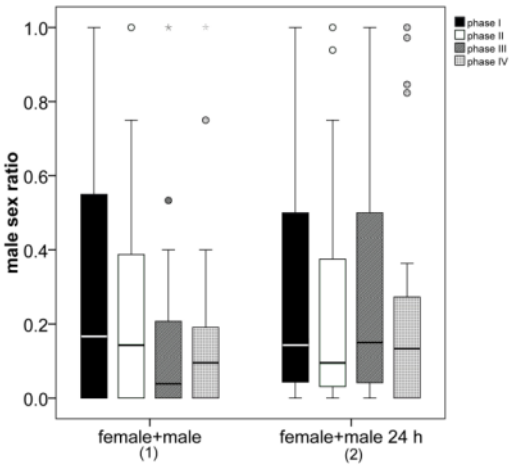


Fig. 4 Change in male sex ratio of progeny over mother`s lifespan depending on male presence

Influence on developmental time

Depending on their mothers' test condition, developmental time of female progeny differed significantly (table 5). Whereas time for embryonic development and pupation do not differ, development of first-instar larvae, second-instar larvae and propupa is significantly shorter, when mother was only 24 h associated with a male. Also male development is different between mothers' test condition (table 5). All developmental stages were significantly shorter in virgin or 24 h mated female condition, than in the permanently associated female condition.

Table 5 Mean developmental time of female and male progeny depending on mothers mating status, means followed by the same letter are not significantly different (Kruskal-Wallis-test, Mann-Whitney-U-test).

design	sex	n	egg in days ±SE	1st larval instar in days ±SE	2nd larval instar in days ±SE	propupae in days ±SE	pupae in days ±SE
(1) 1♀, 1♂	female	808	8,23 ± 0,034a	2,18 ± 0,019a	2,68 ± 0,023a	1,11 ± 0,014a	2,28 ± 0,017a
(2) 1♀, 1♂ 24 h	female	1149	8,19 ± 0,026a	1,89 ± 0,014b	2,41 ± 0,017b	1,06 ± 0,01b	2,25 ± 0,014a
(1) 1♀, 1♂	male	196	8,48 ± 0,071a	2,00 ± 0,043a	2,47 ± 0,05a	1,13 ± 0,03a	2,27 ± 0,038a
(2) 1♀, 1♂ 24 h	male	480	8,34 ± 0,044a	1,68 ± 0,025b	2,22 ± 0,022b	1,05 ± 0,015b	2,18 ± 0,02a
(4) 1♀	male	1296	8,40 ± 0,026b	1,66 ± 0,016b	2,20 ± 0,012b	1,09 ± 0,01ab	2,21 ± 0,012ab

Influence on total developmental time from egg to adult

Total developmental time of female and male progeny differed significantly, depending on mothers' test conditions (fig. 5, table 5). Female offspring develop much faster if their mother was only 24 h associated with a male, than in permanent-associated male condition (Mann–Whitney U-test, U = -10.568, P < 0.0125). Also male offspring development was much shorter if mother was only 24-h male-associated (Mann–Whitney U-test, U = -7.259, P > 0.0125) or virgin (Mann–Whitney U-test, U = -9.856, P < 0.0125), than permanently

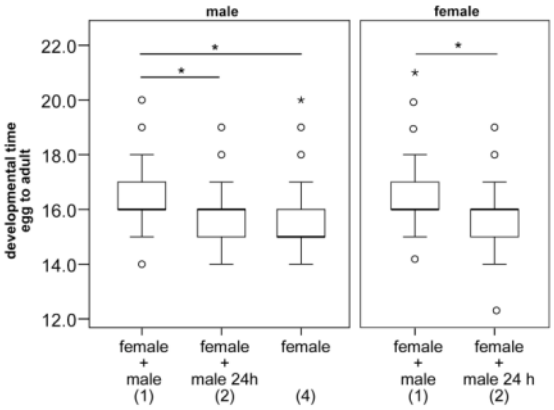


Fig. 5 Mean developmental time in days of male and female progeny from egg to adult depending on male presence. * indicate significant differences.

associated female condition. There was no significant difference in time of development of progeny in mated female condition and virgin female condition (Mann–Whitney U-test, U = -2.816, P > 0.0125).

4.5 Discussion

Longevity male

In our study, access of males to females had a significant influence on longevity of males. Whereas males permanently cohabited with a female had the lowest probability of survival, males with 24-h contact, and therefore possibility to mate, showed the highest probability of survival. Differences in population density and therefore access to food or investment in copulation, courtship and repeated copulation tries could be reasons for decreased longevity of permanently associated males, which is known from wolf spiders (Mappes et al. 1996), leaf beetles (Paukku and Kotiaho 2005), dung beetles (Kotiaho and Simmons 2003) and tsetse flies (Clutton-Brock and Langley 1997). But virgin males, and males with permanent female contact (therefore two individuals per arena), showed no difference in longevity, whereas virgin males and mated males (24 h) differed significantly (kept alone after 24-h female contact). Therefore, differences in population density cannot be considered as cause of these differences. The higher longevity of 24-h female contact males appears to be a product of a positive effect of copulation without a possibility for further re-mating, courtship or male harassment. Courtship is known to reduce longevity in some insects (Cordts and Patridge 1996; Mappes et al. 1996; Clutton-Brock and Langley 1997; Kotiaho 2000, 2001). We could not observe courtship behaviour or re-mating (unpublished data). Also Li et al. (2014) reported an extremely low re-mating frequency. But repeated harassment could be a reason for differences in longevity. Several studies have demonstrated costs for both sexes, including loss of feeding time (Magurran and Seghers 1994; Dadda et al. 2005), energy expenditure (Watson et al. 1998), increased risk of predation (Rowe et al. 1994; Croft et al. 2006) or physical injuries (LeBoeuf and Mesnick 1991). The low longevity of virgin males without any possibility to mate was not expected. A similar phenomenon is described by Cordts and Patridge (1996) where male *Drosophila melanogaster* kept with females which could not mate died sooner, than males kept with females which could mate. In contrast to Li et al. (2014), virgin males of *E. americanus* have a significant higher longevity, than mated males or males kept with one female. But the presence of females did not affect longevity and survival of mated males (Li et al. 2014). Therefore, it lacks an answer to these results. However, mean longevity in Li et al. (2012, 2014) is more than twice as long as in our study. The use of different host plants, temperature and rearing method could be reasons. Thysanoptera respond sensitive to these (a) biotic factors (for instance: Lublinkhof and Foster 1977; Nugaliyadde 1984; Lowry et al. 1992; van Rijn et al. 1995; Oetting and Beshear 1993; Oetting et al. 1993; Anyango 1992; Murai 2000; Stacey and Fellows 2002; Premachandra et al. 2004; Chhagan and Stevens 2007; Fathi et al. 2011; Zhang et al. 2007; Nielsen et al. 2010).

Longevity of females

In contrast, mated or virgin females did not differ in probability of survival. Therefore, mating itself does not have an effect on longevity, which is also known from *Franklinothrips* (Hoddle et al. 2000). However, a permanent male contact had a negative effect on female longevity. Females cohabited with a male had a significantly lower probability of survival, than mated and virgin females. To determine whether the population density or the male presence are decisive factors for this effect, two virgin females were kept together and longevity as well as oviposition were analysed. Cohabited females showed no difference in probability of survival, but a slight difference to permanently male-associated females. So contact to males and probably male harassment are the affecting factors of longevity of females. This phenomenon of lower longevity (and fecundity) of mated females due to the presence of males is known from *E. americanus* (Li et al. 2014), *T. tabaci* (Li et al. 2015), *Lygaeus equestris* and *Callosobruchus* (Rönn et al. 2006; Edvardsson 2007; Gay et al. 2009; den Hollander and Gwynne 2009). Female *D. melanogaster* exposed to males incur costs to longevity as a result of both non-mating effects (Partridge and Fowler 1990; Chapman 1992) and mating itself (Fowler and Partridge 1989; Chapman et al. 1995).

Effect on number of eggs laid and fecundity

Number of eggs laid between virgin and mated females did not differ. Again, we were not able to confirm the postulated decrease in oviposition of virgin females in arrhenotokous species (Wrensch and Young 1975; Li et al. 2012; Rugman-Jones et al. 2012). Females permanently associated with a male laid significantly fewer eggs, than mated females or virgin females. Oviposition of females permanently associated with females occupies an intermediate position. Higher abundance of individuals is known to reduce female oviposition rate in *Frankliniella tenuicornis, Stenchaetothrips biformis*, *Thrips fuscipennis, Hercinothrips femoralis* (Takrony 1973; Nugaliyadde 1984; Malchau 1991; Kirk 1994). But intermediate oviposition in female–female condition suggests the conclusion that male presence also has an important influence. Male harassment can disturb females during oviposition, reducing their overall fitness (McLain and Pratt 1999; den Hollander and Gwynne 2009), longevity (Clutton-Brock and Langley 1997) and fecundity (Gosden et al. 2007; Gay et al. 2009; Rossi et al. 2010) and therefore can be reasonable for a lower number of eggs. However, the hatching rate did not differ.

Amount of progeny and influence of sex ratio

We found a significant difference in progeny sex ratio (tertiary!) depending on the mothers' test conditions. Virgin females produce only male offspring. Also Kumm (2002) and Li et al. (2012) found a purely male-biased sex ratio of progeny of virgin females in contrast to Oetting and Beshear (1993), where virgin females produced a few females. Mothers permanently associated with a male produced significantly more female offspring, than mated females, despite the total number of progeny differing between the conditions. In contrast, Li et al. (2014) found no difference in sex ratio depending on male presence. Kumm (2002) observed a male sex ratio of 0.3 in the same culture under normal breeding conditions. Based on the analysis of sex ratio of offspring over time, an effect of insufficient sperm or insufficient

fertilization capacity can be excluded. A 24 h male contact is enough to produce about 70% females to the end of a mother's lifetime. This is congruent with Walker (1980) concept, where one copulation provides enough sperm to fertilize all eggs of a female. Although there is no difference in the sex ratio of offspring over time, there is a significant difference in overall sex ratios under different conditions. In perspective of sex allocation theory, a female is endeavouring to maximize her fitness. Therefore, she will produce enough sons to fertilize all of her daughters (Hamilton 1967; Crespi 1993). Furthermore, she will increase male proportion under unfavourable environmental factors, like a decline in food availability or change in temperature to assure advantages of sexual reproduction (Wrensch 1993), or in our case a low male density. This suggests a control over sex ratio. Haplo-diploid organisms may generally control sex ratio by controlling sperm access to eggs and fertilization and have adaptive sex allocation strategies in different environmental conditions (Clausen 1939; Charnov 1982; King 1987; Antolin 1993; Wrensch and Ebbert 1993; Godfray 1994; Hoddle et al. 2000; Kumm 2002). In Thysanoptera, the sex determination process and time of decision are unknown. In a few species of Phlaeothripidae, embryogenesis of males happens within the female abdomen and results in ovoviviparity (Bagnall 1921; Crespi 1989). Therefore, decision about progeny should occur within early egg development. In our results, *E. americanus* showed under certain population conditions an altered sex ratio, which suggests a great deal of control. Nevertheless, above all is still the question about the influence of *Wolbachia* or other reproduction manipulating microorganisms. Our laboratory culture of *E. americanus* was known to be highly infected with Wolbachia (Kumm and Moritz 2008), a widely known manipulator of reproduction and offspring sex ratio (e.g. Stouthamer et al. 1999; Werren et al. 2008), but until now a direct impact has not been proven.

Influence on offspring developmental time

Beside changes in sex ratio, developmental time of progeny between the different treatments differed significantly. Whereas male and female shared an equal developmental time if their mothers were permanently male associated, a significant decrease occurred within progeny of 24-h male-associated and virgin mothers. General developmental time is similar to that given by Kumm (2002), whereas in Li et al. (2012) a much longer egg and second-instar larvae stage were observed. Furthermore, they found no difference in offspring development between parthenogenetic (equal to our virgin test condition, male offspring) and sexual (equal to our permanently associated test condition) reproduction mode. But they did not check differences between duration of development depending on sex of offspring. However, assessment of development of Thysanoptera between different publications is difficult because of their sensitivity to biotic and abiotic factors. Differences in development time of progeny seem to be influenced more by maternal stress conditions than mating status. Iwao (1968) suggested a probable relationship between changes in development under laboratory conditions and the characteristic lifestyle of a species. In species that lay single eggs, grouping effects may result in high mortality and extended larval development. In contrast, species with a gregarious mode may react with an accelerated development. Therefore, *E. americanus* seems to prefer lower population densities.

Conclusion for reproduction strategy and damage potential

In case of low-male population conditions, mated females or virgin females have a higher probability of survival. They produce more offspring with a higher proportion of males under such conditions. Additionally, post-embryonic development of these descendants is significantly faster. Therefore, this could produce a feedback system, where mother–son mating occurs to ensure persistence or establishment of a new population. First pre-trial shows that mother–son matings result in female offspring. Such negative feedback system is known for several species (Borgia 1980). The assumption about a lower infestation risk of arrhenotokous species should be reconsidered.

Acknowledgements

For technical help, we have to thank candidate M.Sc. Marcus Jilge and Dr. Katrin Bittrich for support using SPSS Statistics© 22. For critical comments, we thank Dr. Tanja Schwander, University of Lausanne, Switzerland.

4.6 References

Andjus L, Jovic M, Trdan S, 2009. First Record of *Echinothrips americanus* in Serbia. HPPJ, 2, 71–73.

Antolin MF, 1993. Genetics of biased sex ratios in subdivided populations: models, assumptions and evidence. Oxf Surv Evol Biol, 9, 239–281.

Anyango JJ, 1992. The life history of chrysanthemum thrips, *Thrips nigropilosus* Uzel (Thripidae: Thysanoptera) and an evaluation of the effects of temperature on its life cycle. Int J Trop Insect Sci, 13, 41–44.

Bagnall RS, 1921. On Thysanoptera from the Seychelles Islands and Rodriguez. J Nat Hist Mus, 7, 257–293.

Bailey W, Withers P, Endersby M, Gaull K, 1993. The energetic costs of calling in the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae: Listroscelidinae). J Exp Biol, 178, 21–37.

Barnes AI, Partridge L, 2003. Costing reproduction. Anim Behav, 66, 199–204.

Bell G, Koufopanou V, 1986. The costs of reproduction. Oxf Surv Evol Biol, 3, 83–131.

Borgia G, 1980. Evolution of haplodiploidy: models for inbred and outbred systems. Theor Popul Biol, 17, 103–128.

Burk T, 1982. Evolutionary significance of predation on sexually signalling males. Fla Entomol, 65, 90–104.

Chapman T, 1992. A cost of mating with males that do not transfer sperm in female *Drosophila melanogaster*. J Insect Physiol, 38, 223–227.

Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L, 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature, 373, 241–244.

Charnov EL, 1982. The theory of sex allocation. Princeton University Press, New York, NY.

Chhagan A, Stevens P, 2007. Effect of temperature on the development, longevity and oviposition of greenhouse thrips (*Heliothrips haemorhoidalis*) on lemon fruit. N Z Plant Prot, 60, 50–55.

Clausen C, 1939. The effect of host size upon the sex ratio of Hymenopterous parasites and its relation to methods or rearing and colonization. J N Y Entomol Soc, 47, 1–9.

Clutton-Brock T, Langley P, 1997. Persistent courtship reduces male and female longevity in captive tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). Behav Ecol, 8, 392–395.

Collins DW, 1998. Recent interceptions of *Echinothrips americanus* Morgan (Thysanoptera, Thripidae) imported into England. Entomol Mon Mag, 133, 1–3.

Cordts R, Patridge L, 1996. Courtship reduces longevity of male *Drosophila melanogaster*. Anim Behav, 52, 269–278.

Crespi BJ, 1989. Facultative viviparity in a thrips. Nature, 337, 357–358.

Crespi BJ, 1993. Sex Allocation Ratio Selection in Thysanoptera. In: Evolution and diversity of sex ratio in insects and mites. Ed. by Wrensch DL, Ebbert MA, Chapman & Hall, New York, NY, 214–234.

Croft DP, Morrell LJ, Wade AS, Piyapong C, Ioannou CC, Dyer JRG, Chapman BB, Wong Y, Krause J, 2006. Predation risk as a driving force for sexual segregation: a cross-population comparison. Am Nat, 167, 867–878.

Dadda M, Pilastro A, Bisazza A, 2005. Male sexual harassment and female schooling behaviour in the eastern mosquitofish. Anim Behav, 70, 463–471.

Dallai R, Del Bene G, Lupetti P, 1996. Fine structure of spermatheca and accessory gland of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Int J Insect Morphol Embryol, 25, 317–330.

Edvardsson M, 2007. Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle. Anim Behav, 74, 183–188.

Fathi SAA, Gholami F, Nouri-Ganbalani G, Mohiseni A, 2011. Life history parameters of *Thrips tabaci* (Thysanoptera: Thripidae) on six commercial cultivars of canola. Appl Entomol Zool, 46, 505–510.

Ferguson G, Shipp L, 2002. New pests in Ontario greenhouse vegetables. Bull OILB, 25, 69–72.

Flatt T, Promislow DEL, 2007. Still pondering an age-old question. Science, 318, 1255–1256.

Fowler K, Partridge L, 1989. A cost of mating in female fruitflies. Nature, 338, 760–761.

Gay L, Eady PE, Vasudev RA, Hosken DJ, Tregenza TO, 2009. Costly sexual harassment in a beetle. Physiol Entomol, 34, 86–92.

Gems D, Riddle DL, 1996. Longevity in *Caenorhabditis elegans* reduced by mating but not gamete production. Nature, 379, 723–725.

Godfray HCJ, 1994. Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ.

Gosden TP, Svensson EI, Michalak P, 2007. Female sexual polymorphism and fecundity consequences of male mating harassment in the wild. PLoS ONE; doi:10.1371/ journal.pone.0000580.

Hamilton WD, 1967. Extraordinary sex ratios. Science, 156, 477–488.

Harshman LG, Zera AJ, 2007. The cost of reproduction: the devil in the details. Trends Ecol Evol, 22, 80–86.

Heming BS, 1970. Postembryonic development of the female reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Entomol Soc Am, 7, 197–234.

Hoddle MS, Robinson L, Drescher K, Jones J, 2000. Developmental and reproductive biology of a predatory *Franklinothrips* n. sp. (Thysanoptera: Aeolothripidae). Biol Control, 18, 27–38.

den Hollander M, Gwynne DT, 2009. Female fitness consequences of male harassment and copulation in seed beetles, *Callosobruchus maculatus*. Anim Behav, 78, 1061–1070.

Hosken DJ, 2001. Sex and death: microevolutionary tradeoffs between reproductive and immune investment in dung flies. Curr Biol, 11, R379–R380.

Hosken DJ, Bailey WJ, Oshea JE, Roberts JD, 1994. Localization of insect calls by the bat *Nyctophilus geoffroyi* (Chiroptera, Vespertilionidae) – a Laboratory Study. Aust J Zool, 42, 177.

Itoh K, Oguri A, Suzuki A, 2003. Occurrence and control of insect pests in perilla on Aichi Prefecture. Proc Kansai Plant Prot Soc, 45, 71–72.

Iwao S, 1968. Some effects of grouping in lepidopterous insects. CNRS, 173, 185–212.

Kenyon C, 2005. The plasticity of aging: insights from long-lived mutants. Cell, 120, 449–460.

King B, 1987. Offspring sex ratios in parasitoid wasps. Q Rev Biol, 62, 367–396.

Kirk W, 1994. The effects of density on the oviposition rate of flower thrips. Cour Forsch Inst Senckenberg, 178, 69-73.

Kotiaho JS, 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. Behav Ecol Sociobiol, 48, 188– 194.

Kotiaho JS, 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. Biol Rev, 76, 365–376.

Kotiaho JS, Simmons LW, 2003. Longevity cost of reproduction for males but no longevity cost of mating or courtship for females in the male-dimorphic dung beetle *Onthophagus binodis*. J Insect Physiol, 49, 817–822.

Kotiaho JS, Alatalo RV, Mappes J, Nielsen MG, Parri S, Rivero A, 1998a. Energetic costs of size and sexual signalling in a wolf spider. Proc R Soc B, 265, 2203–2209.

Kotiaho JS, Alatalo RV, Mappes J, Parri S, Rivero A, 1998b. Male mating success and risk of predation in a wolf spider: a balance between sexual and natural selection? J Anim Ecol, 67, 287–291.

Kumm S, 2002. Reproduction, progenesis and embryogenesis of thrips (Thysanoptera, Insecta). PhD thesis. Martin-Luther-University Halle-Wittenberg, Halle, Germany.

Kumm S, Moritz G, 2008. First detection of Wolbachia in arrhenotokous populations of thrips species (Thysanoptera: Thripidae and Phlaeothripidae) and its role in reproduction. Environ Entomol, 37, 1422–1428.

LeBoeuf BJ, Mesnick S, 1991. Sexual behavior of male northern elephant seals: I. Lethal injuries to adult females. Behaviour, 116, 143–162.

Lewis T, 1997. Thrips as crop pests. CAB International, New York, NY.

Li XW, Zhang XC, Jiang HX, Feng JN, 2012. Comparisons of developmental and reproductive biology between parthenogenetic and sexual *Echinothrips americanus* (Thysanoptera: Thripidae). Environ Entomol, 41, 706–713.

Li XW, Jiang HX, Zhang XC, Shelton AM, Feng JN, Wicker-Thomas C, 2014. Post-mating interactions and their effects on fitness of female and male *Echinothrips americanus* (Thysanoptera: Thripidae), a new insect pest in China. PLoS ONE; doi:10.1371/journal. pone.0087725.

Li XW, Fail J, Shelton AM, 2015. Female multiple matings and male harassment and their effects on fitness of arrhenotokous *Thrips tabaci* (Thysanoptera: Thripinae). Behav Ecol Sociobiol, 69, 1585–1595.

Lowry V, Smith J, Mitchell F, 1992. Life-fertility tables of *Frankliniella fusca* (Hinds) and *F. occidentalis* (Pergande) (Thysanoptera: Thripidae) on peanut. Ann Entomol Soc Am, 85, 744–754.

Lublinkhof J, Foster D, 1977. Development and reproductive capacity of *Frankliniella occidentalis*. J Kans Entomol Soc, 50, 313–316.

Magurran AE, Seghers BH, 1994. A cost of sexual harassment in the guppy, *Poecilia reticulata*. Proc R Soc Lond B Biol Sci, 258, 89–92.

Malchau W, 1991. Zum Einfluss intraspezifischer Relationen auf die postmetabole Entwicklung von *Frankliniella tenuicornis* (Uzel, 1895) (Thysanoptera, Insecta). Zool Jahrb Syst, 118, 265–276.

Mappes J, Alatalo RV, Kotiaho J, Parri S, 1996. Viability costs of condition-dependent sexual male display in a drumming wolf spider. Proc R Soc B, 263, 785–789.

McKean KA, Nunney L, 2001. Increased sexual activity reduces male immune function in *Drosophila melanogaster*. Proc Natl Acad Sci USA, 98, 7904–7909.

McLain DK, Pratt AE, 1999. The cost of sexual coercion and heterospecific sexual harassment on the fecundity of a host-specific, seed-eating insect (*Neacoryphus bicrucis*). Behav Ecol Sociobiol, 46, 164–170.

Mirab-Balou M, Lu H, Xue-Xin C, 2010. First record of *Echinothrips americanus* Morgan (Thysanoptera, Thripidae) in mainland China, with notes on distribution and host plants. Acta Zool Sin, 35, 674–679.

Moritz G, 1982. Investigation on the morphology and anatomy in *Aeolothrips intermedius* Bagnall, 1934 (Aeolothripidae, Thysanoptera, Insecta), 3. Abdomen (in German). Zool Jahrb Anat, 108, 293–340.

Moritz G, 1989. The ontogenesis of Thysanoptera (Insecta) with special reference to the panchaetothripine *Hercinothrips femoralis* (O.M. Reuter, 1891) (Thysanoptera, Thripidae, Panchaetothripinae), VI. Imago-Abdomen (in German). Zool Jahrb Anat, 119, 157–217.

Moritz G, 1997. Structure, growth and development. In: Thrips as crop pests. Ed. by Lewis T, CAB International, New York, USA, 15–63.

Moritz G, 2006. Thripse. Fransenflügler, Thysanoptera. Westarp Wissenschaften, Hohenwarsleben.

Mound LA, 2000. The aquatic thrips *Organothrips indicus* Bhatti (Thysanoptera: Thripidae) in Queensland, and a new species, *O. wrighti*, from tropical Australia. Aust J Entomol, 39, 10–14.

Mound LA, Ng YF, 2009. An illustrated key to the genera of Thripinae (Thysanoptera) from South East Asia. Zootaxa, 2265, 27–47.

Mound LA, Tree DC, Sartiami D, 2013. The greenhouse pest, *Echinothrips americanus* Morgan, recorded for the first time from Australia and Java. Myrmecia, 49, 54– 57.

Mousseau TA, Dingle H, 1991. Maternal effects in insect life histories. Annu Rev Entomol, 36, 511–534.

Murai T, 2000. Effect of temperature on development and reproduction of the onion thrips, *Thrips tabaci* Lindemann (Thysanoptera: Thripidae), on pollen and honey solution. Appl Entomol Zool, 35, 499–504.

Nielsen MC, Teulon D, Chapman R, Butler RDGPH, 2010. Comparison of life history parameters of two *Frankliniella occidentalis* (Thysanoptera: Thripidae) strains in New Zealand. Environ Entomol, 39, 303–311.

Nugaliyadde L, 1984. Biology of rice thrips, *Stenchaetothrips biformis* (Bagnall) (Thysanoptera: Thripidae) and a greenhouse rearing technique. J Econ Entomol, 77, 1171–1175.

Oetting RD, Beshear RJ, 1993. Biology of the greenhouse pest *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Int J Pure Appl Zool, 4, 307– 315.

Oetting RD, Beshear RJ, Liu TX, Braman SK, Baker JR, 1993. Biology and identification of thrips on greenhouse ornamentals. Geo Agr Exp St Bull, 414, 20.

Partridge L, Fowler K, 1990. Non-mating costs of exposure to males in female *Drosophila melanogaster*. J Insect Physiol, 36, 419–425.

Paukku S, Kotiaho JS, 2005. Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. J Insect Physiol, 51, 1220–1226.

Plant Health Australia, 2010. Poinsettia thrips contingency plan. [WWW document, July 2015] URL http://www.planthealthaustralia.com.au/pests/poinsettia-thrips/

Premachandra W, Borgemeister C, Chabi-Olaye A, Poehling HM, 2004. Influence of temperature on the development, reproduction and longevity of *Ceratothripoides claratris* (Thysanoptera: Thripidae) on tomatoes. Bull Entomol Res, 94, 377–384.

Reynaud P, 1998. *Echinothrips americanus*. Un nouveau thrips des serres importe en France. Phytoma, 507, 36–38.

Reznick D, 1985. Costs of reproduction- an evaluation of the empirical-evidence. Oikos, 44, 257–267.

van Rijn PCJ, Mollema C, Steenhuis-Broers GM, 1995. Comparative life-history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. Bull Entomol Res, 85, 285–297.

Roff D, 1992. Evolution of life histories: theory and analysis. Springer, Berlin.

Rolff J, Siva-Jothy MT, 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. Proc Natl Acad Sci USA, 99, 9916–9918.

Rönn J, Katvala M, Arnqvist G, 2006. The costs of mating and egg production in *Callosobruchus* seed beetles. Anim Behav, 72, 335–342.

Rossi BH, Nonacs P, Pitts-Singer TL, 2010. Sexual harassment by males reduces female fecundity in the alfalfa leafcutting bee, *Megachile rotundata*. Anim Behav, 79, 165–171.

Rowe L, Arnqvist G, Sih A, Krupa J, 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. Trends Ecol Evol, 9, 289–293.

Rugman-Jones PF, Hoddle MS, Amrich R, Heraty JM, Stouthamer-Ingel CE, Stouthamer R, 2012. Phylogeographic structure, outbreeding depression, and reluctant virgin oviposition in the bean thrips, *Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae), in California. Bull Entomol Res, 102, 698–709.

Shipp JL, Gillespie DR, Fry KM, Ferguson GM, 2001. *Echinothrips americanus* (Morgan), *Frankliniella occidentalis* (Pergande), western flower thrips, and *Thrips tabaci* Lindemann, onion thrips (Thysanoptera: Thripidae). In: Biological control programs in Canada. Ed. by Mason PG, Huber JT, CABI Publishing, Wallington, 115–119.

Siva-Jothy MT, Tsubaki Y, Hopper RE, 1998. Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. Physiol Entomol, 23, 274–277.

Stacey AD, Fellows D, 2002. Temperature and development rates of thrips: evidence for a constraint on local adaption? Eur J Entomol, 99, 399–404.

Stannard L, 1968. The thrips, or Thysanoptera, of Illinois. Ill Nat Hist Surv Bull, 29, 215–552.

Stearns SC, 1992. The evolution of life histories. Oxford University Press, Oxford, New York, NY.

Stouthamer R, Breeuwer JAJ, Hurst GD, 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. Annu Rev Microbiol, 53, 71–102.

Takrony MO, 1973. Bionomics and control of sugar beet thrips *Hercinothrips femoralis* (Reuter). PhD thesis, University of Reading, Reading, UK.

Tatar M, 2010. Reproductive aging in invertebrate genetic models. Ann N Y Acad Sci, 1204, 149–155.

Trdan S, Milevoj L, Raspudic E, Zezlina I, 2003. The first record of *Echinothrips americanus* Morgan in Slovenia. Acta Phytopathol Entomol Hung, 38, 157–166.

Varga L, Fedor PJ, 2008. First interception of the greenhouse pest *Echinothrips americanus* Morgan, 1913 (Thysanoptera: Thripidae) in Slovak Republic. Plant Prot Sci, 44, 155–158.

Vierbergen G, 1998. *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). Proc Exp Appl Entomol, 9, 155–160.

Vierbergen G, Cean M, Hatalànè Szellèr I, Jenser G, Masten T, Simala M, 2006. Spread of two thrips pests in Europe: *Echinothrips americanus* and *Microcephalothrips abdominalis* (Thysanoptera: Thripidae). Acta Phytopathol Entomol Hung, 41, 287–296.

van Voorhies WA, 1992. Production of sperm reduces nematode lifespan. Nature, 360, 456–458.

Walker WF, 1980. Sperm utilization strategies in non-social insects. Am Nat, 115, 780–799.

Watson PJ, Arnqvist G, Stallmann RR, 1998. Sexual conflict and the energetic costs of mating and mate choice in water striders. Am Nat, 151, 46–58.

Werren JH, Baldo L, Clark ME, 2008. Wolbachia: master manipulators of invertebrate biology. Nat Rev Microbiol, 6, 741–751.

Wrensch DL, 1993. Evolutionary flexibility through haploid males or how chance favors the prepared genome. In: Evolution and diversity of sex ratio in insects and mites. Ed. by Wrensch DL, Ebbert MA, Chapman and Hall, New York, NY, 118–149.

Wrensch DL, Ebbert MA, 1993. Evolution and diversity of sex ratio in insects and mites. Chapman & Hall, New York, NY.

Wrensch DL, Young SSY, 1975. Effects of quality of resource and fertilization status of some traits in the two spotted spider mite, *Tetranychus urticae* Koch. Oecologica, 18, 259–267.

Zhang ZJ, Wu QJ, Li XF, Zhang YJ, Xu BY, Zhu GR, 2007. Life history of western flower thrips, *Frankliniella occidentalis* (Thysan., Thripidae), on five different vegetable leaves. J Appl Entomol, 131, 347–354.

CHAPTER 5

STERNAL GLANDS IN THYSANOPTERA

Summary

Sternal pore plates are important features for the identification of male thrips, especially within the subfamily Thripinae. They vary in shape, size and distribution even between species of single genera. Little is known about their morphological structure, particularly in species with multiple pore areas. In Section 5.1 the morphology of pore areas and associated secretory gland cells, within three different economically important species with different types of pore plates are examined with light- and electron microscope. In Section 5.2 information is provided on the chemical composition of the gland cells and their secretions through histochemical studies of *Frankliniella occidentalis* and *Echinothrips americanus* (both Thysanoptera: Thripidae).

The functional role of sternal glands has remained unknown. Their structural resemblance to pheromone glands in other insect species, their almost exclusive occurrence in males (except e.g. *Oxythrips, Macrurothrips, Chilothrips*), and their location ventrally on the abdomen has lead several authors to conclude that these glands might be the source of pheromones. But there has been no reliable evidence. In Section 5.2 histochemical investigations of sternal gland structure are reported in relation to male mating status. In Section 5.3 we tested possible functions of glandular secretions as mate-assessment pheromones and anti-aphrodisiacs beside the body size as a typical variable for mate assessment.
These new insights shed light on the mating system in the thripine *E. americanus.*

5.1 Sternal gland structures in males of bean flower thrips, *Megalurothrips sjostedti*, and Poinsettia thrips, *Echinothrips americanus*, in comparison with those of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae)

Arthropod Structure and Development 44, September 2015: 455-467.

Stephanie Krueger[1], Sevgan Subramanian[2], Saliou Niassy[2], Gerald B. Moritz[1]

[1] *Institute of Biology, Faculty of Natural Sciences, Martin Luther University Halle-Wittenberg, Heinrich-Damerow Str. 4; 06120 Halle, Germany*

[2] *Plant Health Division, International Centre of Insect Physiology and Ecology (icipe), PO Box 30772-00100, Nairobi, Kenya*

5.1.1 Summary

Sternal pores are important features for identification of male thrips, especially within the subfamily Thripinae. They vary in shape, size and distribution even between species of one genus. Their functional role is speculated to be that of sex- and/or aggregation pheromone production. Yet, sexual aggregations are not reported in *Echinothrips americanus*, known to have sternal pores, while we observed aggregations in *Megalurothrips sjostedti*, previously reported to lack them.

We examined the sternal glands and pores of the thripine species *E. americanus* and *M. sjostedti* males, in comparison with those of *Frankliniella occidentalis* using light microscopy, as well as scanning and transmission electron microscopy. Pore plates of *F. occidentalis* were ellipsoid and medial on sternites III–VII, while in *E. americanus* they were distributed as multiple micro pore plates on sternites III–VIII. In *M. sjostedti* they appeared as an extremely small pore in front of the posterior margin of each of sternites IV–VII. Pore plate and pore plate area were distributed similarly on sternites III–VII in *F. occidentalis*. However, in *E. americanus* the total pore plate area increased significantly from sternites III to VIII. Ultrastructure of cells associated with sternal glands showed typical characteristics of gland cells that differ in size, shape and number. The function of sternal glands is further discussed on the basis of morphological comparisons with other thrips species.

Keywords:
Sternal glands; Sternal pore plates; Pheromone; *Frankliniella occidentalis; Echinothrips americanus; Megalurothrips sjostedti*

Own contribution: 75 %

Abbreviations
acc gl- accessory gland, ao-aorta, ATER-agranular tubular endoplasmatic reticulum, bsl-basal lamina, cr-cuticular ridge, fc-fat cell, glc-gland cell, gly-glycogen, hc-haemocoel, hg-hind gut, i-intercellular system, m-mitochondria, mbb-microvilli brush border, M l abd dors ext-Musculus longitudinalis abdominis dorsalis exterior, M l abd dors int-Musculus longitudinalis abdominis dorsalis interior, M l abd ventr-Musculus longitudinalis abdominis ventralis, M p-t-Musculus pleuro-tergalis, M t-s-Musculus tergo-sternalis, mg I-fore part of mid gut, mg II-mid gut loop and forward directed part of mid gut, mg III-hind part of mid gut, mt-Malpighian tubules, n-nucleus, nc-nerve cord, ppl-pore plate (= areae porosae), rER-rough endoplasmatic reticulum, recp-rectal papilla, rsb-rod-shaped bacteria, sal gl-salivary gland, stgl-sternal gland, asterisk (*)-extracellular cavity.

5.1.2 Introduction

Only a small number of thrips species, especially those in the family Thripidae is widely known as important pest insects of horticultural and agricultural crops (Lewis, 1973 and Lewis, 1997). They cause significant damage by piercing and sucking out cells of leaf, flower and fruits. Additionally, some species are vectors of tospoviruses, machlomovirus, bacteria and fungi (Ullman et al., 1997). Because of their worldwide distribution, enormous host range, rapid reproduction, and extremely small body size (1 and 3 mm), thrips species have become major pests. For instance, the bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom 1908), can cause losses of 20–100% to legume crops in Africa (Singh and Allen, 1980, Rusoke and Rubaihayo, 1994, Edema and Adipala, 1996 and Tamò et al., 2010). The cryptic life style of BFT, and its ability to develop broad insecticide resistance, necessitates development of alternative approaches for its management (Tamò et al., 2010). Exploiting thrips semiochemicals (such as the aggregation pheromones produced by males) offers the potential for more effective thrips monitoring and optimal pest control (Hamilton and Kirk, 2003). At present, the males of three thrips species are known to produce pheromones; *Frankliniella intonsa* (Trybom 1895) (Hamilton et al., 2005 and Zhang et al., 2011), *Frankliniella occidentalis* (Pergande 1895) (De Kogel and van Deventer, 2003, Hamilton et al., 2005, Kirk and Hamilton, 2010 and Olaniran et al., 2013) and *Thrips palmi* Karny 1925 (Kirk and Hamilton, 2010 and Akella et al., 2014). Recently, active aggregation behaviour of male BFT on cowpea leaves has been observed, indicating a semiochemical- or insect behaviour-mediated aggregation (Niassy et al., in press).

In many thrips species, especially within the subfamily Thripinae, typical sexually dimorphic structures, taxonomically referred to as areae porosae (=pore plates after Heming, 1970), are present on sternites III or IV to VII or VIII of males. Because of their differences in shape, size, number and arrangement (even within one genus), these structures are important character states for identification of male thrips (Stannard, 1968, zur Strassen, 2003 and Mound, 2009). Functionally, the sternal pore plates, observed externally as a porous cover, are associated internally with specialized epidermal gland cells and an extracellular, subcuticular cavity that functions as a reservoir for glandular secretions. Because of the absence of sternal glands in most females, Klocke (1926) and Mound (2009) postulated a sexually determined function for them. Additionally, based on behavioural studies on *Taeniothrips dianthi* Priesner 1921 (=*Pezothrips dianthi*), Pelikan (1951) concluded that the sternal glands secreted a pheromonal substance. Subsequent, ultrastructural and functional investigations indicated that the sternal glands were structurally consistent with pheromone release as initially inferred by Pelikan (Bode, 1978, Moritz, 1997, Moritz, 2006, Sudo and Tsutsumi, 2002, Sudo and Tsutsumi, 2003, Shitatani and Tsutsumi, 2005, Shitatani and Tsutsumi, 2006, El-Ghariani and Kirk, 2008 and Olaniran et al., 2013). Typically, all examined species with sternal glands have, in common, a medially located, more or less circular porous area. Internally, the extracellular, subcuticular cavity is encircled by a batch of typical glandular cells that are characterized by presence of a basal nucleus, elongated mitochondria and apical microvilli. Secretory granules have not investigated in all species; but in those studied; the gland cell secretions are delivered into the cavity and secreted by cuticular pores and/or secretory ductules. Nonetheless, the hypothesis of pheromone production lacks strong evidence, and the exact function of sternal glands is still unknown.

Considering the active aggregation behaviour and the indications for a semiochemical or insect behaviour-mediated aggregation in BFT, a species considered to lack pore plates (Mound, 2009), our study used light- and electron-microscopical observations to search for sternal glands in *M. sjostedti*. Comparative studies on *F. occidentalis* and *Echinothrips americanus* Morgan 1913 with distinctly different pore plate structures were carried out.

F. occidentalis is a polyphagous, worldwide pest of horticulture and agriculture (Kirk and Terry, 2003 and Moritz et al., 2013). The transverse oval pore plates on its sternites III–VII are the most common type within the subfamily Thripinae.

E. americanus is widely distributed in Europe, Eastern North America and Southeast and East Asia (Li et al., 2012). It is a serious pest in greenhouses throughout the Eastern US (Moritz et al., 2009). The species has multiple circular sternal pore plates coating the whole ventral surface of sternites III–VIII, different from most others in Thripinae with pore plates.

M. sjostedti is a key pest of legumes (especially cowpea) in sub-Saharan Africa (Tamò et al., 1993 and Abate and Ampofo, 1996).

5.1.3 Material and Methods

5.1.3.1 Insects

Among the thrips studied, *F. occidentalis* and *E. americanus* were obtained from laboratory cultures maintained at the University of Halle, Germany (at 23 ± 1 °C, a relative humidity of 60 ± 10% and 16:8 h L:D photoperiod). *M. sjostedti* was obtained from laboratory cultures maintained at ICIPE, Kenya (at 25 ± 2 °C, a relative humidity of 60–80% and a 12:12 h L:D photoperiod). *E. americanus* was reared on *Phaseolus vulgaris* and *Gossypium* sp., the population originated on *Hibiscus* sp. in The Netherlands. *F. occidentalis* was reared on *P. vulgaris* and *Chrysanthemum* sp. and the population originated from *Saintpaulia ionantha* hybrid in the United States. *M. sjostedti* was reared on *P. vulgaris* and the population originated from cowpea, *Vigna unguiculata* var. *Kakamega*, in Nairobi, Kenya.

5.1.3.2 Microscopy

Light microscopy (LM)

Specimens were fixed in Carnoy's solution, dehydrated in a graded ethanol series, gradually infiltrated with isopropanol and embedded in Surgipath Paraplast® (Leica, Germany). Sections (6 µm) were cut with a Leica SM 2000R microtome (Leica, Germany), stained with Haematoxylin–Eosin or DAPI. Individuals for semithin sections were fixed in 4% paraformaldehyde in Soerensen-buffer (pH 7.2) and postfixed in 2% osmium tetroxide. After dehydration, specimens were embedded in araldite via propylenoxide. Semithin sections (500 nm) were cut with an Ultracut R microtome (Leica, Germany) and stained with 1% toluidine blue. All sections were examined under a light microscope (Leitz DMRBE, Leica, Germany) fitted with a Canon EOS 600D digital camera. Automontage (Syncroscopy, UK)

software scans and automatically combines the most in-focus regions from a series of source images taken at different focal depths and allows measurement analysis. Finally, all images were processed using Photoshop CS6 (Adobe, Ireland).

Scanning electron microscopy (SEM)

Samples were fixed in Carnoy's solution, dehydrated in a graded ethanol series, gradually infiltrated with acetone and critical point dried (Emitech K850). To make pores visible in *E. americanus*, specimens were treated with 1% KOH at 40 °C for 5 min before dehydration. Later specimens were mounted onto aluminium stubs with double-sided, adhesive pads. After drying, pads were sputter-coated with gold for 200 s at 20 mA (Balzers SCD 004). Samples were examined with a Hitachi SEM S-2400 at 18 kV and documented with ILFORD FP 4 roll film.

Transmission electron microscope (TEM)

Head and thorax of specimens were removed under chilled 0.1 M Soerensen buffer (pH 7.4). Later, abdomens were fixed in 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M Soerensen buffer (pH 7.4) for 1.5 h under vacuum of about 1000 mbar chilled on ice, and postfixed in 2% osmium tetroxide at room temperature. After dehydration specimens were embedded in araldite via propylenoxide. Semithin sections were handled as mentioned under Section 5.1.3.2. Ultrathin sections (silver/gold interference) were cut using a diamond knife and an Ultracut R (Leica, Germany). Samples were stained with uranyl acetate and/or lead citrate. Observations were obtained with a JEOL TEM 1010 (at 80 kV) fitted with a Megaview II camera.

5.1.3.3 Whole mounting and measurement of pore plates and sternite cuticular areas

Ten individuals of *F. occidentalis* and *E. americanus*, and eight individuals of *M. sjostedti* were processed and slide mounted as detailed in Moritz et al. (2004). Observations on the number of pore plates, porous area in μm^2 and cuticular surface area for sternites III to VII (*F. occidentalis*), III to VIII (*E. americanus*) and IV to VII (*M. sjostedti*) were measured and calculated with Automontage (Syncroscopy, UK) and ImageJ (version 2.0.0; Wayne Rasband, http://imagej.nih.gov/ij/) software.

5.1.3.4 Statistical analysis

All tests were performed with WinSTAT® for Microsoft®-Excel (Fitch-Software, Version 2012.1). Prior to analysis, data on number of pore plates, porous area in μm^2 and % of pore plate area per sternite were checked for normal distribution using the non-parametric Shapiro–Wilk test ($p > 0.05$). All data were normally distributed. Proportion of pore plate area to sternite cuticular surface between species and proportion per sternite within species were analyzed with ANOVA and means separated with LSD at $p = 0.05$.

5.1.4 Results

5.1.4.1 *Frankliniella occidentalis*

Phenotype

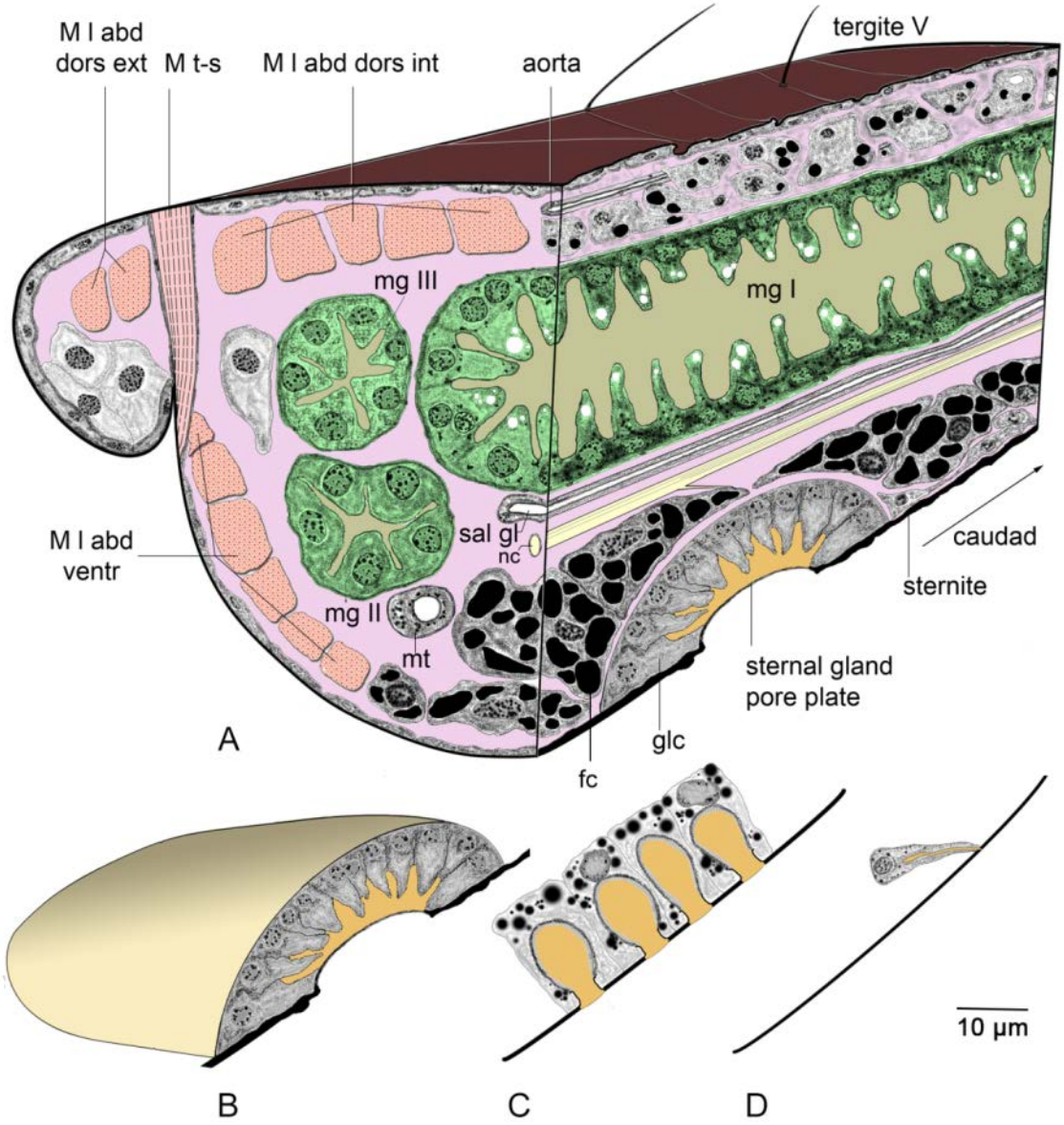
Transverse oval pore plates (Fig. 1 and Fig. 2E), typical of most pore plates reported within Thripinae, were present medially near the anterior edge on sternites III to VII (Fig. 2A). Average surface area of pore plates is about 336 ± 12 μm^2, which is about 3% of whole sternite area. There were no significant differences across sternites in either pore plate area (ANOVA, $F = 0.55$, $df = 4$, $p = 0.7$) or the proportion of pore plate area per sternite (ANOVA, $F = 0.9$, $df = 4$, $p = 0.47$) (Fig. 3 and Fig. 4).

Internal structure

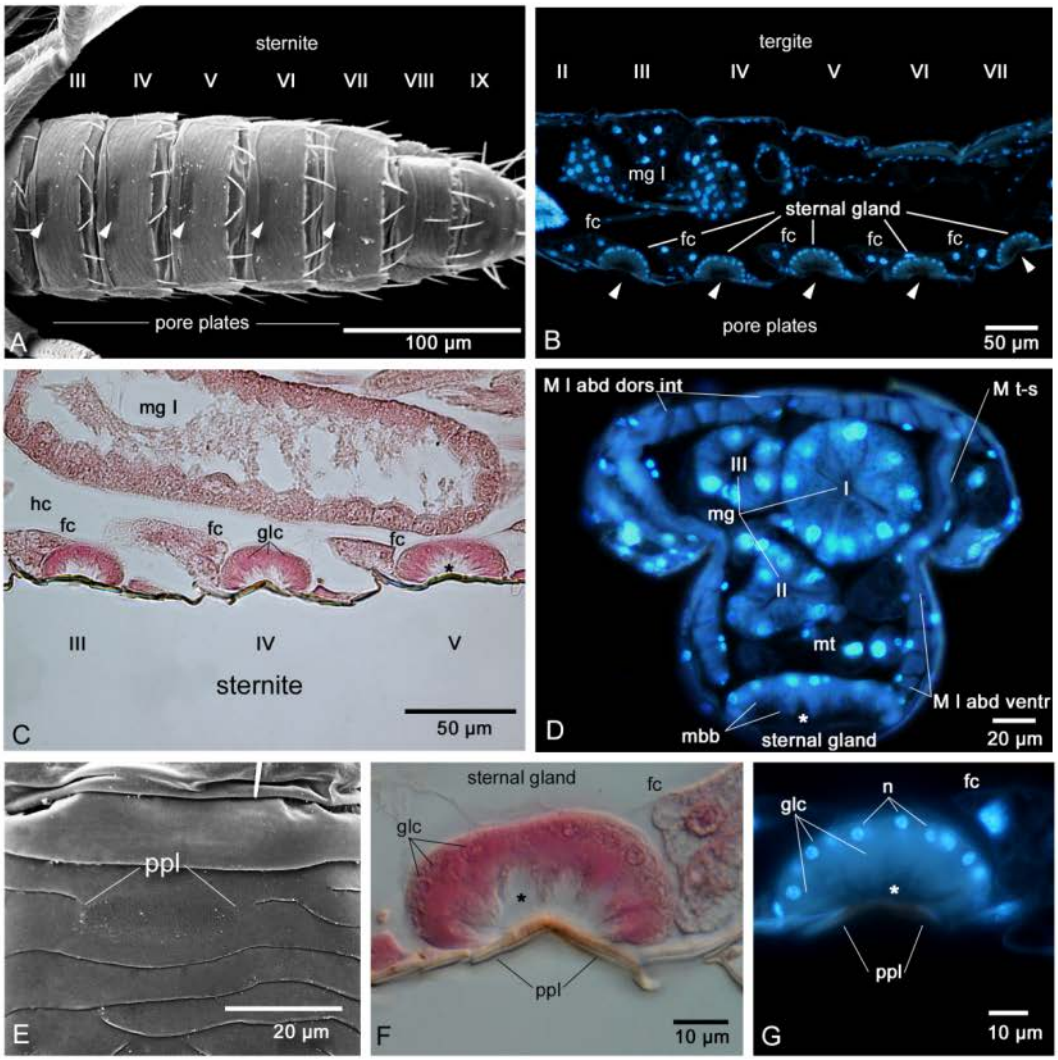
Each pore plate is subtended by numerous gland cells which, in sagittal section, are visible as a C-shaped complex underneath the cuticle (Fig. 2 and Fig. 8B, available eSlide: VM00589). The whole spheroidal gland is covered by a basal lamina, which separates the complex from the haemocoel, but has contact with a few fat cell clusters (Fig. 2B, C, F, G). Columnar shaped gland cells (about 7 µm high × 4 µm × 4 µm) are arranged around an extracellular ellipsoidal cavity (6 µm high × 40 µm × 16 µm) (Fig. 2 and Fig. 8A, available eSlide: VM00592). Cells are connected via desmosomes. The basal membrane is heavily infolded and forms an intercellular system between fat body and gland cells (Fig. 7A, C).

Numerous mitochondria, lipid droplets, aggregations of glycogen and rough endoplasmatic reticulum characterize the fat body (Fig. 7A, C). The cytoplasm of the fat body cells is not as electron-dense, as that one of the gland cells (Fig. 7A). The nerve cord is embedded in the fat body (Fig. 7G), but no direct contacts between the nerve cord and glandular tissue were observed. The basal nuclei of the gland cells had an abundance of euchromatin, while the heterochromatin is only visible in a few areas (Fig. 2 and Fig. 7A). Within the basal region some Golgi complexes were present. Secretion droplets are very rare and are only found in basal regions. Gland cells are densely packed with mitochondria (Fig. 7A). Larger and more oval mitochondria were present in the basal area, whereas in apical regions, they were smaller and more elongate (Fig. 7A).

The apical membranes of cells show a typical microvilli brush border (Fig. 2D, F). Microvilli are permeated by agranular, tubular endoplasmatic reticulum and elongated mitochondria (Fig. 7A, B). A homogeneous substance within the reservoir was detected (Fig. 7A, B). A small cuticular ridge surrounds the pore plate. The cuticle of the pore plate differs in development of endocuticle compared to the remaining ventral cuticle. The epicuticle forms secretory ducts through the cuticula. No feltwork (term referred first in Bode, 1978) at the inner end of the secretion ducts could be found.



Figures 1 A-D: Abdominal segment 5 and sternal glands, male:
1A *Frankliniella occidentalis*, abdominal segment 5, sagittal and transverse overview;
1B *Frankliniella occidentalis*, sternal gland, sagittal section;
1C *Echinothrips americanus*, sternal gland,
1D *Megalurothrips sjostedti*, sternal gland, sagittal section



Figures 2 A-G: *Frankliniella occidentalis*, male: **2A** Abdomen, sternites III to VII with medial, ellipsoid pore plates indicated by arrowheads, SEM;
2B Sagittal section of abdomen segments II-VII (note regular distribution of universal gland complexes of sternites III-VII), DAPI;
2C Sagittal section of ventral part of abdomen segments 3-5 with close association of gland complexes with fat cell clusters, HE;
2D Transverse section of abdominal segment 4 with mid gut loop (I - fore, II - mid and III - hind part of mid gut), the large extracellular reservoir of the sternal gland is indicated by an asterisk), DAPI;
2E Pore plate (areae porosae) of sternite V, SEM;
2F Sternal gland complex of sternite V (the secretion cavity is indicated by an asterisk), sagittal section, HE;
2G Columnar cell clusters with basal nuclei of a sternal gland (the cell secretion reservoir is indicated by an asterisk), sagittal section, DAPI

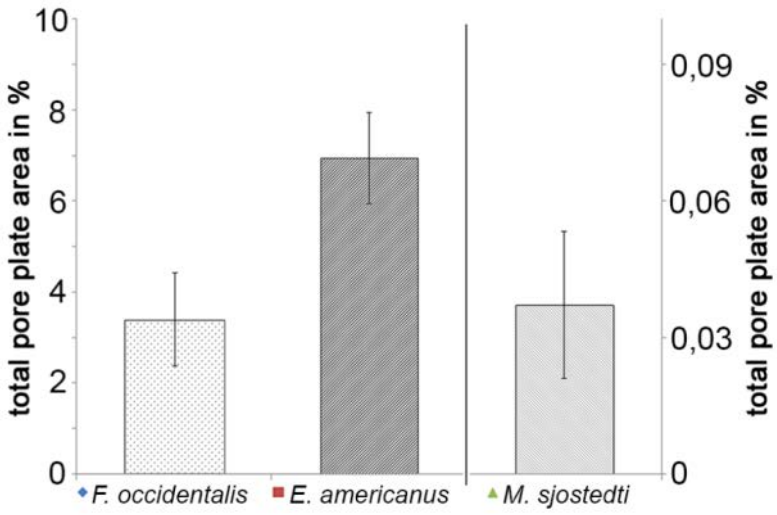


Fig 3 Percentage of total pore plate area per sternite in *Frankliniella occidentalis, Echinothrips americanus* and *Megalurothrips sjostedti* (plotted on secondary axis)

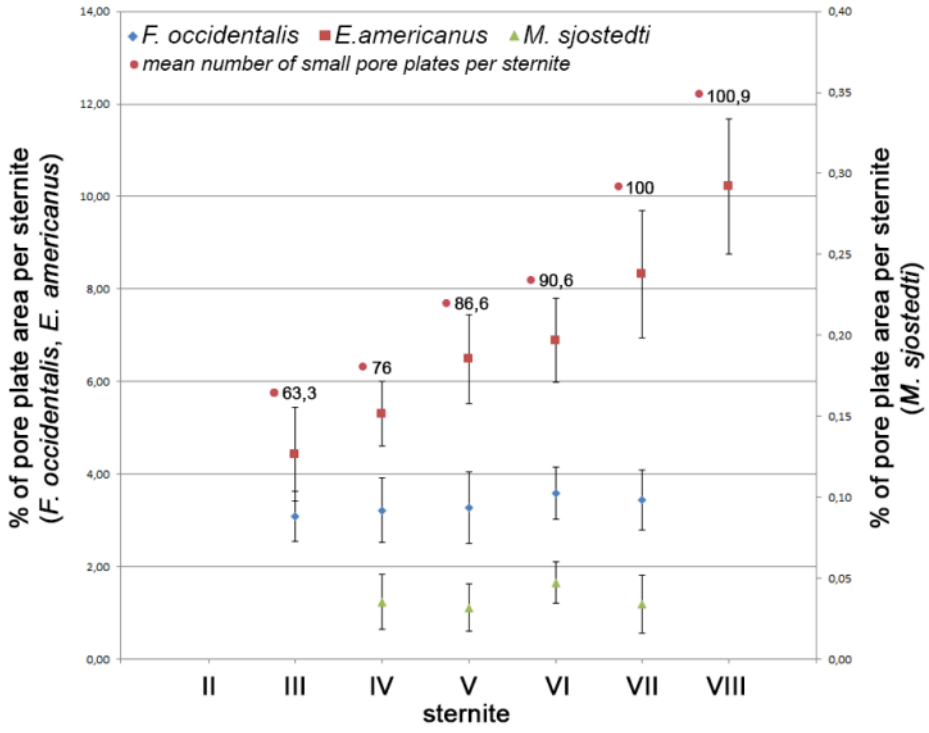


Fig 4 Differences in percentage of pore plate area per sternite of sternite III/IV – VII/VIII among *F. occidentalis, E. americanus* (with mean number of minute pore plates per sternite) and *M. sjostedti* (plotted on secondary axis)

5.1.4.2 *Echinothrips americanus*

Phenotype

Among many species with a single sternal pore plate per sternite, *E. americanus* had an enormous number of small eruptive pale plates on sternites III–VIII (Fig. 1 and Fig. 5A). Each translucent micro plate is about 4–6 µm in diameter. Scanning electron micrographs show, after KOH-treatment, typical pores on each pale micro pore plate. The micro pore plates were randomly distributed on sternites III–VIII. Total number of micro pore plates (ANOVA, $F = 12.7$, $df = 5$, $p < 0.001$) and total pore area (ANOVA, $F = 17.4$, $df = 5$, $p < 0.001$) increased significantly from sternites III to VIII (Table 1). Furthermore, the mean proportion of micro pore areas per sternite was significantly different among sternites (ANOVA, $F = 36.1$, $df = 5$, $p < 0.001$, Fig. 3 and Fig. 4).

Table 1 Mean number of micro pore plates per sternite and mean porous area of pore plates in *Echinothrips americanus* males. *Means followed by the same letters are not significantly different over sternites (n=10, p=0.001).

pore plate features	sternites*					
	III	IV	V	VI	VII	VIII
mean number	62.9 ± 12.8^{a}	76 ± 10.6^{bc}	86.6 ± 14.8^{bcd}	90.6 ± 11.4^{cdef}	100 ± 12.6^{def}	100.9 ± 16.9^{def}
porous area (µm²)	512.5 ± 126^{a}	633.7 ± 72.5^{b}	745.1 ± 107.3^{c}	788.6 ± 101.3^{cde}	875 ± 132.5^{def}	930.6 ± 141.7^{ef}

Internal structure

Sternites III to VIII had many gland cell clusters that cover nearly the whole sternal sclerite below the haemocoel. Each of the numerous, translucent, minute circles on each sternite represents a pore plate and each of these pore plates is associated with single gland cell and an extracellular secretory cavity. With an increasing number of pore plates the gland complexes build a remarkable gland cell cluster layer, which surrounds the ventral segmental longitudinal muscles (Fig. 1 and Fig. 8C, available eSlide: VM00591). Cells are approximately columnar in shape (20–30 × 10 × 10 µm). A basal lamina and an apical secretion zone provide the polarity of these gland cell groups (Fig. 5C, D, G). Dorsally, well developed fat cell tissue was in close contact with these gland cells (Fig. 5, Fig. 7 and Fig. 8C, available eSlide: VM00591, and Fig. 8D, available eSlide: VM00590). Intercellular spaces connect as a bridge with the fat body, haemocoel and gland cells (Fig. 7D). The nerve cord is embedded within the fat body and is located directly above the glandular cells (Fig. 7F). However, no direct contact between the nerve cord and glandular cells was observed. In the gland cells the basal nuclei had an abundance of euchromatin. Heterochromatin was only visible in a few areas (Fig. 5 and Fig. 7D). The cytoplasm of gland cells was relatively electron dense compared to that of fat body cells (Fig. 7D). Large droplets of lipids or vesicles and inclusions of different sizes (up to 7 µm) were characteristically seen basally (Fig. 5 and Fig. 7D, E). Droplets were surrounded by mitochondria of different sizes and shapes and rough endoplasmic reticulum (Fig. 7D, E).

Apically, cells were bounded by a well-developed microvilli brush border. Microvilli were in close contact with membranous system, though not as distinctively as in *F. occidentalis* (Fig. 7B, E). Glycogen granules were also found in this region (Fig. 7E). Most microvilli line in an extracellular cavity that is located at the opposite side of each gland cell (Fig. 5 and Fig. 7 D, E). The substance within the extracellular cavity (reservoir) was electron dense and homogeneous. The reservoir leads to an extrusion area, which is bounded by a cuticular ridge (Fig. 7D, E). The cuticle of the extrusion areas had poorly developed endocuticle and was perforated with pores for release of glandular product (Fig. 5 and Fig. 7E). The gland cell cluster of sternite VIII was much larger than those of other sternites (Fig. 5 and Fig. 8D, available eSlide: VM00590).

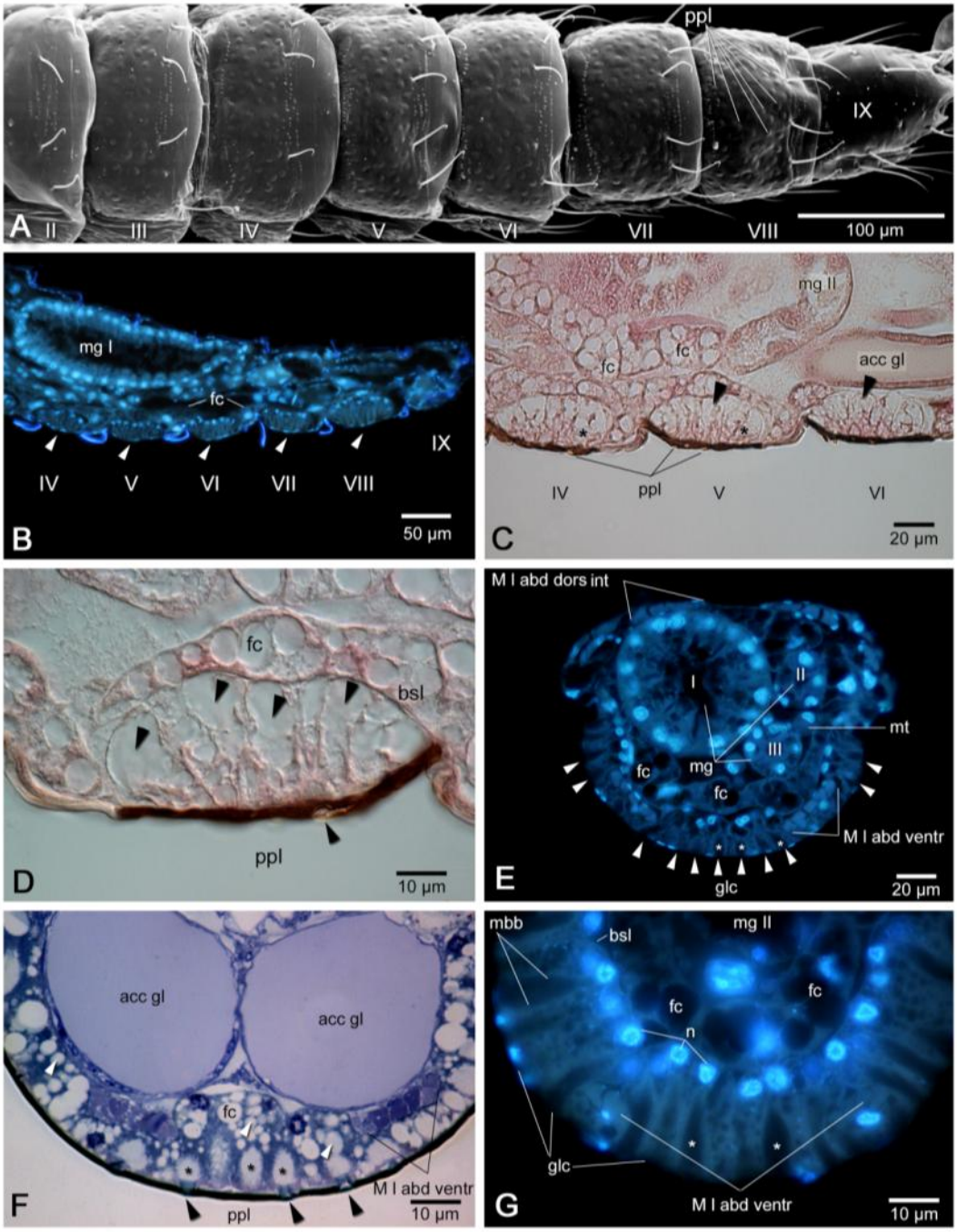
5.1.4.3 *Megalurothrips sjostedti*

Phenotype

An extremely small pore region in front of the posterior margin of abdominal sternite IV–VII is present, but often difficult to recognize, especially with bright field microscopy (Fig. 1 and Fig. 6A, B). Transversely oriented sculpture lines of the sternite mark the minute pore region of the gland (Fig. 6B). The elliptic gland opening area is about 0.8 µm across and 0.45 µm long. No other additional structures are noticeable (Fig. 6B). The entire pore area is about $3.9 \pm 1.8\ \mu m^2$. No difference of pore area was recognized between sternites IV–VII (ANOVA, $F = 0.9$, $df = 3$, $p = 0.44$). However, the percentage of total pore area per sternite is extremely low in *M. sjostedti* (about 0.03–0.04% in comparison to >6–7% in *E. americanus* and >3–4% in *F. occidentalis*) (Fig. 3 and Fig. 4).

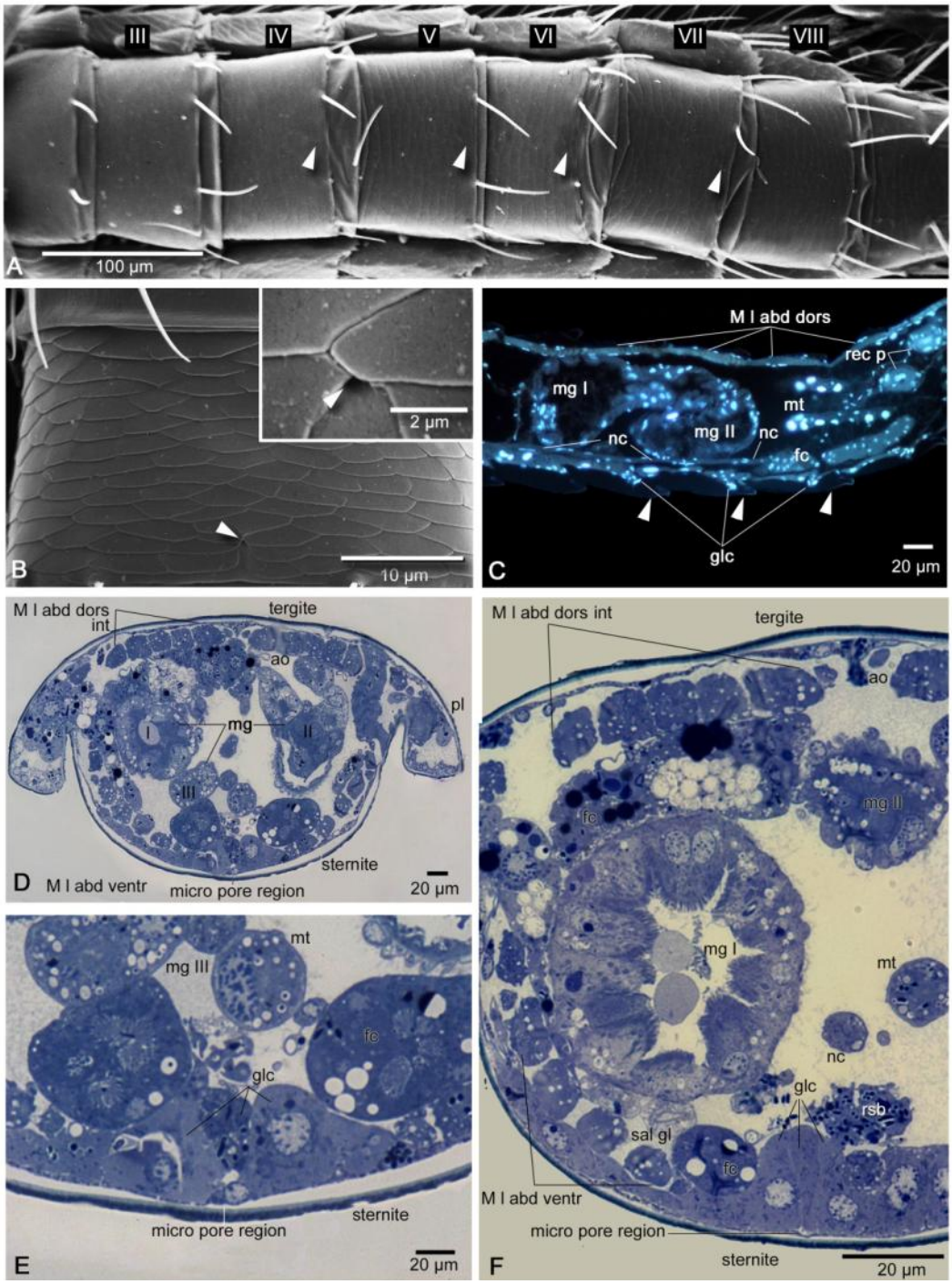
Internal structure

In contrast to the large, multiple sternal gland complexes in *E. americanus*, *M. sjostedti* has a highly reduced sternal gland system, which is partly recessed deep in the haemocoel, but which clearly contacts the minute openings in the centre of each sternite. Typical columnar gland cells are not visible. Interestingly, rod-shaped bacteria are also common within these modified gland and fat cell complexes (Fig. 6E, F). DAPI stained sections show sternal gland cell clusters with filament-like contacts to each of the sternite areas. Additionally, in semithin sections, a close contact with fat cells is recognizable as in other species (Fig. 6C, E, F). However, the small opening of the sternite pore plates leads to an atypical medial cell cluster formation between the longitudinal skeletal muscles. A typical cavity to store secretions immediately beneath the sternal sclerite is absent (Fig. 6D–F).



Figures 5A-G: *Echinothrips americanus*, male

Figures 5A-G: *Echinothrips americanus*, male:
5A Abdomen, sternites III to VIII with typical pattern of many small pore plates, SEM;
5B Sagittal section of abdominal segments IV-IX, regular distribution of gland complexes from sternite III to VII, but conspicuous larger gland complex on sternite VIII, DAPI;
5C Sagittal section of abdominal sternites IV-VI, arrows: basal inclusions and vesicles (secretory cavity is indicated by asterisks), HE;
5D Sagittal section of sternite V with sternal gland, showing a close relation to fat cell clusters, inner arrow points at basal gland cell reservoir, outer arrow marks a small pore plate, HE;
5E Transverse section of abdominal segment 5 with enormous gland tissue, in which the ventral longitudinal muscles are integrated, DAPI;
5F Transverse semithin section of posterior region of abdominal sternite VI, with paired accessory glands and many inclusions and vesicles within the sternal gland tissue (secretion reservoirs with microvilli membranes are indicated by asterisks, arrowheads mark the gland secretion vesicles), toluidine blue;
5G Transverse section of prominent sternal gland complex of sternite VII, formed by large columnar cells with basal nuclei and apically-oriented secretion reservoirs, DAPI



Figures 6 A-F: *Megalurothrips sjostedti*, male:

6A Abdomen, sternites IV to VII with extremely small pore area in the middle of each sternite, SEM;

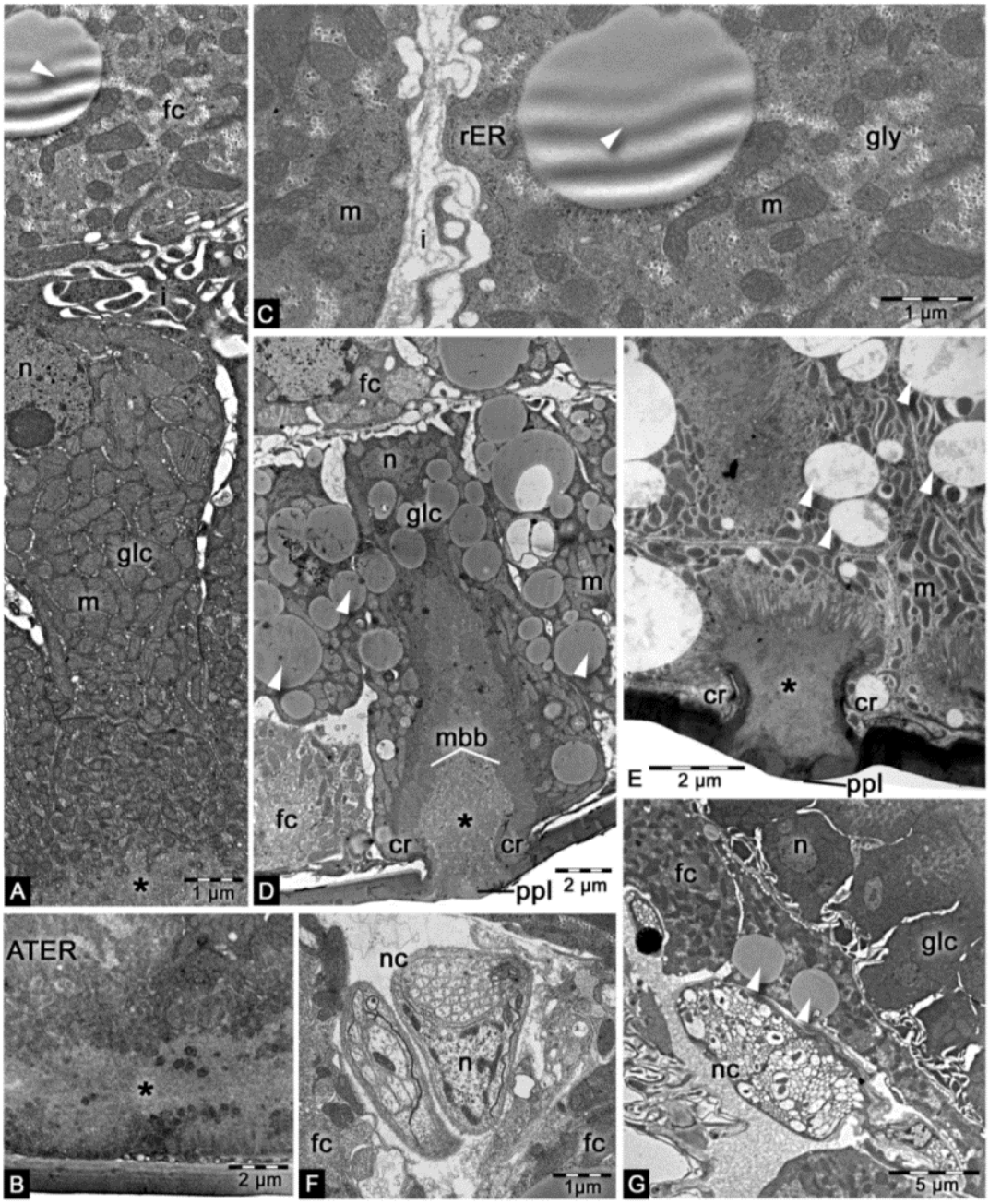
6B Sternite V with typical oriented transverse sculpture lines pointing at the pore region, Inlay: micro pore region of sternal gland, SEM;

6C Sagittal section of abdomen with mid gut loop (with descendent and ascendant part I and II of the mid gut), ventrally small, but well organized sternal gland complexes beneath the ventral nerve chord and accumulated with fat cell clusters, DAPI;

6D Transverse section of anterior segments with sternal gland cells ventrally, toluidine blue;

6E Detail of Fig. 6D, transverse section of anterior segments, note the close arrangement with fat cells, toluidine blue;

6F Transverse section of posterior region of abdomen with ventral sternal gland cell clusters, toluidine blue



Figures 7A-G: Ultrastructure of sternal glands of *Frankliniella occidentalis* and *Echinothrips americanus*, males, TEM:

7A *Frankliniella occidentalis*, columnar shaped gland cells with basal intercellular system and close contact to fat body, cell apices with microvilli and homogenous material in reservoir;

7B *Frankliniella occidentalis,* passage of microvilli with ATER- system to reservoir (note the electron dense and homologous substance within reservoir);

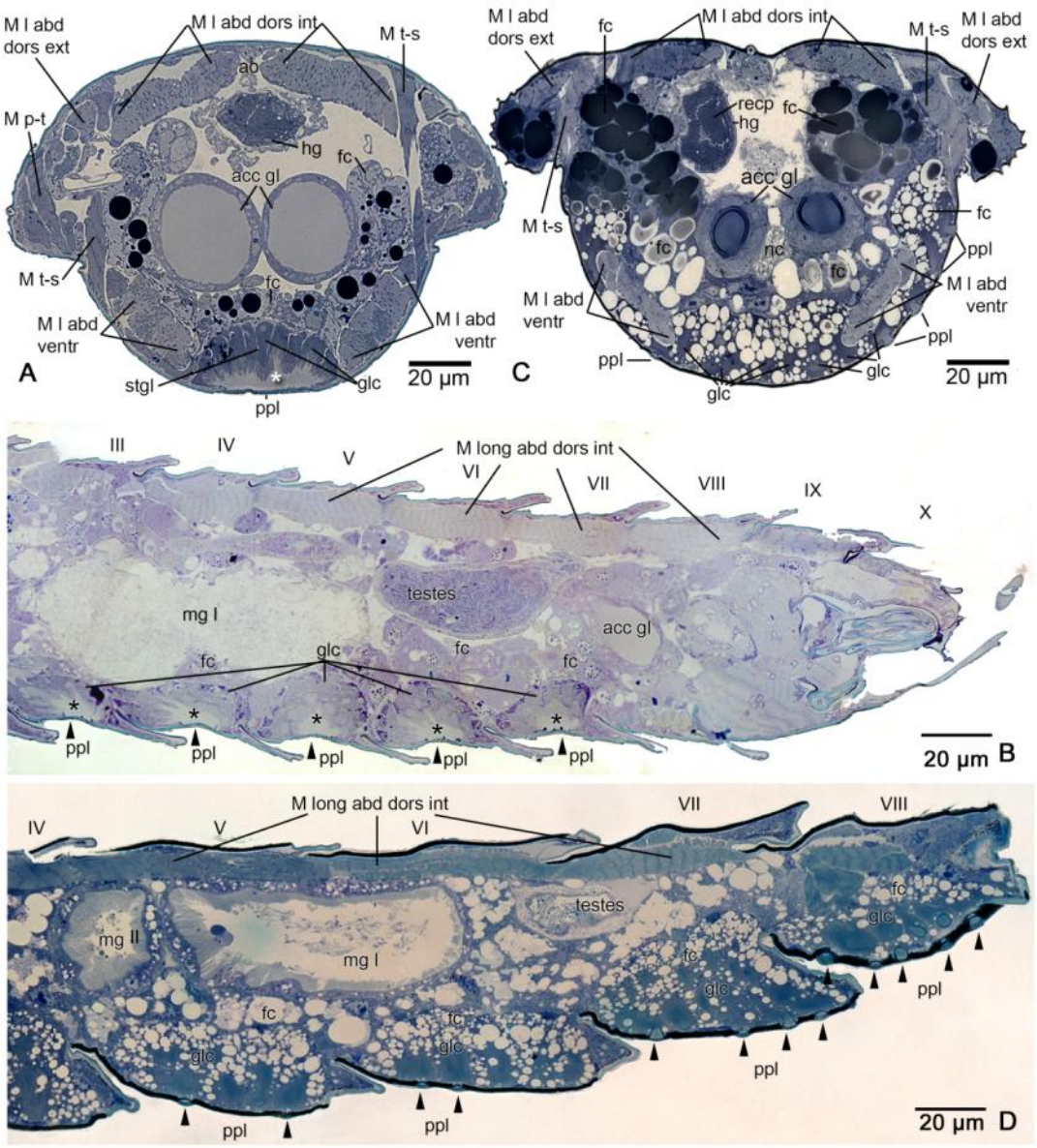
7C *Frankliniella occidentalis*, fat cell clusters above gland cells with large lipid droplets, numerous mitochondria and glycogen;

7D *Echinothrips americanus*, columnar shaped gland cell with an abundance of secretion droplets and mitochondria, cell apices with microvilli brush border, reservoir and pore plate;

7E *Echinothrips americanus,* detail of reservoir and transition to extern pore plate area (note the poor developed endocuticle in extrusion area);

7F *Echinothrips americanus*, fat body- embedded nerve cord above glandular cells;

7G *Frankliniella occidentalis*, position of different cell types around gland cell clusters, intercellular system connects gland cells and fat body, fat body surrounds nerve cord (note the different electron density within the different cell types)



Figures 8A-D: Sagittal and transverse sections of abdominal segments (males, see virtual microscope):
8A *Frankliniella occidentalis*, abdomen, transverse section of segment 6, semithin section, toluidine blue;
8B *Frankliniella occidentalis*, abdomen, sagittal section of segment 3 to 9, semithin section, toluidine blue;
8C *Echinothrips americanus*, abdomen, transverse section of segment 5, semithin section, toluidine blue;
8D *Echinothrips americanus*, abdomen, sagittal section of segment 4 to 8, semithin section, toluidine blue.

5.1.5 Discussion

Phenotype

The so-called “areae porosae”, or sternal pore plates, are typical sexually dimorphic structures of many thrips males (Heming, 1970), especially in the suborder Terebrantia. These structures are present in males of more than 60% of genera of the family Thripidae (Mound and Walker, 1986 and Mound, 2009). Several studies suggest that these porous structures are associated with the release of pheromones with different functions (aggregation: Kirk and Hamilton, 2004 and Hamilton et al., 2005; sex: Milne et al., 2002, Hamilton and Kirk, 2003, Webster et al., 2006 and Akella et al., 2014; contact: Olaniran et al., 2013). In other terebrantian families sternal pore plates are present in males of Stenurothripidae, Fauriellidae, and Heterothripidae, but located in front of the antecostal ridge of the sternite (Mound et al., 1980). In the ancestral families Merothripidae, Melanthripidae and Aeolothripidae sternal pore plates are absent (Mound, 2009) and only a few males in the genus *Merothrips* have a similar pore plate on the vertex of the head with a comparable subcuticular histological gland structure (Moritz, 1984). However, males of closely related species within the same genus can either have typical sternal glands (*Limothrips cerealium*) or lack this feature altogether (*Limothrips denticornis* Haliday 1836), which makes physiological interpretations difficult (Mound, 2009).
In the suborder Tubulifera, comparable but structurally different pore plates (Mound and Walker, 1986 and Cavalleri and Kaminski, 2007) or reticulate markings (Okajima, 1981 and Mound and Palmer, 1983) are present in several species, but these are limited to sternite VII and VIII.

The three thripine species we examined (*F. occidentalis*, *E. americanus* and *M. sjostedti*) provide an overview of principal pore plate variability and gland structure. In *F. occidentalis*, the general type of c-shaped, semi-circular gland complexes was seen to have clearly arranged columnar cells, as described by Moritz (1997) for *Taeniothrips inconsequens* (Uzel 1895), Sudo and Tsutsumi (2002) for *Thrips hawaiiensis* Morgan 1913 and *F. intonsa*, and by El-Ghariani and Kirk (2008) for *F. occidentalis*. *E. americanus* provided an example of a very different glandular model, with a single gland cell behind each pore plate whose numbers ranged from 60 to 100. The number of these plates increased from segment III–VIII with a maximum on sternite VIII. In *M. sjostedti*, particularly remarkable was the presence of an extremely minute sternal pore plate of about 0.8 × 0.45 µm on each abdominal segment IV–VII, which were extremely difficult to visualize using bright field microscopy. This may have been responsible for earlier reports which concluded that pore plates were absent in males of *M. sjostedti* (Mound and Kibby, 1998 and Mound, 2009). However, scanning electron microscopic observations in the present study revealed a clearly visible pattern of cuticular ridge marks and the minute release area of the pore plates. Based on these observations, Laurence Mound (2014, personal communication) examined males of *M. sjostedti* from Kenya, South Africa and South East Asia and observed that while the Kenyan specimens had the small pore plates, those from Zululand lacked smaller pore plates along as did males of other *Megalurothrips* species from SE Asia. It is possible that males of other species described without pore plates, like *L. cerealium*, could actually have such minute pores. This needs further ultrastructural investigation. Comparative ecological studies on male aggregation

among populations of *M. sjostedti* with or without sternal glands could provide valuable information concerning the role of sternal glands.

Internal structure

Within the Thysanoptera, reports of the presence of sternal pore plates and sternal glands are widely common, but studies on their internal structure and function are rare. The earlier remarkable studies of sternal gland histology were done with species all belonging to the family Thripidae such as, *Thrips physapus* L. 1758 (Klocke, 1926) and *P. dianthi* (Pelikan, 1951). These were followed by ultrastructural studies using transmission electron microscopy on *Thrips validus* Uzel 1895 (Bode, 1978), *F. intonsa* and *T. hawaiiensis* (Sudo and Tsutsumi, 2002), *Tenothrips frici* (Uzel 1895) (Shitatani and Tsutsumi, 2005), *Hydatothrips abdominalis* (Kurosawa 1937) and *Neohydatothrips* gracilicornis (Williams 1916) (Shitatani and Tsutsumi, 2006). Only one species of the suborder Tubulifera (*Psalidothrips simplus* Haga 1973) was studied by Sudo and Tsutsumi (2002).

In our study, *F. occidentalis* was observed to have a c-shaped (semi-circular) gland complex with typical columnar cells as reported for several other species. However, for *E. americanus* we observed a completely different structure of inner sternal gland complexes together with multiple pore plates. Multiple pore plates are known from males of a few other species, including *Pezothrips kellyanus* and *Yoshinothrips pasekamui* (Mound, 2009) and *Ceratothripoides brunneus* (Mound and Nickle, 2009 and Moritz et al., 2013). Until now, for multiple pore plates only external morphological descriptions are available. The condition of extremely reduced sternal pore plates and their associated, modified gland cells in *M. sjostedti* is reported for the first time. However, the presence of secretory granules as reported in this study is similar to observations made on species in the genera *Thrips* and *Frankliniella*, and in *T. frici* which belongs to the *Megalurothrips* genus group (Shitatani and Tsutsumi, 2005). Table 2 gives an overview and comparison of ultrastructural features of the sternal glands of known thrips species.

Table 2: Overview of structural features of sternal glands in examined species; [1] Bode, 1978; [2] Sudo and Tsutsumi, 2002; [3] Shitatani and Tsutsumi, 2005; [4] Shitatani and Tsutsumi, 2006; [5] El-Ghariani and Kirk, 2008; [#] observed in this study.

family	subfamily	species	sternites	gland form	subcuticular space	mitochondria	secretory granules	filamentous material through cuticle	cuticular ridge
Thripidae	Thripinae	*Echinothrips americanus*[#]	III-VIII	multiple pore plates	wide	oval, elongated	present	absent	present (small)
		Frankliniella intonsa [2]	III- VII	c- shaped	wide	oval, elongated	absent	absent	present (small)
		Frankliniella occidentalis[5,#]	III- VII	c- shaped	wide	oval, elongated	very rare	absent	present (small)
		Megalurothrips sjostedti[#]	IV-VII	distorted in few cell clusters	extremely small	oval	absent	absent	absent
		Thrips hawaiiensis[2]	III- VII	c-shaped	wide	oval, elongated	absent	absent	present (large)
		Thrips validus[1]	III- VII	c-shaped	wide	oval, elongated	absent	absent	present (large)
		Tenothrips frici[3]	III- VII	c-shaped	extremely narrow	oval, elongated	present	present	absent
	Sericothripinae	*Hydatothrips abdominalis*[4]	IV- VII	c-shaped	Wide	oval, elongated	present	absent	present
		Hydatothrips gracilicornis[4] (=*Neohydatothrips gracilicornis*)	IV- VII	c-shaped	Present	oval, elongated	present	present	present
Phlaeothripidae	Phlaeothripinae	*Psalidothrips simplus*[2]	VIII	distorted semicircular	present	oval	absent	developed	absent

Comparison with sternal glands within the hemipteroid assemblage

Noirot and Quennedey, 1974 and Noirot and Quennedey, 1991 and Quennedey (1998) classified epidermal glands into 3 classes. Epidermal gland cells in class 1 are simply covered by cuticle, which is secreted by the cells themselves and which allow the secretion fluid to flow. More advanced glands are not in direct contact with the cuticle and are surrounded by epidermal cells (class 2). Class 3 cells resemble those of class 2 but, in addition, have a canal or cuticular duct through which the secreted fluid flows. In thrips, a slightly modified class 1 gland cell is found, forming a simple glandular complex with each pore plate associated with gland cells and an extracellular cavity. Such modified class 1 glands are found in *E. americanus*, *T. inconsequens* (Moritz, 1997), *F. intonsa*, *T. hawaiiensis*, *P. simplus* (Sudo and Tsutsumi, 2002 and Sudo and Tsutsumi, 2003) and *F. occidentalis* (El-Ghariani and Kirk, 2008).

Within the hemipteroid assemblage, like the Thysanoptera, not much is known about these features in Psocodea (Ma and Ramaswamy, 2003). In contrast, the Hemiptera are probably the best studied Paraneoptera in this regard. Heteroptera possess numerous different gland types (e.g. dorsal abdominal scent gland, metathoracic scent gland), mostly with much higher complexity (Cobben, 1978, Schuh and Slater, 1995 and Weirauch, 2006) than in thrips. Within the Pentatomorpha females of Lygaeidae, Largidae, Pyrrhocoridae, Stenocephalidae, Coreidae, Alydidae, Rhopalidae, and males of Lygaeidae, Coreidae, Stenocephalidae possess ventral abdominal glands (paragenital glands, uradenies). These multicellular and complex glands have a single opening in the intersegmental membrane between sternites VII and VIII, VIII and IX or more rarely IX and X (Thouvenin, 1965). In Rhopalidae, the produced secretions play a role in mate recognition (Aldrich et al., 1990). Within the Anthocoridae, some males of the tribe Scolopini possess internally paired glands with a single or double orifice opening onto abdominal sternite IV or V. This structure seems to be homologous to the ventral abdominal gland of Pentatomorpha. At present, nothing is known about the secretion or its function (Schuh and Slater, 1995).

As so far, within the Heteroptera, only ventral abdominal glands (sternal glands) and dermal glands are sex specific. Paired, simple saclike glands are known from the Elasmodeminae, Holoptilinae and Phymatinae. These sternal glands or Carayon's glands are located in a position similar to the metathoracic scent gland, but each individual gland opens ventrally into the membrane between the thorax and abdomen. The secretions and their functions of these glands are unknown (Carayon, 1958 and Staddon, 1979). In addition to complex gland structures, unicellular glands are also known. Males (and some females) of most groups of Pentatomoidea possess pheromone producing, unicellular integumentary glands laterally on 3 or more abdominal sternites (Carayon, 1981). Males in several genera of Scutelleridae have a special type of such glands. Each gland consists of a single blind cell set in an alveolus and contains aphrodisiacs, which are produced by an endocuticular cell (Carayon, 1984). Two isolated instances of female-specific sternal glands are known from females of some Reduviinae and Acanthosomatidae (Carayon, 1987 and Staddon, 1990). Coccidea mainly have wax and dermal glands. Although disc shaped wax glands on the ventral abdominal segments of females of Pseudococcids, Coccids and Eriococcids form an arrangement of class 1 gland cells around a reservoir (Foldi, 1981), that is superficially similar to those in thrips species.

However, they differ in ultrastructural details from all Thysanoptera that have been studied. Wax glands produce several kinds of waxes for protection against environmental influences and to avoid egg sticking (Schmutterer, 2008). Within the Auchenorrhyncha, wax glands have similar structures (Strümpel, 2010) as described in Coccidea.

Little is known about sex pheromones in scale insects. In *Diaspidiotus perniciosus* a male attractant is produced in the female pygidial gland (Moreno, 1972 and Moreno and Fargerlund, 1975). It is assumed, that special glands in dorsal and ventral abdominal areas produce sex pheromones within the Matsucoccidae (Young and Hong-Ren, 1986). However, these have not been characterized in detail.

In contrast, Aphids have no sternal glands, but oviparous females possess sex pheromone glands on their hind tibia, which form plaques. Despite belonging to class 3 gland cells, they have a unicellular origin (Pettersson, 1971 and Harrington, 1985) and could therefore represent a transitional form of gland type. Evidence of innervation of the sternal gland complex has never observed in thrips (Bode, 1978 and Moritz, 1984). Hormonal stimulation is probably responsible for glandular activity in thrips, as has been shown for the higher termite species (Noirot and Quennedey, 1974).

Evolutionary considerations

In view of the medio-ventral position of sternal pore plates and exocrine gland cells in thrips, their glandular system may have developed from paired ectodermal derivatives through fusion and invagination. This could have produced single pore plates per sternite or, in contrast, partition or early degeneration leading to multiple minor pore plates or complete disappearance of this feature. Beside this general genesis of this feature, several differences in the gland histology, even between the two suborders of thrips, like variety in cuticular ridges, presence of secretory ducts or types of mitochondria indicate an independent and different origin (Sudo and Tsutsumi, 2002 and Mound, 2009).

Quality and quantity of pheromone components are depending on different characteristics, as on chemical properties of volatiles and their induced biological activity, of permanent or successive synthesis and efficiency of perception. Moreover, the functions of sternal glands (production of aggregation-, contact- and/or sex-pheromones) need further investigations on pheromone identification and biosynthesis together with physiological, behavioural and molecular studies to make any further conclusions and to reject or support existing sternal glands as a derived character state (Mound and Masumoto, 2005 and Mound, 2009).

Acknowledgements

This study was funded by the BMZ (The German Federal Ministry for Economic Cooperation and Development) through GIZ (Deutsche Gesellschaft für Internationale Zusammenarbeit) through a project grant entitled “Implementation of integrated thrips and tospovirus management strategies in small-holder vegetable cropping systems of Eastern Africa” (Project number: 11.7860.7-001.00, Contract number: 81141840) and the African Union through the African Union Research Grant Contract No: AURG/108/2012 to whom we are grateful. The funders had no role in study design, data collection and analysis, decision to publish, or

preparation of the manuscript. The authors would like to acknowledge the technical assistance of Mr. Shem Ochieng, ICIPE, Nairobi, and editorial assistance of Dr. Robert Copeland and Mrs. Dolorosa Osoga.

5.1.6 References

Abate, T. and Ampofo, J.K.O., 1996. Insect pests of beans in Africa: their ecology and management. Annu. Rev. Entomol. 41, 45-73.

Akella, S.V.S., Kirk, W.D.J., Lu, Y.B., Murai, T., Walters, K.F.A., Hamilton, J.G.C. and Renou, M., 2014. Identification of the aggregation pheromone of the melon thrips, *Thrips palmi*. PLoS ONE 9, e103315.

Aldrich, J.R.; Caroll, S.P.; Oliver, J.E.; Lusby, W.R., Rudmann, A.A. and Waters, R.M., 1990. Exocrine secretions of scentless plant bugs: *Jadera, Boisea* and *Niesthrea* (Hemiptera: Heteroptera: Rhopalidae). Biochem. Syst. Ecol. 18, 369-376.

Bode, W., 1978. Ultrastructure of the sternal glands in *Thrips validus* Uzel (Thysanoptera, Terebrantia). Zoomorphologie 90, 53-65.

Carayon, J., 1958. Etudes sur les Hémiptères Cimicoidea. 1. Position des genres *Bilia, Bilioa, Bilianella* et *Wollastoniella* dans une tribe nouvelle (Oriini) des Anthrocoridae; differences entre deniers et les Miridae Isometopinae (Heteroptera). Mém. Mus. Natl. Hist. Nat. Ser. A, Zool. 16, 141-172.

Carayon, J. ,1981. Dimorphisme sexuel des glandes tégumentaires et production de phéromones ches les Hémiptères Pentatomoidea. C.r. hebd. Séanc. Acad. Sci. Paris 292, 867-870.

Carayon, J., 1984. Les androconies de certains hémiptères Scutelleridae. Ann. Soc. Entomol. Fr. 20, 113-134.

Carayon, J., 1987. *Reduvius (Pseudoreduvius) dicki* n. sp. et ses allies. Premiere mention de glandes a pheromones chez des hemipteres reduviides. Rev. Fr. Entomol. 9, 190-196.

Cavalleri, A. and Kaminski, L.A., 2007. A new *Holopothrips* species (Thysanoptera: Phlaeothripidae) damaging *Mollinedia* (Monimiaceae) leaves in southern Brazil. Zootaxa 1625, 61-68.

Cobben, R.H., 1978. Evolutionary trends in Heteroptera Part II: Mouthpart-structures and feeding strategies. Medelingen Landbouwhogeschool, Wageningen 78-5, 407pp.

De Kogel, W.J. and van Deventer, P., 2003. Intraspecific attraction in the western flower thrips *Frankliniella occidentalis*, indications for a male sex pheromone. Entomol. Exp. Appl. 107, 87-89.

Edema, R. and Adipala, E., 1996. Effect of crop protection management practice on yield of seven cowpea varieties in Uganda. Int. J. Pest Manage. 42, 317-320.

El-Ghariani, I.M. and Kirk, W.D.J., 2008. The structure of the male sternal glands of the western flower thrips, *Frankliniella occidentalis* (Pergande). Acta Phytopathol. Entomol. Hung. 43, 257-266.

Foldi, I., 1981. Ultrastructure of the wax-gland system in subterranean scale insects (Homoptera, Coccoidea, Margarodidae). J. Morphol. 168, 159-170.

Hamilton, J.G.C. and Kirk, W.D.J., 2003. Method of Monitoring/Controlling Thrips. Priority date: 21 December 2001. International patent application published under the Patent Cooperation Treaty WO 2003/055309, http://www3.syngenta.com/global/Bioline/en/ products/allproducts/Pages/Thriplineams.aspx)

Hamilton, J.G.C., Hall, D.R. and Kirk, W.D.J., 2005. Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem. Ecol. 31, 1369-1379.

Harrington, R. 1985. A comparison of the external morphology of scent plaques on the hind tibiae of oviparous aphids (Homoptera: Aphididae). Syst. Entomol. 10, 135-144.

Heming, B.S., 1970. Postembryonic development of the male reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc. Publ. Entomol. Soc. Amer. 7, 237-272.

Kirk, W.D.J. and Hamilton, J.G.C., 2004. Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. J. Chem. Ecol. 30, 167-174.

Kirk, W.D.J., and Hamilton, J.G.C., 2010. The aggregation pheromones in thrips. In: Persley, D., Wilson, C., Thomas, J., Sharman, M. and Tree, D. (Eds), IXth International Symposium on Thysanoptera and Tospoviruses, J. Insect Sci. 10, 166.

Kirk, W.D.J. and Terry, I.L., 2003. The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). Agric. Forest Entomol. 5, 301-310.

Klocke, F., 1926. Beiträge zur Anatomie und Histologie der Thysanopteren. Z. wiss. Zool., Abt. A 128, 1-36.

Lewis, T., 1973. Thrips: Their Biology, Ecology and Economic Importance. Academic Press London, New York, 349 pp.

Lewis, T., 1997. Chemical control. In: Lewis, T. (Eds), Thrips as Crop Pests. CAB International, Oxon and New York, pp. 567-593.

Li, X.-W., Zhang, X.-C., Jiang, H.-X. and Feng, J.-N., 2012. Comparisons of developmental and reproductive biology between parthenogenetic and sexual *Echinothrips americanus* (Thysanoptera: Thripidae). Environ. Entomol. 41, 706-713.

Ma, P.W.K. and Ramaswamy, S.B. 2003. Biology and ultrastructure of sex pheromone-producing tissue. In: Blomquist, G. J. and Vogt, R. G. (Eds), Insect Pheromone Biochemistry and Molecular Biology: The Biosynthesis and Detection of Insect Pheromones and Plant Volatiles, Elsevier Academic Press, London, pp. 19–52.

Milne, M., Walter, G.H., Milne, J.R., 2002. Mating aggregations and mating success in the flower thrips, *Frankliniella schultzei* (Thysanoptera: Thripidae), and a possible role for pheromones. J. Insect Behav. 15, 351-368.

Moreno, D.S., 1972. Location of the site of production of the sex pheromone in the yellow scale and the california red scale. Ann. Entomol. Soc. Am. 65, 1283-1286.

Moreno D.S., Fargerlund J., 1975. Histology of the sex pheromone glands in the yellow scale and the California red scale. Ann. Entomol. Soc. Am. 68, 425-428.

Moritz, G., 1984. Exocrine vertex gland in males of the genus *Merothrips* Hood, 1914 (Merothripidae, Thysanoptera, Insecta). Zool. Jb. Anat. 111, 1-13.

Moritz, G., 1997. Structure, growth and development. In: Lewis, T. (Eds), Thrips as Crop Pests. CAB International, Oxon and New York, pp. 15-63.

Moritz, G., 2006. Thripse- Fransenflügler, Thysanoptera, Pflanzensaftsaugende Insekten Bd. 1, Die Neue Brehm-Bücherei Bd. 663,Westarp-Verlag, Hohenwarsleben.

Moritz, G., Brandt, S., Triapitsyn, S. and Subramanian, S., 2013. Identification and information tools for pest thrips in East Africa. QAAFI Biological Information Technology (QBIT), the University of Queensland, Brisbane, Australia. ISBN 978-1-74272-0687-8: http://thripsnet.zoologie.uni-halle.de/key-server-neu/

Moritz, G., O´Donnell, C. and Parrella, M., 2009. Pest thrips of North America associated with domestic and imported crops. QAAFI Biological Information Technology (QBIT), the University of Queensland. ISBN 978-1-86499-940-2: http://thripsnet.zoologie.uni-halle.de/key-server-neu/

Moritz, G., Mound, L.A., Morris, D. and Goldarazena, A., 2004. Pest thrips of the world - an identification and information system using molecular and microscopial methods. CBIT, University of Queensland, ISBN 1-86499-781-8: http://thripsnet.zoologie.uni-halle.de/key-server-neu/

Mound, L.A., 2009. Sternal pore plates (glandular areas) of male Thripidae (Thysanoptera). Zootaxa 2129, 29-46.

Mound, L.A., Heming, B.S. and Palmer, J.M. 1980. Phylogenetic relationships between the families of recent Thysanoptera (Insecta). Zool. J. Linn. Soc. 69, 111-141.

Mound, L.A. and Kibby G., 1998. Thysanoptera - An Identification Guide. 2ed edition. CAB International, Wallingford, Oxon.

Mound, L.A. and Masumoto, M., 2005. The genus *Thrips* (Thysanoptera, Thripidae) in Australia, New Caledonia and New Zealand. Zootaxa 1020, 1-64.

Mound, L.A. and Nickle, D.A., 2009. The Old-World genus Ceratothripoides (Thysanoptera: Thripidae) with a new genus for related New-World species. Zootaxa 2230, 57–63.

Mound, L.A. and Palmer, J.M., 1983. The generic and tribal classification of spore-feeding Thysanoptera (Phlaeothripidae, Idolothripinae). Bull. Br. Mus. Nat. Hist., Entomol. 46, 1-174.

Mound, L.A. and Walker, A.K. 1986. Fauna of New Zealand Number 10 Tubulifera (Insecta: Thysanoptera). Fauna of New Zealand 10, 1-140.

Niassy, S., Ekesi, S., Maniania, N.K., Moritz, G.B., De Kogel, W.J. and Subramanian, S., (in press). Evidence of active aggregation among sexes in bean flower thrips (*Megalurothrips sjostedti*) on cowpea (*Vigna unguiculata*). Entomol Exp Appl.

Noirot, C. and Quennedey, A., 1974. Fine structure of insect epidermal glands. Annu. Rev. Entomol. 19, 61-80.

Noirot, C., and Quennedey, A., 1991. Glands, gland cells, glandular units: some comments on terminology and classification. Ann. Soc. Entomol. Fr. 27, 123-128.

Okajima, S., 1981. A revision of the tribe Plectrothripini of fungus-feeding Thysanoptera (Phlaeothripidae: Phlaeothripinae). Syst. Entomol. 6, 291-336.

Olaniran, O.A.; Sudhakar, A.V.S.; Drijfhout, F.P.; Dublon, I.A.N.; Hall, D.R.; Hamilton, J.G.C. and Kirk, W.D.J., 2013. A male-predominant cuticular hydrocarbon, 7-methyltricosane, is used as a contact pheromone in the western flower thrips *Frankliniella occidentalis*. J. Chem. Ecol. 39, 559-568.

Pelikan, J., 1951. On carnation thrips, *Taeniothrips dianthi* Priesner. Folia Entomol. 14, 5-38.

Pettersson, J. 1971. An aphid sex attractant. II. Histological, ethological, and comparative studies. Entomol. Scand. 2, 81-93.

Quennedey, A., 1998. Insect epidermal gland cells: ultrastructure and morphogenesis. In: Harrison F.W. and Locke, M. (Eds), Microscopic Anatomy of Invertebrates, Vol. 11A. Wiley, New York, pp. 177-207.

Rusoke, D.G. and Rubaihayo, P.R. 1994. The influence of some crop protection management practices on yield stability of cowpeas. Afr. Crop Sci. J. 2, 43-48.

Schmutterer, H., 2008. Schildläuse. Pflanzensaftsaugende Insekten Bd. 4. Die Neue Brehm-Bücherei Bd. 666, Westarp-Verlag, Hohenwarsleben.

Schuh, R.T. and Slater, J.A. 1995. True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History. Cornell University Press, Ithaca, New York.

Shitatani, S. and Tsutsumi, T. 2005. Ultrastructure of sternal glands in *Tenothrips frici* (Uzel) (Thysanoptera: Terebrantia). Proc. Arthropod. Embryol. Soc. Jpn. 40, 35-39.

Shitatani, S. and Tsutsumi, T., 2006. Ultrastructure of sternal glands in *Hydatothrips* (*Hydatothrips*) *abdominalis* (Kurosawa) and *H.* (*Neohydatothrips*) *gracilicornis* (Williams) (Thysanoptera: Terebrantia). Proc. Arthropod. Embryol. Soc. Jpn. 41, 59-65.

Singh, S.R. and Allen, D.J., 1980. Pests, diseases, resistance and protection of (*Vigna unguiculata* (L.) Walp. In: Summerfield, R.J., Bunting, A.H.K. (Eds), Advances in Legume Science, Royal Botanic Gardens, London, and Ministry of Agriculture, Fish and Food, MAFF, pp. 419-443.

Staddon, B.W., 1979. The scent glands of Heteroptera. Adv. Insect Physiol. 14, 351-418.

Staddon, B.W., 1990. Male sternal pheromone glands in acanthosomatid shield bugs from Britain. J. Chem. Ecol. 16, 2195-2201.

Stannard, L.J., 1968. The Thrips, or Thysanoptera, of Illinois. Bull. Ill. Nat. Hist. Surv. 29, 214-552.

Strümpel, H., 2010. Zikaden. Pflanzensaftsaugende Insekten Bd. 6. Die Neue Brehm-Bücherei Bd. 668, Westarp-Verlag, Hohenwarsleben.

Sudo, M. and Tsutsumi, T., 2002. Ultrastructure of sternal glands in two thripine thrips and one plaeothripinae thrips (Thysanoptera: Insecta). Proc. Arthropod. Embryol. Soc. Jpn. 37, 35-41.

Sudo, M. and Tsutsumi, T., 2003. Ultrastructure of the primordia of sternal glands in the second-instar pupal stage of *Frankliniella intonsa* (Thysanoptera: Insecta). Proc. Arthropod. Embryol. Soc. Jpn. 38, 27-29.

Tamò, M., Baumgartner, J., Delluchil, V. and Herren, H.R., 1993. Assessment of key factors responsible for the pest status of the bean flower thrips, *Megalurothrips sjostedti* Tryb. (Thysanoptera: Thripidae) in West Africa. Bull. Entomol. Res. 83, 251-258.

Tamò, M., Srinivasan, R., Dannon, E., Agboton, C., Datinon, B., Dabire, C., Baoua, I., Ba, M., Haruna, B., and Pittendrigh, B.R., 2010. Biological control: a major component for the long term cowpea pest management strategy. In: Boukar, O., Coulibaly, O., Fatokun, C., Lopez, K., and Tamò, M. (Eds), Enhancing Cowpea Value Chains through Research Advances. Proceedings of the 5th World Cowpea Research Conference, IITA, Benin, Senegal, pp. 249-259.

Thouvenin, M., 1965. Etude préliminaire de uradénies chez certains Hétéroptères pentatomomorphes. Ann. Soc. Entomol. Fr. 1, 973-988.

Ullman, D., Sherwood, J.L., and German, T.L. 1997. Thrips as vectors of plant pathogens. In: Lewis, T. (Ed), Thrips as Crop Pests. CAB International, Oxon and New York, pp. 539-565.

Webster, K.W., Cooper, P. and Mound, L.A., 2006. Studies on Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae): sex attractants, host associations and country of origin. Aust. J. Entomol. 45, 67-74.

Weirauch, C., 2006. Dorsal abdominal glands in adult Reduviidae (Heteroptera, Cimicomorpha). Mitt. Mus. Nat.kd. Berl., Dtsch. entomol. Z. 53, 91-102.

Young, B. and Hong-Ren, Y., 1986. Studies on ultrastructure and function of epidermal glands on female *Matsucoccus matsumurae* (Kuwana) (Coccoidea: Margarodidae). Bull. Lab. Entom. Agrar. Portici 43, 79-82.

Zhang, P.-J., Zhu, X.-Y. and Lu, Y.-B., 2011. Behavioural and chemical evidence of a male-produced aggregation pheromone in the flower thrips *Frankliniella intonsa*. Physiol. Entomol. 36, 317-320.

zur Strassen, R., 2003. Die terebranten Thysanopteren Europas. 1st edition, Goecke and Evers, Keltern.

SECTION 5.2

HISTOCHEMICAL STUDIES ON STERNAL GLANDS IN ECONOMICALLY IMPORTANT PEST THRIPS

Stephanie Krueger, Marcus Jilge, Gerald B. Moritz

Martin Luther University Halle- Wittenberg, Institute of Biology, Faculty of Natural Science I, Heinrich-Damerow Str. 4, 06120 Halle, Germany

5.2.1 Summary

Very little is known about the structure of, and substance production by, the sternal glands of male Thysanoptera. Whereas *Frankliniella occidentalis* (Thysanoptera, Thripidae) has oval pore plates with c-shaped secretory cells, *Echinothrips americanus* (Thysanoptera, Thripidae) has multiple pore plates with unicellular gland units, but their functional role remains unknown. Given the absence of these structures in most females, and their location in males ventrally on the abdomen, some authors deduce that there is a sexually determined function, but until now there has been no reliable evidence.
This study investigated sternal glands and their secretions using histochemical methods to characterize the structure. In addition, the mating status of the thrips was determined to get some indication of the possible function of the glands in reproduction.

As well as the different distribution patterns of proteins within the reservoirs and gland cells, lipid content also differs greatly between the two species. Both contain only small amounts of periodic acid-Schiffs reagent- and aldehyde fuchsine-alcian blue active substances. The results are discussed with respect to substances known to be emitted by both species.

Keywords:
pheromone, reproduction, mating, Thysanoptera, Poinsettia thrips, Western Flower thrips

Own contribution: 80 %

Abbreviations:
acc gl-accessory gland, fc-fat cell, glc-gland cell, M l abd ventr-Musculus longitudinalis abdominis ventralis, mbb-microvilli brush border, mt-Malpighian tubules, ppl-pore plate (= areae porosae, arrow-region of special interest, asterisk (*) -extracellular cavity

5.2.2 Introduction

In many thrips species, especially within the Thripidae and the subfamily Thripinae, males have typical sexually dimorphic glandular areas on some abdominal sternites. These sternal areas comprise an external pore plate (=area porosa) on sternites III or IV to VII or VIII and an internal gland complex. Because of their differences in shape, size, number and arrangement (even within one genus), these structures provide important character states for identifying the species of male thrips (Stannard, 1968, zur Strassen, 2003 and Mound, 2009).

The most common type is an oval to round pore plate with a c-shaped gland complex inside (Mound, 2009 and Krueger et al., 2015a). A batch of secretory cells is arranged around an extracellular cavity, which leads apically to the pore plate as the extrusion area.
However, in some species up to 100 or more small circular pore plates are located on each sternite. Each of these is associated with a secretory cell and an extracellular cavity. In a third type of structure, the sternal glands can be greatly reduced in some species with only a few gland cells associated with a simple pore (Krueger et al., 2015a).

Males of the Thripinae species *Frankliniella occidentalis* bear the common type of c-shaped gland cells with an oval pore plate on sternites III to VII, whereas *E. americanus* bears multiple pore plates (Mound, 2009 and Krueger et al., 2015a). Both species of economic importance, with a worldwide distribution and an enormous host range (summarized in Krueger et al. 2015a).

Due to the absence of these structures in most females, and their location in males ventrally on the abdomen, some authors have deduced a sexually related function (Klocke, 1926 and Mound, 2009). Also, their morphological similarity to pheromone producing glands in other insects suggests a pheromonal function (Pelikan, 1951, Bode, 1978, Moritz, 1997, 2006, Sudo and Tsutsumi, 2002, Sudo and Tsutsumi 2003, Shitatani and Tsutsumi, 2005, Shitatani and Tsutsumi, 2006, El-Ghariani and Kirk, 2008 and Olaniran et al., 2013). However, up to know, there has been no reliable evidence.

Receptive females are the limiting factor in reproduction in both *E. americanus* and *F. occidentalis*. Females mate only once (*E. americanus*, Li et al., 2014 and Krueger et al., 2015b) or re-mate with a very low frequency (*F. occidentalis,* Terry and Schneider, 1993). The high local competition for mates may lead to a male mating strategy, where females are choosy and males develop for self-advertisement and courting behavior (summarized in Bradbury and Vehrencamp, 2011). The mating behavior of these two species suggests the need for pheromonal substances to assist in mate recognition and mate assessment or to act as an anti- aphrodisiac.

In this study, sternal glands and their secretions were investigated using histochemical methods to characterize their structure. In addition, mating status was considered to get indication about the possible function of the sternal glands in reproduction.

5.2.3 Material and Methods

Insects

F. occidentalis and *E. americanus* were obtained from laboratory cultures maintained at the University of Halle, Germany. They were reared at 23 ± 1 °C, 60 ± 10 % RH, and a photoperiod of 16: 8 h L:D at 5000 Lux during photophase. *Phaseolus vulgaris* served as the host plants, but *E. americanus* was also fed on *Gossypium* sp..

Mated individuals were collected from the main culture. To obtain virgin males and females, pupae were collected and raised individually in 12-well Greiner Plates (see Krueger et al., 2015b). Newly hatched virgin females were allowed to lay unfertilized eggs. Because of their arrhenotokous reproduction mode, only male offspring occur from such eggs. Thrips were selected for use at age of 5 days post eclosion.

Histochemical Study

Protein and polysaccharides staining
Specimens were fixed in Carnoy´s solution, dehydrated in a graded ethanol series and embedded in Surgipath Paraplast® via isopropanol. Sections were cut (6 µm) with a Leica SM 200R microtome (Leica, Germany). The following staining procedures were carried out: Coomassie staining for proteins, adjusted after Kiernan (2012), counterstained with haematoxylin; Periodic acid-Schiff`s reagent (PAS) for polysaccharides, modified after Kiernan (2012), counterstained with haematoxylin (H) and combined method of aldehyde-fuchsine-alcian blue (AF-AB) for mucins, adjusted after Spicer and Meyer (1960).

Lipid-staining
Specimens for oilred-O staining were 2 h prefixed in 4 % paraformaldehyde in 0.1 M Soerensen buffer (pH 7.4), chilled on ice. Afterwards samples were washed for 4 x 15 min in buffer and embedded in cryomoulds (Tissue Tek©, Sakura, 25 mm x 20 mm x 5 mm) with Tissue Tek© (Sakura). Cryosections (6-8 µm) were cut with a Leica CM1900. Oilred-O-staining for neutral lipids was modified after Kiernan (2012).

Individuals for nile blue sulphate staining were first embedded and cut, than postfixed with 4 % paraformaldehyde in 0.1 M Soerensen-buffer (pH 7.4) for 20 min. Nile blue sulphate staining for acidic and neutral lipids was then applied according to Bancroft (2008).

For a more detailed description of all fixation and staining procedures see Supplementary Material S5.2 on CD.

All sections were examined under a light microscope (Leitz DMBRE, Leica, Germany) fitted with a Canon EOS 600D or a Leica DFC 450C (Leica, Germany). All images were processed using Photoshop CS5 and CS6 (Adobe, Ireland).

5.2.4 Results

Although staining procedures produced very different staining patterns between species, there were only slight differences in coloration within species in association with mating status (Tab. 1,2, Fig. 1,2).

Coomassie- staining for proteins

In *E. americanus,* the reservoir contents stained blue, but not the gland cell itself (Fig. 1A, B). Staining within the reservoirs of mated males seemed to be more constant and evenly distributed (Fig. 1B), but blue-coloration in virgin males was associated more closely with the basal region of the extracellular cavity (Fig. 1A).

In contrast, the reservoir contents of *F. occidentalis* did not contain dye after procedure, but the secretory cells did. Mated males showed an equal blue coloration in each individual, but it was individually different among virgin males (Fig. 2A).

PASH-staining for polysaccharides

In *E.* americanus, staining with PASH resulted in weak magenta coloration of the reservoir. Mated males showed an increased coloring at the microvilli brush border (Fig. 1D). However, the sternal gland region contains only very few PAS-positive structures, compared to the accessory gland (Fig. 1C) and fat cells (Fig. 1C, D).

In *F. occidentalis* no PAS-positive structures were detectable. Only haematoxylin-counterstain was visible (Fig. 2C,D).

AF-AB staining for mucins

In order to detect possible mucine substances AF-AB staining was carried out. In *E. americanus,* staining resulted in blue coloring of fat cells, and a slight fuchsine-color around the pore plate area (Fig. 1E). Also *F. occidentalis* had a fuchsine-colored pore plate region (Fig. 2E,F).

Oilred-O and nile blue-sulphate staining for lipids

While a strong coloring of lipids occurred in *E. americanus*, no lipids were detected in *F. occidentalis,* irrespective of mating status (Fig. 1G,H; 2G,H).

Table 2 Results of histochemical staining in *Echinothrips americanus* in relation to mating status, ++ = strongly positive reaction, + = positive reaction, (+) = weakly positive reaction, not in all samples, - = no reaction

	E. americanus, virgin male			*E. americanus, mated male*		
	Cytoplasm of secretory cells	Secretion in reservoir	Microvilli brush border	Cytoplasm of secretory cells	Secretion in reservoir	Microvilli brush border
For proteins						
Coomassie	-	++	-	-	++	-
For polysaccharides						
PASH	-	(+)	-	-	(+)	+
For mucins						
AF-AB	(+)	+	-	(+)	-	-
For lipids						
Oilred-O	++	+	-	++	+	-
Nil blue sulphate	++	+	-	++	+	-

Table 3 Results of histochemical staining in *Frankliniella occidentalis* in relation to mating status, ++ = strongly positive reaction, + = positive reaction, (+) = weakly positive reaction, not in all samples, - = no reaction

	F. occidentalis, virgin male			*F. occidentalis, mated male*		
	Cytoplasm of secretory cells	Secretion in reservoir	Microvilli brush border	Cytoplasm of secretory cells	Secretion in reservoir	Microvilli brush border
For proteins						
Coomassie	++ (-)	-	-	++	-	-
For polysaccharides						
PASH	-	-	-	-	-	-
For mucins						
AF-AB	-	(+)	-	-	(+)	-
For lipids						
Oilred-O	-	-	-	-	-	-
Nil blue sulphate	-	-	-	-	-	-

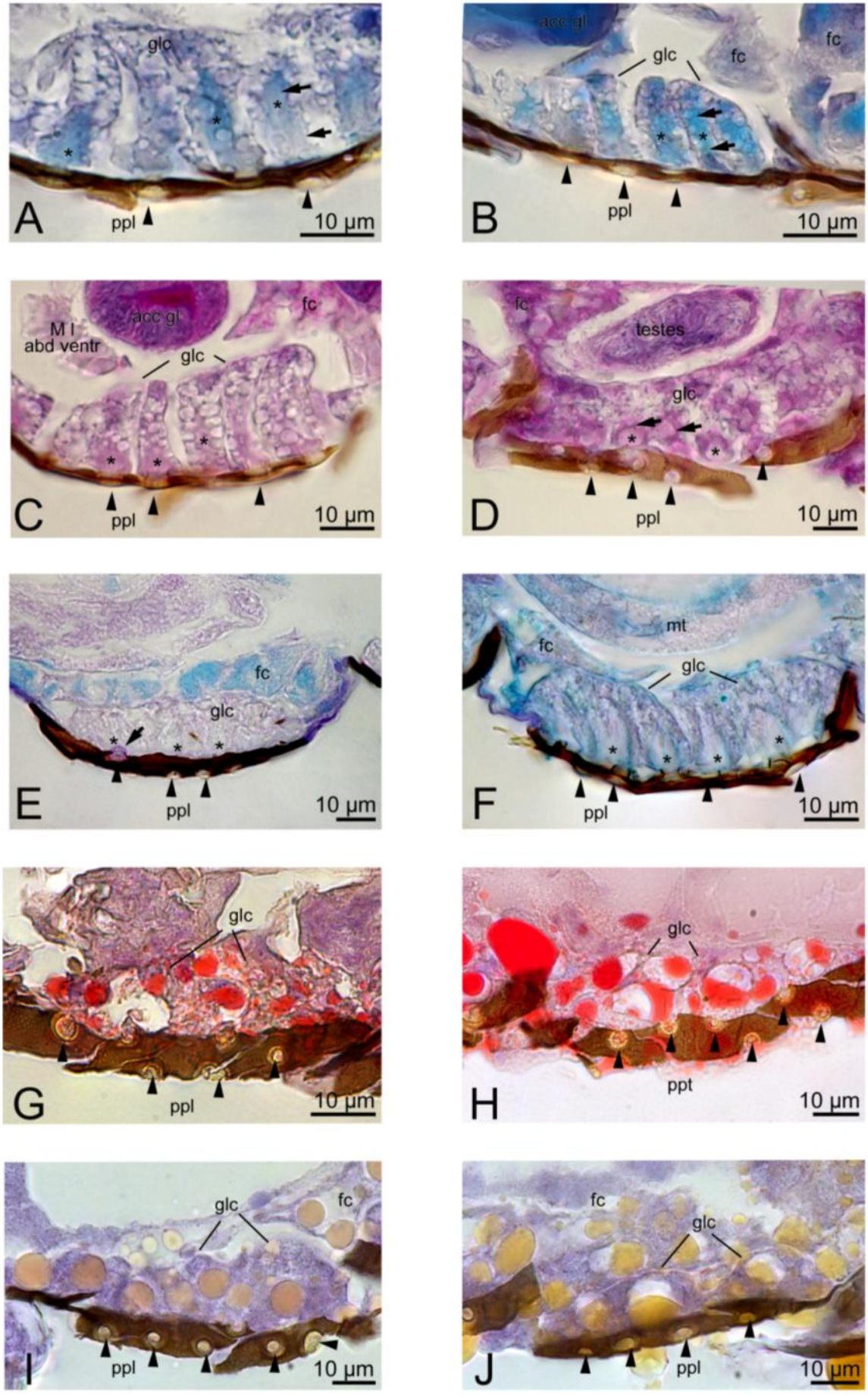


Fig. 1 Histochemical staining of sternal glands in *Echinothrips americanus*

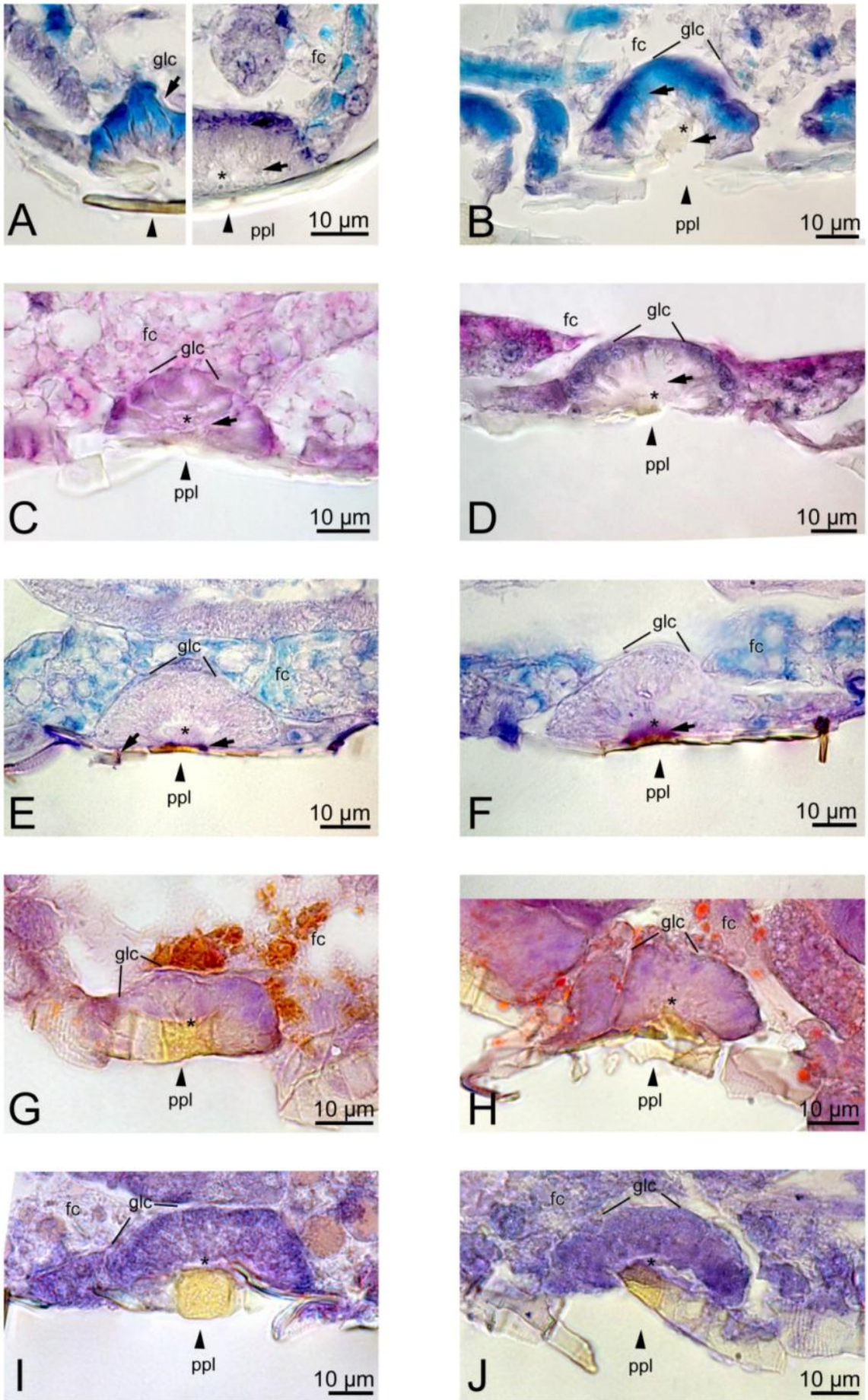


Fig. 2 Histochemical staining of sternal glands in *Frankliniella occidentalis*

Fig. 1 Histochemical staining of sternal glands in *Echinothrips americanus,* light microscopy, sagittal section of abdomen, left row virgin males, right row mated males
A-B Coomassie- staining, note the unequal blue staining within reservoir of virgin males
C-D PASH staining, note the increased coloring of microvilli brush border in mated males
E-F AF-AB staining, note the increased coloring near internal pore plate
G-H Oilred-O staining, note the abundance of red-stained lipids
I-J Nile blue sulphate staining, note the abundance orange stained lipids

Fig. 2 Histochemical staining of sternal glands in *Frankliniella occidentalis,* light microscopy, sagittal section of abdomen (except Fig 2A), left row virgin males, right row mated males
A-B Coomassie- staining, A transversal section, note the different blue staining pattern between individuals, B sagittal section
C-D PASH staining, note the fuchsine colored fat cells, but no coloration of gland cells
E-F AF-AB staining, note the fuchsine-coloring of internal pore plate area
G-H Oilred-O staining, no lipids were observable within gland cells, only in fat cells
I-J Nile blue sulphate staining, no lipids were observable in gland cells

5.2.5 Discussion

Sternal glands are presumed to be pheromone glands. But neither secreted substances nor their function are known. With histochemical studies, two important pest species were examined in order to determine the nature of their secretion products.

Large lipid droplets were observed in *E. americanus,* which is also known from ultrastructural study (Krueger et al., 2015a). Within the reservoir, proteins are prevalent. A completely different staining pattern is observed in *F. occidentalis*. Proteins are prevalent within secretory gland cells, which may indicate the high amounts of mitochondria in the gland cells (Krueger et al., 2015a). Secretion within the reservoir showed only a positive signal for mucins near the pore plate.

Until now, histochemical studies on sternal glands in Thysanoptera have been available only for *Thrips validus* (Bode, 1978). This species has rounded pore plates on sternites III to VII, with c-shaped sternal glands, which is comparable to the structures in *F. occidentalis*. Staining pattern in both species is quite similar, except the presence of lipids within the reservoir and gland cells in *T. validus*.

The absence of lipids and the only slight coloring of the secretion in *F. occidentalis* raise questions about the suggested pheromone- secretion function. Until now, characterized aggregation pheromone substances in *F. occidentalis* (Hamilton et al., 2005) belong to fatty acids and their derivates. Thus, lipids were expected within the gland cells and their secretion, but staining pattern implies a nonproteinaceous and PASH –negative substance. This corresponds more to the postulated contact pheromone, a cuticular hydrocarbon, with the sternal glands considered as production site (Olaniran et al., 2013). However, cuticular hydrocarbons are normally produced via oenocytes associated with the fat body or epidermal cells (Howard and Blomquist, 2005 and Makki et al., 2014); only sometimes they are taken up from the hemolymph and released from localized pheromone glands (Bagnéres and Blomquist, 2010). Olaniran et al. (2013) already suspected such a hemolymph uptake and concentrated release in *F. occidentalis.*

The secretion in *E. americanus* consists of protein and lipid components, which are typical components of pheromone substances (Yew and Chung, 2015) and are also known from termites (Quennedey, 1971 and Quennedey, 1972). Lipophorine-like material could also be indicated by this staining pattern. Lipophorines are presumed to play an important role in pheromone transport (Schal et al., 2001). Alternatively, large amounts of proteins within the reservoir may indicate an enzymatic postsecretory modification within the reservoir as a reaction chamber, as is known for the beetles *Eleodes longicollis* and *Tribolium castaneum* (Happ, 1968).

As expected due to the different ultrastructure of gland cells in *E. americanus* and *F. occidentalis* (Krueger et al., 2015a), the histochemical staining patterns of the gland cell and secretion are also different. The variation in staining within the reservoir tends to suggest that different substances were produced in the two species. This phenomenon of variation in the chemical constitution and physiological function of secretions of similar glands in different species is also known from Heteroptera, a suborder of Hemiptera, the possible sister group of Thysanoptera (Staddon, 1979).

Beside the characterization of the gland product, differences in staining pattern with regard to mating status were investigated: males from our main population were stained. These males had contact with both males and females. This condition seems to have a small impact on the staining pattern and therefore on substance production. In both examined species, protein staining is much more equally distributed within the extracellular cavity in mated males, than in virgin ones. Additionally in *E. americanus,* slight differences are visible on the microvilli brush border (Tab. 1). Hence presence of other individuals seems to have a stabilising effect on male substance production. A similar effect is known from some Orthoptera species, where male-specific pheromone substances were emitted only under crowded conditions (Seidelmann et al., 2000, Stahr et al., 2013).

However, the function of sternal glands remains unresolved. Presence of both lipid and protein components in the secretion suggests that a close-range pheromone function is possible, and this should be examined more closely with GC-MS and bioassay-analyses. These results prove that in *F. occidentalis* gland secretions and the contact pheromone have similar or even equal characteristics. Further studies with labeled tracers should confirm whether the production site of the contact pheromone and the sternal gland secrete are identical.

5.2.6 References

Bagnéres, A.-G. and Blomquist, G. J., 2010. Site of synthesis, mechanism of transport and selective deposition of hydrocarbons. In: Bagnéres, A.-G. and Blomquist, G.J. (Eds.), Insect Hydrocarbons. Cambridge University Press, Cambridge, pp. 75–99.

Bancroft, J.D. and Gamble, M., 2008. Theory and Practice of Histological Techniques. 6th edition. Churchill Livingstone/Elsevier, Philadelphia.

Bode, W., 1978. Ultrastructure of the sternal glands in *Thrips validus* Uzel (Thysanoptera, Terebrantia). Zoomorphologie 90, 53-65.

Bradbury, J.W. and Vehrencamp, S.L., 2011. Principals of Animal communication. 2nd edition. Sinauer Associates, Sunderland Massachusetts.

El-Ghariani, I.M. and Kirk, W.D.J., 2008. The structure of the male sternal glands of the western flower thrips, *Frankliniella occidentalis* (Pergande). Acta Phytopathol. Entomol. Hung. 43, 257-266.

Hamilton, J.G.C., Hall, D.R. and Kirk, W.D.J., 2005. Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem. Ecol. 31, 1369-1379.

Happ, G.M., 1968. Quinone and hydrocarbon production in the defensive gland of *Eleodes longicollis* and *Tribolium castaneum*. J. Insect Physiol. 14, 1821-1837.

Howard, R.W., and Blomquist, G.J., 2005. Ecological, Behavioral, and Biochemical Aspects of Insect Hydrocarbons. Annu. Rev. Entomol. 50, 371-393.

Kiernan, J.A., 2012. Histological and histochemical methods. 4th edition. Scion Publishing Ltd, Oxford.

Klocke, F., 1926. Beiträge zur Anatomie und Histologie der Thysanopteren. Z. wiss. Zool., Abt. A 128, 1-36.

Krueger, S., Subramanian, S., Niassy, S. and Moritz, G.B., 2015a. Sternal gland structures in males of bean flower thrips, *Megalurothrips sjostedti*, and Poinsettia thrips, *Echinothrips americanus*, in comparison with those of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Arth. Struct. & Dev. 44, 455-467.

Krueger, S., Mound, L.A. and Moritz, G.B., 2015b. Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* MORGAN 1913 (Thysanoptera). J. Appl. Entomol. doi: 10.1111/jen.12280

Li, X-W., Jiang, H-X., Zhang, X-C., Shelton, A.M., Feng, J-N., 2014. Post-mating interactions and their effects on fitness of female and male *Echinothrips americanus* (Thysanoptera: Thripidae), a new insect pest in China. PLOS One 9, 1-7.

Makki, R., Cinnamon, E., and Gould, A.P., 2014. The Development and Functions of Oenocytes. Annu. Rev. Entomol. 59, 405-425.

Moritz, G., 1997. Structure, growth and development. In: Lewis, T. (Ed), Thrips as Crop Pests. CAB International, Oxon and New York, pp. 15-63.

Moritz, G., 2006. Thripse- Fransenflügler, Thysanoptera, Pflanzensaftsaugende Insekten Bd. 1, Die Neue Brehm-Bücherei Bd. 663,Westarp-Verlag, Hohenwarsleben.

Mound, L.A., 2009. Sternal pore plates (glandular areas) of male Thripidae (Thysanoptera). Zootaxa 2129, 29-46.

Olaniran, O.A.; Sudhakar, A.V.S.; Drijfhout, F.P.; Dublon, I.A.N.; Hall, D.R.; Hamilton, J.G.C. and Kirk, W.D.J., 2013. A male-predominant cuticular hydrocarbon, 7-methyltricosane, is used as a contact pheromone in the western flower thrips *Frankliniella occidentalis*. J. Chem. Ecol. 39, 559-568.

Pelikan, J., 1951. On carnation thrips, *Taeniothrips dianthi* Priesner. Folia Entomol. 14, 5-38.

Quennedey, A., 1971. Les glandes exocrines des Termites. I. Etude histochimique et ultrastructurale de la glande sternale de *Kalotermes flavicollis* Fab. (Isoptera, Kalotermitidae). Z. Zellforsch. 121, 27-47.

Quennedey, A., 1972. Les glandes exocrines des Termites. III Structure finde de la glande sternale de *Trinervitermes geminates* Wasman (Termitidae, Nasututermitinae). Z. Zellforsch. 130, 205-218.

Schal, C., Sevala, V. and Cardè, R.T., 2001. Tissue distribution and lipophorin transport of hydrocarbon and sex pheromones in the housefly, Musca domestica. J. Insect Sci. 1, 12.

Seidelmann, K., Luber, K. and Ferenz, H.-J., 2000. Analysis of release and role of benzyl cyanide in male desert locusts, *Schistocerca gregaria*. J. Chem. Ecol. 26, 1897-1910.

Shitatani, S. and Tsutsumi, T., 2005. Ultrastructure of sternal glands in *Tenothrips frici* (Uzel) (Thysanoptera: Terebrantia). Proc. Arthropod. Embryol. Soc. Jpn. 40, 35-39.

Shitatani, S. and Tsutsumi, T., 2006. Ultrastructure of sternal glands in *Hydatothrips* (*Hydatothrips*) *abdominalis* (Kurosawa) and *H.* (*Neohydatothrips*) *gracilicornis* (Williams) (Thysanoptera: Terebrantia). Proc. Arthropod. Embryol. Soc. Jpn. 41, 59-65.

Spicer, S.S. and Meyer, D.B., 1960. Histochemical differentiation of acid mucopolysaccharides by means of combined aldehyde fuchsine-alcian blue staining. Amer. J. Clin. Path. 83, 453460.

Staddon, B.W., 1979. The scent glands of Heteroptera. Adv. Insect Physiol. 14, 351-418.

Stahr, C., Stavos, A. and Seidelmann, K., 2013. Chemical Identification, Emission Pattern and Function of Male-Specific Pheromones Released by a Rarely Swarming Locust, *Schistocerca americana*. J. Chem. Ecol, 39, 15-27.

Stannard, L.J., 1968. The Thrips, or Thysanoptera, of Illinois. Bull. Ill. Nat. Hist. Surv. 29, 214-552.

Sudo, M. and Tsutsumi, T., 2002. Ultrastructure of sternal glands in two thripine thrips and one plaeothripinae thrips (Thysanoptera: Insecta). Proc. Arthropod. Embryol. Soc. Jpn. 37, 35-41.

Sudo, M. and Tsutsumi, T., 2003. Ultrastructure of the primordia of sternal glands in the second-instar pupal stage of *Frankliniella intonsa* (Thysanoptera: Insecta). Proc. Arthropod. Embryol. Soc. Jpn. 38, 27-29.

Terry, I. and Schneider, M., 1993. Copulatory behavior and mating frequency of the Western Flower thrips, *Frankliniella occidentalis* (Insecta: Thysanoptera). J. Pure Appl. Zool. 4, 339-354.

Yew, J.Y. and Chung, H., 2015. Insect pheromones: An overview of function, form, and discovery. Progress in Lipid Research 59, 88-105.

zur Strassen, R., 2003. Die terebranten Thysanopteren Europas. 1st edition, Goecke and Evers, Keltern.

SECTION 5.3

Correlation of sternal gland, body size and mating success in *Echinothrips americanus* (Thysanoptera: Thripidae)

Stephanie Krueger, Christin Hesse, Gerald B. Moritz

Martin Luther University Halle- Wittenberg, Institute of Biology, Faculty of Natural Science I, Heinrich-Damerow Str. 4, 06120 Halle, Germany

5.3.1 Summary

In many insect species, females cue in on male signals for selection of mates. Mate choice can benefit fecundity and survival of females, or have an indirect effect by providing better genes. Assortative mating by size is one of the most commonly observed sexual behaviors. Additionally, chemical cues may be detected by females, as these can be indicators of body size and condition.

Little is known about mate choice behavior among Thysanoptera. The mating system of *Echinothrips americanus* (Thysanoptera, Thripidae) is characterized by a strong competition for mates, leading to assortative mating. In this species very prominent gland structures are located on sternites III to VIII, of which the function remains unknown. However, given the individual differences in expression of the gland release areas, a probable role in mate selection is postulated.

In this study, we are interested in possible correlations between male body size, gland release areas, glandular substances, and mating success. Additionally, we used an experimental approach to test for a possible function in mate- assessment, or as an anti-aphrodisiac.

Whereas pronotum length, distance between 1st and 2nd coxae, as well as total sternite area shows a significant correlation with mating success, no direct correlation with sternal gland structures was observed. Furthermore, we failed to constitute a mate-assessment or anti-aphrodisiac-like function of sternal gland products.

Keywords:
pheromone, anti-aphrodisiac, mate choice, assortative mating

Own contribution: 80 %

5.3.2 Introduction

Sexual selection is a process that arises from competition among members of one sex for access to the limiting sex to maximise their own fitness (Darwin, 1871). Whereas males could maximise their reproductive effort by maximising the number of fertilized gametes, females might benefit from extended access to resources (reviewed in Shuker and Simmons, 2014). The way in which competition for mates occurs, and the form it takes, is strongly influenced by an organism´s mating system (e.g. Trivers, 1972, Emlen and Oring, 1977, Shuster and Wade, 2003, and Kokko et al., 2006). One component of sexual selection models is precopulatory mate choice, and this can operate at the levels of intraspecific- (male-male competition) and interspecific competition (female choice) (Kuijper et al., 2012).

Usually, females use male signals to select mates. Their mate choice may confer direct benefits by affecting their fecundity or survival, or indirect benefits by providing good genes and other advantages to their offspring (summed up in Bradbury and Vehrencamp, 2011).

Assortative mating by size is one of the most common mating patterns observed (Arak, 1983 and Ridley, 1983), a consequence being the correlation of body sizes of mating males and females in a population in the absence of inbreeding (Lewontin et al., 1968). Additionally female mate choice can also be influenced by chemical cues, via mate assessment pheromones (Conner et al., 1990 and Clark et al., 1997).

For Thysanoptera, little is known about mate selection or morphological adaptions associated mating or fighting success (Lewis, 1973, Ananthakrishnan, 1990 and Terry and Dyreson, 1996).

Adult male thrips of mycophagous species within the suborder Tubulifera vary in foreleg size, an extreme being males that have forelegs of exaggerated sizes bearing various armatures. Crespi (1986, a,b and 1988, a,b) found a relationship between behavior and size of forelegs: larger males are guards, protecting territories of reproductive females and/or oviposition sites and have greater success in mating with females before oviposition. In contrast, subordinate, non-guarding males sometimes mate successfully by sneak mating.

Among Terebrantia, only a few species (*Merothrips* and *Perissothrips* species) show exaggerated forelegs (Ananthakrishnan, 1990), but nothing is known about their behavioral context. However, in *Frankliniella occidentalis* (Thysanoptera: Thripidae) larger males are known to be fighters in male mating swarms (Terry and Dyreson, 1996).

As well as the now ubiquitous pest species, *F. occidentalis*, several other species have spread widely due to the global trade in agricultural products. *Echinothrips americanus*, a species native in the eastern part of the United States (Stannard, 1968), has spread within the last 30 years over Canada, and Europe, to Asia and Northern Australia (see Krueger et al., 2015b). With its broad host range and feeding damage (Vierbergen, 1998, Oetting and Beshear, 1993, Oetting et al., 1993 and Trdan et al., 2003) this species is potentially a major pest (Plant Health Australia, 2010). However, very little is known about the species, especially its reproductive biology.

E. americanus reproduces via arrhenotoky: fertilized eggs develop into females and males arise from unfertilized eggs. Populations are characterized by a male sex ratio of 0.3 (Kumm, 2002 and Krueger et al., 2015b). Additionally females mate only once or with a very low frequency (Li et al., 2014, Krueger et al., 2015b and Chapter 3), leading to strong competition for successful copulations. Female mate choice and assortative mating are probable results. This

species has no exaggerated forelegs or other additional structures, such as seen in the Phlaeothripidae *Elaphrothrips tuberculatus* (Hood, 1908) and *Hoplothrips karnyi* (Hood, 1914). In preliminary experiments with *E. americanus* it was noted that some males are repeatedly rejected by females. Therefore body size or pheromonal substance might be the cues for this assortative mating. Males of *E. americanus* have a large array of multiple pore plates on the abdominal sternites that are presumed to produce and release sex pheromones (see Krueger et al., 2015a). This suggestion is supported by histochemical investigations (Section 5.2). Lipid and proteinous substances were detected, and these are typical components of pheromone substances (Yew and Chung, 2015).

In this study, we are interested in possible correlations between male body size, gland release area, glandular substances and mating success. For that reason we first recorded mating success in association with selected characters of body size in terms of assortative mating by size.

Second, we analysed structure of multiple pore plates, recorded the presence of sex pheromonal substances and their possible role in mating success as an assortative mating pheromone or as an anti-aphrodisiac. Since it is not possible to measure individual differences of substance release directly, due to the small quantities (Chapter 6), the structure of the multiple pore plates is used as a key criterion for presumed substance release. Pore plate arrangement is individually variable. Each pore plate is associated with one gland cell. Therefore, it was assumed, that more pore plates and a larger release area means a greater volume of emitted substance. Sex pheromonal function was tested by mechanical obstruction of the sternal glands and a subsequent behavioral assay.

5.3.3 Material and Methods

Insects

Echinothrips americanus specimens were maintained at the University of Halle, Germany. They were reared in cages of acrylic glass (50 cm x 50 cm), coated with gauze on two sides. Rearing conditions were as follows: 23 ± 1 °C, 60 ± 10 % RH, and a photoperiod of 16: 8 h L:D at 5000 Lux during photophase. *Phaseolus vulgaris* and *Gossypium* sp. served as host plants.

To obtain virgin individuals, pupae were collected from our laboratory culture and transferred individually to a well of a 12-well Greiner-plate (Sigma-Aldrich), filled with 1.5 ml of 1.4 % (w/v) agar and topped with a leaf discs (Ø 1.6 cm) of *P. vulgaris.* Plates were closed with a glass-lid, sealed with Parafilm M © (Pechiney Plastic Packaging), and kept in a climate chamber under the same rearing condition as the main culture.

1) Assortative mating by size

Mating trials were carried out in similarly prepared Greiner-plates. Each well was loaded with one male and three females at an age of 2 to 5 days. The observation arena was observed 1 h with a HD Webcam C525 (Logitech, Switzerland). Afterwards males were grouped according to their mating success (mating and non-mating) and processed further as mentioned in the text below. Mating success was defined by successful copulation with a female. Sperm transfer was checked by histological examination of spermatheca.

Whole mounts were made of each individual male after Moritz et al. (2004) (for a detailed instruction see Supplementary Material S5.3) in order to measure body size. Preparations were observed with a Leitz DMRBE (Leica, Germany), fitted with a Leica DFC 450C (Leica, Germany). Measurements were conducted with ImageJ software (version 2.0.0; Wayne Rasband, http://imagej.nih.gov/ij/). As indicators for specific body parts mainly cuticular characteristics were used, as these are largely independent of an individual´s physiological conditions and might have possible impact on mating success (Fig. 1, A-P, R).

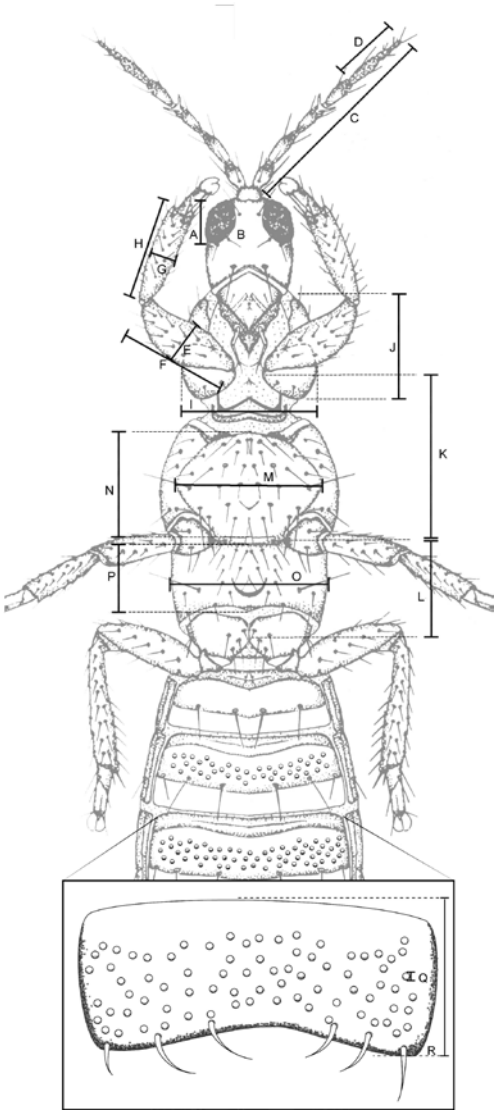


Fig. 1 Scheme of body measurements,

- **A** Compound eye length
- **B** Number of brown ommatidia
- **C** Antenna total length
- **D** Length antennae segment 6 to 8
- **E** Forefemur width
- **F** Forefemur length
- **G** Foretibia width
- **H** Foretibia length
- **I** Pronotum width
- **J** Pronotum length
- **K** Distance 1st coxa to 2nd coxa
- **L** Distance 2nd coxa to 3rd coxa
- **M** Mesosternum width
- **N** Mesosternum length
- **O** Metasternum width
- **P** Metasternum length
- **Q** Area and number of poreplate(s) on sternites III to VIII
- **R** Area of sternites III to VIII,

image modified after Moritz 2011

2) Assortative mating by pheromonal cues

To test a possible effect of assortative mating by pheromonal cues, size of release areas (area of pore plates) was measured. Whole mounts and technical equipment from 1) Assortative mating by size was used to determine number of pore plates per sternite, mean size of pore plate per sternite, and proportion of pore plate area per sternite (Fig. 1, Q-R).

In a subsequent experiment, multiple pore plates of virgin males were mechanically obstructed, followed by a behavioral assay: Virgin males were chilled on ice. Either nail polish diluted with acetone (1: 10) (treatment), water (control 1), or acetone (control 2, solvent) were applied on abdomens ventrally with a very thin brush under a stereomicroscope Leica S8 APO. After 24 h, treated males were offered virgin females (mating trial for assortative mating by pheromonal cues) as mentioned above under point 1: assortative mating by size. Successful matings were counted. In order to test for a possible anti-aphrodisiac function of sternal gland substances, mated females (from prior mating trial) were presented again to virgin males every 48 h for 30 min (re-mating trial) (Fig. 2). Two behaviors were counted: mating attempts and mating. Observation stopped with female death.

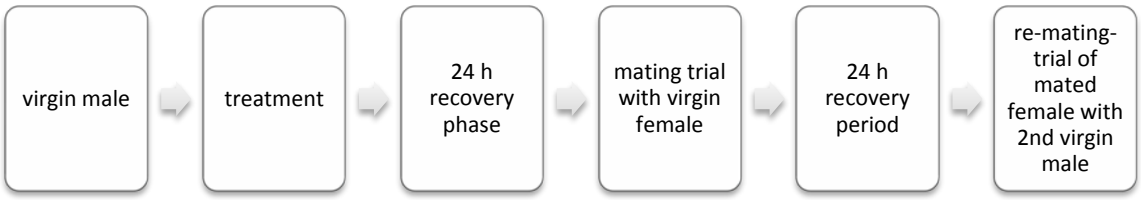


Fig. 2 Scheme of mating and re-mating trial in *E. americanus*

Statistical analysis

1) Prior to analysis, data were checked for normal distribution using the Kolmogorov-Smirnov-test ($p > 0.05$). Data were found to be distributed normally, and therefore statistical analyses were conducted using a t-test ($p \leq 0.05$) to check for difference between mating and non-mating group. The usual log-transformation to equalize variances and to reduce correlation between the mean and variance revealed no stronger results. Therefore the original data were used for analysis. All test were performed with WinSTAT® for Microsoft®Excel (Fitch Software, version 2012.1) and SPSS Statistics © 22 (IBM).

2) Data on multiple pore plate size were handled as for measurements of body size above. Behavioral data were checked for normal distribution using the Kolmogorov-Smirnov-test ($p > 0.05$) prior to analysis. Data were not normally distributed, therefore nonparametric tests were applied. Frequency of the particular behaviors was analysed with 3 x 2 Fishers-Exact-test ($p < 0.05$) (Joosse, 2012), mean number of behaviors using the Kruskal-Wallis-test ($p < 0.05$).

5.3.4 Results

1) Assortative mating by size

Male body size characteristics were measured and analysed depending on mating success. All complete data are given in Supplementary Material S5.3.

Males of the mated and non-mated group show significant differences in pronotum length (t-test, t = 2.763, df = 16, p = 0.014), distance between 1st and 2nd coxa (t-test, t = 1.904, df = 16, p = 0.007) and total sternite area (t-test, t = 2.286, df = 19, t = 0.034) of mated and non mated males (Fig. 3).

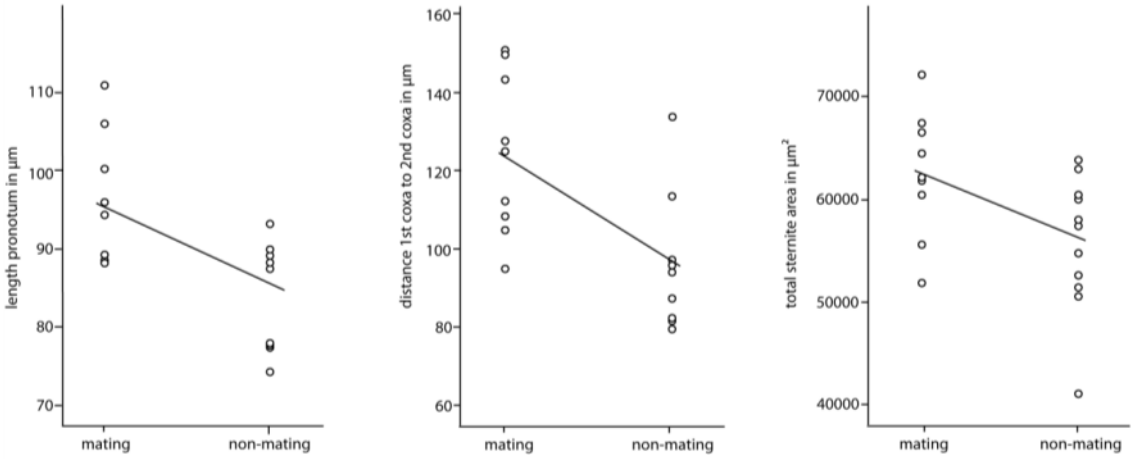


Fig. 3 Relationship of body size characters and mating sucess in male *E. americanus*

2) Assortative mating by pheromonal cues

No differences could be observed between mating and non-mating males in number of pore plates per sternite, mean area of pore plates per sternite, total area of pore plates per sternite, sternite area, or proportion of pore plate area per sternite (Supplementary Material S5.3).

Additionally, in the mating trial, there was no significant difference in mating success between the different groups (Fishers-Exact-test, p = 0.289)(Tab. 1).

Table 1 Mating success of *E. americanus* males in mating trail

Mating success	Control 1	Control 2 (solvent)	Treatment
Mating success in %	86.6	88.8	66.7
Number of treatments (n)	15	18	12

In the following re-mating trials of mated females that were offered treated or control males first, there was neither a significant difference in frequency of behaviors (mating attempts, 3 x 2 Fishers-Exact test, p = 0.1851; mating, 3 x 2 Fishers-Exact-test, p = 0.428) (Fig. 4) nor a significant difference in number of different behaviors observed (Kruskal-Wallis-test, p = 0.0509) (Tab. 2).

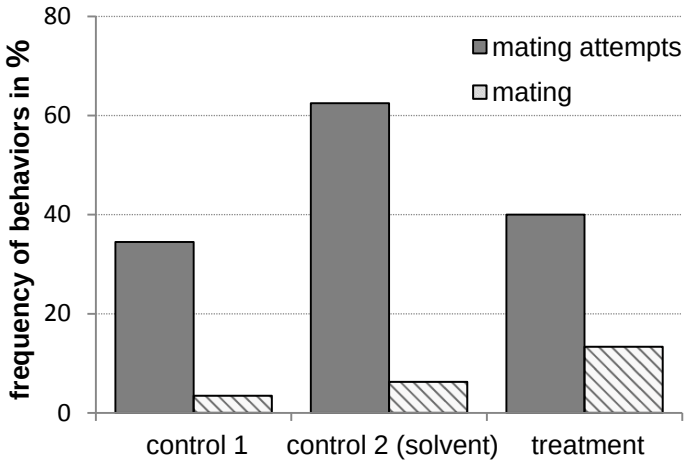


Fig. 4 Frequency of behaviors of virgin male *E. americanus* in re-mating-trials

However, an increase in mating attempts in control group 2 compared with control group 1 was detectable, even if this was not significant (Tab. 2).

Table 2 Mean number of behaviors ± SE of virgin male *E. americanus* in re-mating trials

Mean number of behavior ± SE	Control 1	Control 2 (solvent)	Treatment
Mating attempts	0.517 ± 0.189	1.688 ± 0.425	1.033 ± 0.333
Mating	0.034 ± 0.034	0.063 ± 0.063	0.133 ± 0.063

5.3.5 Discussion

One of the most common mating strategies is assortative mating in order to maximize individual fertility (e.g. Kuijper et al., 2012 and Shuker and Simmons, 2014). Both sexes want to benefit from direct or indirect advantages for their offspring, for instance reduced mating costs, access to resources or to good genes (Thornhill and Alcock, 1972 and Kuijper et al., 2012). Due to the high competition for mates, male performance is more important in terms of assortative mating, which is why females have been neglected in this study.

To find a suitable mate, individuals need to be able to discriminate via honest and individually different signals (Johannson and Jones, 2007). Both male body size and multiple pore plate patterns tested in this study were analysed initially for individuality. Both characteristics met this requirement.

In our study only a few features of male stature are correlated with male mating success. The longer pronotum, and therefore presumably also longer prothorax, and distance between 1st and 2nd coxae enable a more stable mounting position. After mounting, a male *E. americanus* grasps a female with the forelegs, and stroke the female thorax with the mesothoracic legs (Chapter 3). A similar behavior is known in *F. occidentalis* (Terry and Schneider, 1993). Furthermore, the extended thorax offers advantages for palpation of the antennae of the female, an important behavior in the initial phase of copulation (Terry and Schneider, 1993). These authors also reported rejected males being unable to reach the female antennae because of their smaller body size, and suggested that assortative mating occurs in this species.

Moreover, successfully mated males are larger and/or heavier. In addition the total sternite area differs between mated and non-mated groups. Total sternite area can be used as an indicator of the overall body size of thrips, since body length cannot be measured satisfactorily due to variation influenced by physiological conditions. Larger size and greater weight enable better performance in male-male competitions (Crespi, 1989). Males of *E. americanus* guard and defend female pupae against other males (Chapter 6). After eclosion, females mate with the successfully defending and more competitive males. In terms of Fisherian model, they opt for indirect benefits to their offspring (Fisher, 1915 and Fisher, 1930).
Terry used weight in *F. occidentalis* as the indicator of body size. Males that were successful in mating were 2.2 x heavier than males that were unsuccessful (unpublished, mentioned in Terry and Schneider, 1993). In *F. occidentalis,* size is related to fighting activity and access to mating sites in mating swarms (Terry and Dyreson, 1996).

In the second part of the experiment, pheromonal cues were checked for mate assessment. Chemical signals often vary with body size, and are used as a proxy for condition (Bradbury and Vehrencamp, 2011). *Utetheisa* moths use the quantity of male-emitted pheromone to assess male size, and this is an indicator for larval diet quality (Conner et al., 1990). Females of the cockroach *Nauphoeta cinerea* detect the amount and ratio of compounds of male pheromone to select mates that are in good condition (Clark et al., 1997). A similar mechanism is known for *Drosophila serrata* (Blows et al., 2004).
In preliminary experiments with *E. americanus* it was noted that some males are repeatedly rejected by females. Given the individual variation in distribution of the multiple pore plates, we postulate a relationship between this pattern and mating success. The lipid and proteinaceous substances produced (Section 5.2) probable have a short-range function.
However, no correlation between the morphological structure of the pheromonal release area and mating success could be detected. Additionally, obstruction of this area had no significant impact. Moreover, investigation of a possible anti-aphrodisiac of the sternal gland secretion failed. The slightly increased frequency of mating attempts in control group 2 can be explained by the enhanced wettability of the acetone solvent applied. During application, other parts of the body, beside sternites, may have come into contact with the solvent, possibly washing away the active substances. Presumably the known anti-aphrodisiac pheromone DBE-6, found in male *E. americanus* head and prothorax, was affected (Chapter 6).
In section 5.2 we reported a stabilizing effect on the production of sternal gland substances by the presence of females. Due to the individual rearing conditions to obtain virgin males, these males might have failed to produce complete secretions thus affecting the further mating trials. Below threshold production might account for the lack of difference in mating success between the controls and treated males. A bioassay with virgin males which have olfactory but no physical contact to females could be used to test this in a future trial.
Even, if male sternal glands have no influence on mating success, a correlation has been demonstrated between male body and mating success.

Acknowledgement
We would like to thank candidate M.Sc. Marcus Jilge for technical assistance.

5.3.6 References

Ananthakrishnan, T.N., 1990. The reproductive biology of thrips. Indira Publishing House, Oak Park.

Arak, A., 1983. Male-male competition and mate choice in anuran amphibians. In: Bateson, P. (Ed), Mate Choice. Cambridge University Press, Cambridge, pp. 181-210.

Blows, M.W., Chenoweth, S.F. and Hine, E., 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Amer. Nat. 163, 329-340.

Bradbury, J.W. and Vehrencamp, S.L., 2011. Principals of Animal communication. 2nd edition. Sinauer Associates, Sunderland Massachusetts.

Clark, D.C., DeBano, S.J. and Moore, A.J., 1997. The influence of environmental quality on sexual selection in *Nauphoeta cinerea* (Dictyoptera: Blaberidae). Behav. Ecol. 8, 46-53.

Conner, W.E., Roach, B., Benedict, E., Meinwald, J. and Eisner, T., 1990. Courtship pheromone production and body size as correlates of larval diet in males of the arctiid moth, *Utetheisa-ornatrix*. J. Chem. Ecol. 16, 543-552.

Crespi, B.J., 1986a. Territoriality and fighting in a colonial thrips, *Hoplothrips pedicularis*, and sexual dimorphism in Thysanoptera. Ecol. Entomol. 11, 119-130.

Crespi, B.J., 1986b. Size assessment and alternative fighting tactics in *Elaphrothrips tuberculatus* (Insecta: Thysanoptera). Anim. Behav. 34, 1324-1335.

Crespi, B.J., 1988a. Alternative mating tactics in thrips: effects of sex ratio variation and body size. Am. Midl. Nat. 122, 142-150.

Crespi, B.J., 1988b. Risks and benefits from lethal male fighting in colonial *Hoplothrips karnyi* (Insecta: Thysanoptera). Behav. Ecol. Sociobiol. 23, 93-104.

Crespi, B.J., 1989. Causes of assortative mating in arthropods. Anim. Behav. 38, 980-1000.

Darwin, C. 1871. The Descent of Man and Sexual Selection in Relation to Sex .J Murray, London.

Emlen, S.T., and Oring, L.W., 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197, 215-223.

Fisher, R., 1915. The evolution of sexual preferences. Eugen. Rev. 7, 184-192.

Fisher, R., 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

Hood, J.D., 1908. Two new species of *Idolothrips.* Ann. Entomol. Soc. Am. 1, 285-289.

Hood, J.D., 1914. Notes on new North American Thysanoptera, with descriptions of a new family and two new species. Insecutor inscitiae menstruus 2, 17-22.

Johansson, B.G., and Jones, T.M., 2007. The role of chemical communication in mate choice. Biol. Rev. 82, 265-289.

Joosse, S., 2012. Fisher´s exact test. [WWW document, October 2015]
URL http://in-silico.net/tools/statistics/fisher_exact_test

Kokko, H., Klug, H., and Brooks, R., 2006. Unifying and testing models of sexual selection. Annu. Rev. Ecol. Evol. Syst. 37, 43-66.

Krueger, S., Subramanian, S., Niassy, S. and Moritz, G.B., 2015a. Sternal gland structures in males of bean flower thrips, *Megalurothrips sjostedti*, and Poinsettia thrips, *Echinothrips americanus*, in comparison with those of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Arth. Struct. & Dev. 44, 455-467.

Krueger, S., Mound, L.A. and Moritz, G.B., (2015b). Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* MORGAN 1913 (Thysanoptera). J. Appl. Entomol., doi: 10.1111/jen.12280

Kuijper, B., Pen, I., and Weissing, F.J., 2012. A Guide to Sexual Selection Theory. Annu. Rev. Ecol. Evol. Syst. 43, 287-311.

Kumm, S., 2002. Reproduction, progenesis and embryogenesis of thrips (Thysanoptera, Insecta). PhD thesis. Martin-Luther-University Halle-Wittenberg, Germany.

Lewis, T., 1973. Thrips: Their Biology, Ecology and Economic Importance. Academic Press London, New York.

Lewontin, R., Kirk, D. and Crow, J., 1968. Selective mating, assortative mating, and inbreeding: definitions and implications. Eugenics Quart. 15, 141-143.

Li, X-W., Jiang, H-X., Zhang, X-C., Shelton, A.M. and Feng, J-N., 2014. Post-mating interactions and their effects on fitness of female and male *Echinothrips americanus* (Thysanoptera: Thripidae), a new insect pest in China. PLOS One 9, 1-7.

Moritz, G.B., 2011. Thysanoptera – Fransenflügler (Thripse). In: Klausnitzer, B. (Eds), Stresemann Exkursionsfauna von Deutschland, Band 2, Wirbellose: Insekten. Springer Spektrum Akademischer Verlag, Heidelberg, pp. 164-171.

Moritz, G.B., Mound, L.A., Morris, D. and Goldarazena, A., 2004. Pest thrips of the world - an identification and information system using molecular and microscopial methods. Centre for Biological Information Technology, University of Queensland, Australia, CD-ROM ISBN 1 86499 781 8, online available:
http://thripsnet.zoologie.uni-halle.de/key-server-neu/

Oetting, R.D. and Beshear, R.J., 1993. Biology of the greenhouse pest *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). Int. J. Pure Appl. Zool. 4, 307-315.

Oetting, R.D., Beshear, R.J., Liu, T.X., Braman, S.K. and Baker, J.R., 1993. Biology and identification of thrips on greenhouse ornamentals. Geo. Agr. Exp. St. Bull. 414, 20.

Plant Health Australia, 2010. Poinsettia thrips contingency plan. [WWW document, July 2015] URL http://www.planthealthaustralia.com.au/pests/poinsettia-thrips/.

Ridley, M., 1983. The explanation of Organic Diversity: the Comparative Method and Adaptions for Mating. Clarendon Press, Oxford.

Shuker, D.M., and Simmons, L.W., 2014. The Evolution of Insect Mating Systems. Oxford University Press, Oxford.

Shuster, S.M., and Wade, M.J., 2003. Mating Systems and Strategies. Princeton University Press, Princeton.

Stannard, L.J., 1968. The Thrips, or Thysanoptera, of Illinois. Bull. Ill. Nat. Hist. Surv. 29, 214-552.

Terry, L.I. and Schneider, M., 1993. Copulatory behaviour and mating frequency of the western flower thrips, *Frankliniella occidentalis* (Insecta: Thysanoptera). IJPAZ 4, 339-354.

Terry, L.I., and Dyreson, E., 1996. Behavior of *Frankliniella occidentalis* (Thysanoptera: Thripidae) within aggregations, and morphometric correlates of fighting. Ann. Entomol. Soc. Am. 89, 589-602.

Thornhill, R., and Alcock, J., 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge.

Trdan, S., Milevoj, L., Raspudic, E. and Zezlina, I., 2003. The First Record of *Echinothrips americanus* Morgan in Slovenia. Acta Phytopathol. Hun. 38, 157-166.

Trivers, R., 1972. Parental investment and sexual selection. In: Campbell, R. (Ed), Sexual Selection and the Descent of Man 1871-1971. Aline Press, Chicago, 139-179.

Vierbergen, G., 1998. *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). Proc. Exp. Appl. Entomol 9, 155-160.

Yew, J.Y. and Chung, H., 2015. Insect pheromones: An overview of function, form, and discovery. Progress in Lipid Research 59, 88-105.

CHAPTER 6

MALE PHEROMONES INFLUENCE THE MATING BEHAVIOR OF *ECHINOTHRIPS AMERICANUS* (INSECTA, THYSANOPTERA: THRIPIDAE)

Journal of Chemical Ecology
under rereview

Stephanie Krueger[1], Gerald Moritz[1], Peter Lindemann[2], Diana Radisch[1], Gunther Tschuch[1*]

[1] *Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences I, Institute of Biology, Heinrich-Damerow-Str. 4, 06120 Halle, Germany*
[2] *Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences I, Institute of Pharmacy, Hoher Weg 8, 06120 Halle, Germany*

6.1 Summary

Two dibasic esters, the dimethyl ester of hexanedioic acid (dimethyl hexanedioate, dimethyl adipate, DBE-6) and the dimethyl ester of pentanedioic acid (dimethyl pentanedioate, dimethyl glutarate, DBE-5) were found in head-thorax extracts of male *Echinothrips americanus*. DBE-5 induced abdomen wagging and raising in males and females, which is typical behavior when encountering a male. DBE-6 is avoided by males and is detectable on mated females, but not on virgin females. Both substances applied to virgin females, leads to the females are being ignored by the males. The role of both substances is discussed from the perspective of the male mating system.

Keywords: Poinsettia thrips, antiaphrodisiac, sternal glands, male recognition, mate competition

Own contribution: 70 %

6.2 Introduction

Thysanoptera cause millions of US dollars of crop loss each year (Lewis 1973, 1997). These 1–2 mm long insects produce crop damage by piercing and sucking out cells of leaves, flowers and fruit. Additionally, some thrips species are propagative vectors of tospoviruses, and cause indirect damage by transmitting other plant viruses (*Machlomovirus*, *Ilarvirus*, *Carmovirus*, *Sobemovirus*), bacteria and fungi (Ullman et al. 1997; Gitaitis et al. 2003; Jones 2005). As a result of the global trade in agricultural products, some pest thrips species have now spread worldwide. Their cryptic life-style, adaptability to different environmental conditions and ability to develop broad insecticide resistance, necessitate alternative approaches for their management. The use of thrips semiochemicals (such as aggregation pheromones) offers the potential for more effective thrips monitoring and optimal pest control (Hamilton and Kirk 2003).

Currently, male-produced pheromones are known from three species; *Frankliniella intonsa* (Zhang et al. 2011), *Frankliniella occidentalis* (De Kogel and van Deventer 2003; Hamilton et al. 2005; Kirk and Hamilton 2010; Olaniran et al. 2013) and *Thrips palmi* (Kirk and Hamilton 2010; Akella et al. 2014).

The possible source and release area of pheromones could be the sternal glands. The typical sexually dimorphic structures, taxonomically referred to as areae porosae, are mainly present on sternites III/IV to VII/VIII of thripine males. Males of the thripine species *Echinothrips americanus* Morgan 1913 (Poinsettia thrips) possess large sternal glands (Krueger et al. 2015a). Their resemblance to external structures in other insect species and their presence almost exclusively in males, leads several authors to the conclusion that sternal glands might be the source of pheromones (Pelikan 1951; Bode 1978; Moritz 1997, 2006; Sudo and Tsutsumi 2002, 2003; Shitatani and Tsutsumi 2005, 2006; El-Ghariani and Kirk 2008; Mound 2009; Olaniran et al. 2013).

E. americanus is native to eastern parts of the USA (Stannard 1968) and since 2002 it has spread to the whole of the north-eastern USA and Canada (Shipp et al. 2001; Ferguson and Shipp 2002). This species was intercepted in Europe for the first time in 1989 (Collins 1998) and it spread rapidly to more than 20 European countries (Vierbergen et al. 2006; Varga and Fedor 2008; Andjus et al. 2009). It has now been found also in Asia and northern Australia (see Krueger et al. 2015b). *E. americanus* is a phytophagous species with a host range of about 24 plant families (Vierbergen 1998). Feeding activity causes small chlorotic areas and shallow feeding punctures, such that the plants appear to be shrunken (Oetting and Beshear 1993; Oetting et al. 1993; Trdan et al. 2003). Because of the damage, the rapid spread, wide host range and the ability to adapt the duration of their development (Krueger et al. 2015b), this thrips has the potential to become a major pest, and so there is a need for further investigation and development of new control measures (Plant Health Australia 2010).

The aim of this study was to advance the understanding of the biology of this species by identifying male pheromone substances and characterizing their source and function.

6.3 Methods and Materials

Insect rearing

E. americanus were taken from laboratory cultures maintained at the University of Halle, Germany (at 23 ± 1 °C, a relative humidity of 60 ± 10 %, and 16:8 h L:D photoperiod, light on 6:00 a.m. CET). *Phaseolus vulgaris* and *Gossypium* sp. were used as host plants.

To obtain virgin females and males, pupae were collected from the laboratory culture and reared individually in 12-well Greiner plates (Sigma-Aldrich), with each well part-filled with 1.5 ml of 1.4 % (w/v) agar and with a leaf disc of *P. vulgaris* placed on the surface of the agar. Plates were covered with a glass sheet and sealed with Parafilm M © (Pechiney Plastic Packaging). Plates were kept in a climate chamber under the same conditions as the main culture.

GC-MS analysis

Virgin males, virgin females, mated males and mated females were analysed. Whereas mated individuals were obtained from the main culture, virgins were derived from individual rearing as described above. Individuals were manipulated with ethanol-cleaned insect pins and razor blades to avoid contamination.

For chemical analysis, 50 thrips per sample were pooled, extracted in 100 µl distilled methanol (Aldrich, 49,429-1), or alternatively in 2-methoxy-2-methylpropane (Sigma-Aldrich MTBE, 34875), to find out whether any secondary substances were produced by reactions with the solvent. Investigation takes place within 2 h. Pooling was necessary because of the minute size of the animals and the small amount of substances they produced. Preliminary test show the clearest peaks when using 50 individuals per sample. Animals were used either as extraction of 50 insects as whole or as dissected material, where head-thorax and abdomen extracted separately. For coupled gas chromatography/mass spectrometry analyses, 10 µl samples were injected into a Shimadzu GCMS-QP2010 that was operated in electron impact mode at 70 eV.

The compounds of interest were separated on a Shimadzu GC-2010 fitted with a FS Supreme 5 ms ID–MS column (30 m × 0.32 mm ID; stationary phase: 0.10 µm, CS-Chromatographie-Service, Langerwehe, Germany) using a Gerstel Cooled Injection System CIS6 (Mühlheim, Germany) with an injector temperature of -20 °C, a solvent vent mode of 1 min 90 ml H_2/min, splitless injection (320 °C), a temperature gradient programmed from 40 °C to 300 °C at 7 °C /min, and hydrogen (H_2) with a flow rate of 1.0 ml/min. Ion masses were determined using a GCMS-QP2010 (detector: MS source at 250 °C).

The NIST database, literature data, the software AMDIS (NIST, version 2.71) and NIST MS SEARCH were used to analyze mass spectra. For comparison with authentic compounds, the following substances were used: DBE-5 (Aldrich, D158801) and DBE-6 (Aldrich, 186252).

Bioassays

1. Behavioral bioassay (adapted from Dublon 2009)

A filter paper (Ø 90 mm) was placed in a petri dish (Ø 90 mm) and divided into 2 parts, a substance area and a control area. Each area contained a semicircular lane of 40 µl of

substance or distilled water as a control (Figure 1). Substances were used in a concentration of 27 mM DBE-5 and 14 mM DBE-6 in distilled water, which corresponds to a 10 % saturated solution. 10 males and 10 females, collected from the main culture, were placed individually in the middle of the filter paper. The duration of the stay in each half was recorded for 15 minutes. Observation started after initial walking of the individual. The filter paper and the side of substance application were changed after every 5 tests.

2. Effect on mating behavior

Substances (same concentration as above) and control (distilled water, referred to as untreated) were applied on virgin males, virgin females and mated females (obtained from the main culture). Virgin insects were 2 to 7 days old at the time of the experiment. Each treated/untreated animal was placed in one well of a 6-well Greiner plate (each well Ø 35 mm, Sigma-Aldrich) on a filter paper with a fresh virgin male (also 2 to 7 days old). For each treatment 15 individuals were tested. Over a period of 15 min, we registered whether or not there was contact and whether or not mating took place for each pair of animals.

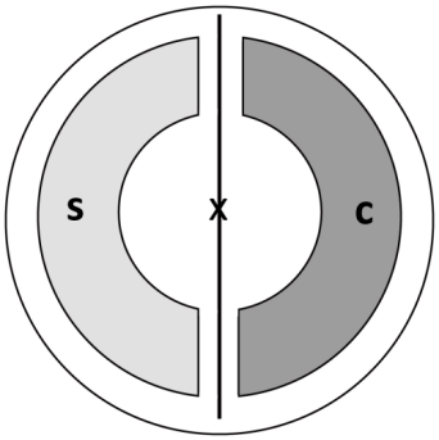


Fig 1: Diagram of the behavioral bioassay. The duration of stay of *E. americanus* individuals in each half of the test area was recorded for 15 minutes; s-substance-area, c-control-area, x-starting position of the animal

3. Statistical tests

Data analyses were performed with WinSTAT© for Excel (Fitch Software). The data from the behavioral bioassay were checked for normal distribution using the non-parametric Kolmogorov-Smirnov test ($p > 0.05$). Afterwards, the data were analyzed with a paired t-test ($p < 0.05$). Effect size d was calculated via d_z = mean difference of values / standard deviation of differences. The frequency of behaviors was compared with a 3 x 3 Fisher's exact test ($p < 0.05$) (Joosse 2012).

6.4 Results

DBE-6 and DBE-5 were both found in methanol extracts as well as in MTBE extracts of whole virgin and mated males. Extracts of virgin females contained no dibasic esters. In contrast, extracts of females reared together with males contained only traces of DBE-6. The peaks for these traces could be found easily on the GC because of their known retention times and by detection of the mass peak 143, which is not common in the mass spectra of other substances. No traces of DBE-5 could be detected with the help of the mass peak 129. In dissected virgin males, both dibasic esters were found in head-thorax extracts only, and not in abdomen extracts (Figure 2). The substances in the abdomen extracts with retention times close to where DBE-5 and DBE-6 would occur have very different mass spectra. No mass peaks of 129 or 143 could be found. The highest peak in the abdomen extracts at 14.49 min retention time is, according to the NIST database, most likely methyl benzoate. This peak was detected in both sexes and will thus not be considered here.

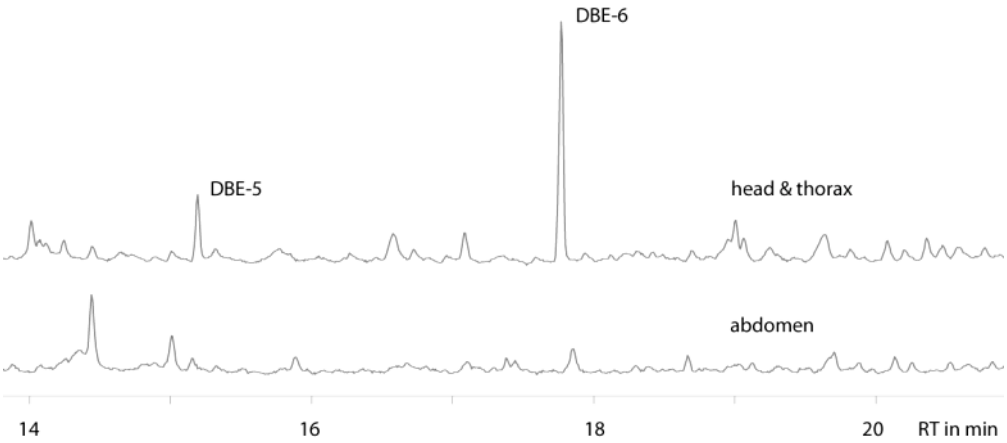


Fig 2: Typical chromatograms of methanol extracts of head-thorax (above) and abdomen (below) of 50 male *E. americanus*. X-axis: retention time in minutes. Y-axis: abundances at the same scale (DBE-5: dimethyl ester of pentanedioic acid, DBE-6: dimethyl ester of hexanedioic acid).

To test the response of the animals to the substances DBE-5 and DBE-6, animals, obtained from the main culture, were observed in the bioassay arena. Males stayed 5.38 ± 1.43 min (mean ± SE) and females 9.22 ± 1.51 min within the DBE-5 substance area. The duration of stay in the substance area (DBE-5) and the control area did not differ significantly for males or females (paired t-test, male: T = -1.088, df = 9, p = 0.305, d_z = 0.344; female: T = 0.988, df = 9, p = 0.349, d_z = 0.33). In the bioassay test all females were observed to raise their abdomen, and males to wag their abdomen, during walking over DBE-5 solutions in water.

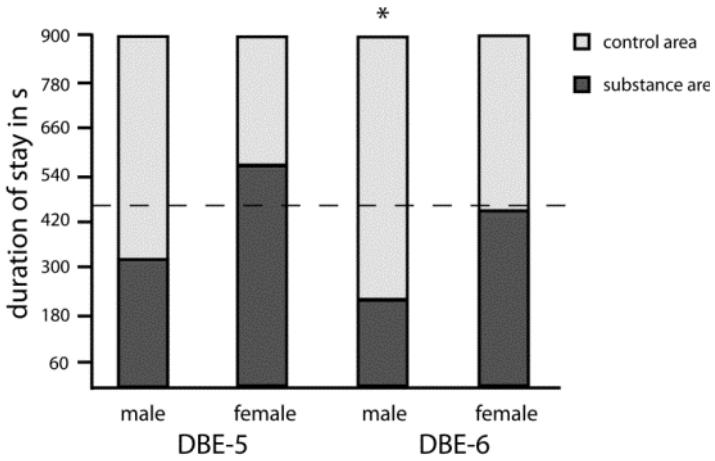


Fig 3: Response to DBE-5 and DBE-6 in the behavioral bioassay. The duration of stay in the substance and control area in min for male and female *E. americanus* (n = 10), asterisk indicate for significance

Females stayed 7.26 ± 1.4 min (mean ± SE) and males stayed 3.35 ± 1.32 min on average within the DBE-6 substance area. The duration of stay of females in the substance area (DBE-6) and the control area were not significantly different (paired t-test, T = -0.036, df = 9 p = 0.972, d_z = -0.01), but males stayed significantly longer within the control area (paired t-test, T = -2.533, df = 9, p = 0.032, d_z = -0.8). Two males and four females out of 10 wagged and raised their abdomen while walking over the substance area.

In order to test for an effect on the mating behavior, response of virgin males to treated (brushed with DBE-5 or DBE-6) or untreated (brushed with distilled water) virgin males, mated

females and virgin females was recorded. No difference in the frequency of contact was found between DBE-5, DBE-6 or water treated virgin males (Fisher´s exact test 3x3, p = 0.096) and mated females (Fisher´s exact test 3x3, p = 1).

Brushing virgin females with DBE-6 or DBE-5 caused a significant reduction in contacts with the virgin male as compared to the water control (Fisher`s exact test 3x3, p = 0.0175). This is the only condition where copulations occurred (Table 1).

Tab 1: Frequency of behaviors of virgin male in response to treated/untreated virgin males, mated female or virgin female of *E. americanus* during a 15 min test (n=15 tests each). DBE-6 and DBE-5: animal brushed with DBE-6 or DBE-5; control: animal brushed with distilled water.

behavior	virgin males			mated females			virgin females		
	DBE-6	DBE-5	control	DBE-6	DBE-5	control	DBE-6	DBE-5	control
no contact	12	15	15	14	15	14	12	12	5
contact	3	0	0	1	0	1	3	3	7
mating	-	-	-	0	0	0	0	0	3

6.5 Discussion

In the behavioural bioassay carried out, no attractive or repellent effects of DBE-5 were observed in males or females. Females raised their abdomen during walking over the substance area, whereas males instead wagged their abdomen. This behaviour is also observed by females rejecting mating attempts (Terry and Schneider 1993; Li et al. 2014) or in male-male aggression (Crespi 1986; Terry and Gardner 1990; Terry and Dyreson 1996). The application of DBE-5 on virgin females leads to them being ignored by males.

The substance DBE-5 initiated similar reaction as the short-range male-recognition pheromone 7-methyltricosane found in *Frankliniella occidentalis* (Thysanoptera: Thripidae) (Olaniran et al. 2013). Apart from *F. occidentalis,* male-produced contact compounds have only been demonstrated in a few species of Diptera (Carlson and Schlein 1991; Nelson et al. 1981; Lacaille et al. 2007), Blattodea (Takahashi and Fukui 1983) and ants (Smith et al. 2014).

Females and males of *E. americanus* seem to be able to discriminate between the sexes, probably due to the absence or presence of DBE-5. In the lab culture, males have frequently being observed guarding female pupae, by sitting near her, and mating with them immediately after eclosion (unpublished) *E. americanus* females mate only once or remate with just a very low frequency (Li et al. 2014; Krueger et al. 2015b), finding and monopolising receptive females will enhance male reproductive effort a trait, often found in species with strong local mate competition (Simmons and Siva-Jothy 1998).

The second substance, DBE-6, causes abdomen wagging with a much lower frequency in males and females than is caused by DBE-5. However, it was avoided by males (Figure 3). Additionally, traces of DBE-6 were found on mated females (but not on virgin females). Since they have a low volatility (boiling point 215–225 °C), these substances could be transferred from males to females during mating. Application of DBE-5 or DBE-6 on virgin females resulted in an avoidance behavior by males, whereas in the control group mating occurred (Table 1).

Therefore, these dibasic esters presumably have an antiaphrodisiac effect in the concentration tested here. They may mask receptive females during copulation to limit take-overs from other males and mark females with their mating status to avoid sexual competition (reviewed in Simmons 2001). Furthermore the substances prevent females from receiving further male harassment, which is known to reduce longevity and fertility in this species (Krueger et al. 2015b). Pheromones transferred from males to females help to mark the female mating status (Thomas 2011) and may work as antiaphrodisiacs (Ayasse et al. 2001). This is known in many arthropods (Happ 1969; Gilbert 1976; Tompkins and Hall 1981ab; Kukuk 1985; Andersson et al. 2000; Schulz et al. 2008, Brent and Byers 2011).

Both substances play an important role in the male mating strategy of *E. americanus.* This species may have developed a system of precopulatory (DBE-5) and postcopulatory (DBE-6) traits minimizing sexual competition within this mating system with high local mate competition.

However, the site of biosynthesis and deposition of DBE-5 and DBE-6 remains unknown. We did not find any signal from abdominal parts (Figure 2), which is not what we expected. The origin of the substances is somewhere in the head or thorax and still remains unknown. A vertex gland has been described only in the genus *Merothrips* (Moritz 1984), and not in *E. americanus.* Possibly fat body cells or oenocytes in the head and prothorax could be the source. In many insects, oenocytes are known to play a role in hydrocarbon pheromone synthesis (reviewed in Martins and Ramalho-Ortigao 2012; Makki et al. 2014). Furthermore, it remains unclear why only one of the substances is transferred to females. Possibly, the amounts of DBE-5 were below the detection limit or both substances are not released at the same place or DBE-6 is actively rather than passively transmitted from males to females.

Additional studies are needed to identify the exact source of the pheromonal substances and the transmission path.

Acknowledgements This study was funded by the University of Halle-Wittenberg. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We gratefully acknowledge William Kirk, Keele University, Staffordshire, and two unknown reviewers for discussion, comments, and valuable information.

6.6 References

Akella SVS, Kirk WDJ, Lu Y-B, Murai T, Walters KFA, Hamilton JGC (2014) Identification of the aggregation pheromone of the melon thrips, *Thrips palmi*. PLOS ONE 9:e103315

Andersson J, Borg-Karlson AK, Wiklund C (2000) Sexual cooperation and conflict in butterflies: a male transferred anti-aphrodisiac reduces harassment of recently mated females. Proc Roy Soc B 267:1271–1275

Andjus L, Jovis M, Trdan S (2009) First record of *Echinothrips americanus* in Serbia. Hellenic Plant Protection Journal 2:71–73

Ayasse M, Paxton RJ, Tengö J (2001) Mating behavior and chemical communication in the order Hymenoptera. Annu Rev Entomol 46:31–78

Bode W (1978) Ultrastructure of the sternal glands in *Thrips validus* Uzel (Thysanoptera, Terebrantia). Zoomorphologie 90:53–65

Brent CS, Byers JA (2011) Female attractiveness modulated by a male-derived antiaphrodisiac pheromone in a plant bug. Anim Behav 82:937-943

Carlson DA, Schlein Y (1991) Unusual polymethyl alkenes in tsetse flies acting as abstinon in *Glossina morsitans*. J Chem Ecol 17:267-284

Collins DW (1998) Recent interceptions of *Echinothrips americanus* Morgan (Thysanoptera, Thripidae) imported into England. The Entomologist's Monthly Magazine 133:1–3

Crespi BJ (1986) Size assessment and alternative fighting tactics in *Elaphrothrips tuberculatus* (Insecta: Thysanoptera). Anim Behav 34:1324–1335

De Kogel WJ, van Deventer P (2003) Intraspecific attraction in the western flower thrips *Frankliniella occidentalis*, indications for a male sex pheromone. Entomol Exp Appl 107:87–89

Dublon IAN (2009) The aggregation pheromone of the western flower thrips. PhD Dissertation, Keele University, UK.

El-Ghariani IM, Kirk WDJ (2008) The structure of the male sternal glands of the western flower thrips, *Frankliniella occidentalis* (Pergande). Acta Phytopathologica Entomologica Hungarica 43:257–266

Ferguson G, Shipp L (2002) New pests in Ontario greenhouse vegetables. Bull OILB 25:69–72

Gilbert L (1976) Postmating female odor in *Heliconius* butterflies: a male-contributed antiaphrodisiac? Science 193:419–420

Gitaitis, RD, Walcott, RR, Wells, ML, Diaz Perez JC, Sanders FH (2003) Transmission of *Pantoea ananatis*, causal agent of center rot of onion, by tobacco thrips, *Frankliniella fusca*. Plant Dis 87:675–678.

Hamilton JGC, Kirk WDJ (2003) Method of monitoring/controlling thysanoptera. Patent WO 2003055309 A1

Hamilton JGC, Hall DR, Kirk WDJ (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem Ecol 31:1369–1379

Happ GM (1969) Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor* L. Nature 222:180–181

Jones DR (2005) Plant viruses transmitted by thrips. Eur J Plant Pathol 113:119–157

Joosse S (2012) http://in-silico.net/tools/statistics/fisher_exact_test

Kirk WDJ, Hamilton JGC (2010) The aggregation pheromones of thrips. In: Persley D, Wilson C, Thomas J, Sharman M, Tree D (ed) IXth International Symposium on Thysanoptera and Tospoviruses, J Insect Sci 10:166

Krueger S, Subramanian S, Niassy S, Moritz GB (2015a) Sternal gland structures in males of bean flower thrips, *Megalurothrips sjostedti*, and Poinsettia thrips, *Echinothrips americanus*, in comparison with those of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Arth Struct & Dev doi:10.1016/j.asd.2015.07.002

Krueger S, Mound LA, Moritz GB (2015b) Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* MORGAN 1913 (Thysanoptera). J Appl Entomol (accepted)

Kukuk P (1985) Evidence for an antiaphrodisiac in the sweat bee *Lasioglossum* (*Dialictus*) *zephyrum*. Science 227:656–657

Lacaille F, Hiroi M, Twele R, Inoshita T, Umemoto D, Maniere G, Marion-Poll F, Ozaki M, Francke W, Cobb M, Everaerts C, Tamimura F, Ferveur JF (2007) An inhibitory sex pheromone tastes bitter for *Drosophila* males. PLoS ONE 2:e661

Lewis T (1973) Thrips: Their biology, ecology and economic importance. Academic Press London, New York

Lewis T (1997) Chemical control. In: Lewis T (ed) Thrips as Crop Pests. CAB International, Oxon and New York, pp. 567–593

Li X-W, Jiang H-X, Zhang X-C, Shelton AM, Feng J-N (2014) Post-mating interactions and their effects on fitness of female and male *Echinothrips americanus* (Thysanoptera: Thripidae), a new insect pest in China. PLoS ONE 9:1–7

Makki R, Cinnamon E, Gould AP (2014) The development and functions of oenocytes. Annu Rev Entomol 59:405–425

Martins GF, Ramalho-Ortigao JM (2012) Oenocytes in insects. Invertebrate Survival Journal 9:139–152

Moritz G (1984) Exocrine vertex gland in males of the genus *Merothrips* Hood, 1914 (Merothripidae, Thysanoptera, Insecta). Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere 111:1–13

Moritz G (1997) Structure, growth and development. In: Lewis T (ed) Thrips as Crop Pests. CAB International, Oxon and New York, pp. 15–63

Moritz G (2006) Thripse - Fransenflügler, Thysanoptera, Pflanzensaftsaugende Insekten Bd. 1, Die Neue Brehm-Bücherei Bd. 663,Westarp-Verlag, Hohenwarsleben

Mound LA (2009) Sternal pore plates (glandular areas) of male Thripidae (Thysanoptera). Zootaxa 2129:29–46

Nelson DR, Dillwith JW, Blomquist GJ (1981) Cuticular hydrocarbons of the house fly, *Musca domestica*. Insect Biochem 11:187-197

Oetting RD, Beshear RJ (1993) Biology of the greenhouse pest *Echinothrips americanus* Morgan (Thysanoptera: Thripidae). International Journal of Pure and Applied Zoology 4:307–315

Oetting RD, Beshear RJ, Liu TX, Braman SK, Baker JR (1993) Biology and identification of thrips on greenhouse ornamentals. Georgia Agricultural Experiment Station Research Bulletin 414:20

Olaniran OA, Sudhakar AVS, Drijfhout FP, Dublon IAN, Hall DR, Hamilton JGC, Kirk WDJ (2013) A male-predominant cuticular hydrocarbon, 7-methyltricosane, is used as a contact pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem Ecol 39:559–568

Pelikan J (1951) On carnation thrips, *Taeniothrips dianthi* Priesner. Folia Entomologica Hungarica 14:5–38

Plant Health Australia (2010) Poinsettia thrips contingency plan. Plant Health Australia. http://www.planthealthaustralia.com.au/pests/poinsettia-thrips/. Accessed 26 July 2015

Schulz S, Estrada C, Yildizhan S, Boppè M, Gilbert LE (2008) An antiaphrodisiac in *Heliconius melpomene* butterflies. J Chem Ecol 34:82–93

Shipp JL, Gillespie DR, Fry KM, Ferguson GM (2001) *Echinothrips americanus* (Morgan), *Frankliniella occidentalis* (Pergande), western flower thrips, and *Thrips tabaci* Lindemann, onion thrips (Thysanoptera: Thripidae). In: Mason PG, Huber JT (eds) Biological control programs in Canada. CABI Publishing, Wallington, pp. 115–119

Shitatani S, Tsutsumi T (2005) Ultrastructure of sternal glands in *Tenothrips frici* (Uzel) (Thysanoptera: Terebrantia). Proc Arthropod Embryol Soc Jpn 40:35–39

Shitatani S, Tsutsumi T (2006) Ultrastructure of sternal glands in *Hydatothrips* (*Hydatothrips*) *abdominalis* (Kurosawa) and *H.* (*Neohydatothrips*) *gracilicornis* (Williams) (Thysanoptera: Terebrantia). Proc Arthropod Embryol Soc Jpn 41:59–65

Simmons LW (2001) Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton.

Simmons LW, Siva-Jothy MT (1998) Sperm competition in insects: mechanisms and the potential for selection. In: Birkhead TR, Møller AP (Eds) Sperm competition and sexual selection. Academic Press, San Diego.

Smith AA, Vanderpool W, Millar JG, Hanks LM, Suarez AV (2014) Conserved male-specific cuticular hydrocarbon patterns in the trap-jaw ant *Odontomachus brunneus*. Chemoecology 24:29-34.

Stannard LJ (1968) The thrips, or Thysanoptera, of Illinois. Bull Ill Nat Hist Surv 29:214–552

Sudo M, Tsutsumi T (2002) Ultrastructure of sternal glands in two thripine thrips and one phlaeothripine thrips (Thysanoptera: Insecta). Proc Arthropod Embryol Soc Jpn 37:35–41.

Sudo M, Tsutsumi T (2003) Ultrastructure of the primordia of sternal glands in the second-instar pupal stage of *Frankliniella intonsa* (Thysanoptera: Insecta). Proc Arthropod Embryol Soc Jpn 38:27–29

Takahashi S, Fukui M (1983) Studies on the mating behavior of the cockroach *Nauphoeta cinerea* (Oliver) (Dictyoptera: Blaberidae). III. Isolation and identification of intermale recognition pheromone. Appl Entomol Zool 18:351-356.

Terry I, Gardner D (1990) Male mating swarms in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). J Insect Behav 3:133–141

Terry I, Schneider M (1993) Copulatory behavior and mating frequency of the western flower thrips, *Frankliniella occidentalis* (Insecta: Thysanoptera). International Journal of Pure and Applied Zoology 4:339–354

Terry I, Dyreson E (1996) Behavior of *Frankliniella occidentalis* (Thysanoptera: Thripidae) within aggregations, and morphometric correlates of fighting. Ann Entomol Soc Am 89:589–602

Thomas ML (2011) Detection of female mating status using chemical signals and cues. Biol Rev 86:1–13

Tompkins L, Hall JC (1981a) *Drosophila* males produce a pheromone which inhibits courtship. Z Naturforsch C 36:694–696

Tompkins L, Hall JC (1981b) The different effects on courtship of volatile compounds from mated and virgin *Drosophila* females. J Insect Physiol 27:17–21

Trdan S, Milevoj L, Raspudic E, Zezlina I (2003) The first record of *Echinothrips americanus* Morgan in Slovenia. Acta Phytopathol Hun 38:157–166

Ullman D, Sherwood JL, German TL (1997) Thrips as vectors of plant pathogens. In: Lewis T (ed) Thrips as Crop Pests. CAB International, Oxon and New York, pp. 539–565

Varga L, Fedor PJ (2008) First interception of the greenhouse pest *Echinothrips americanus* Morgan, 1913 (Thysanoptera: Thripidae) in Slovak Republic. Plant Protection Science 44:155–158

Vierbergen G (1998) *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society 9:155–160

Vierbergen G, Cean M, Hatalànè Szellèr I, Jenser G, Masten T, Simala M (2006) Spread of two thrips pests in Europe: *Echinothrips americanus* and *Microcephalothrips abdominalis* (Thysanoptera: Thripidae). Acta Phytopathologica et Entomologica Hungarica 41:287–296

Zhang P-J, Zhu X-Y, Lu Y-B (2011) Behavioural and chemical evidence of a male produced aggregation pheromone in the flower thrips *Frankliniella intonsa*. Physiol Entomol 36:317–320

CHAPTER 7

GENERAL DISCUSSION ON REPRODUCTIVE STRATEGIES OF *ECHINOTHRIPS AMERICANUS*

The ability to adapt rapidly to different environmental conditions and various habitats has made some thrips highly successful as serious pests of agriculture. Effective pest management necessitates a comprehensive knowledge of pest species, especially when a pest quickly develops resistance to chemical control or becomes an efficient vector of pathogens. However, often missing when planning suitable management strategies is a broad and accurate knowledge of the morphology, function, physiology and development of the reproductive system, germ cells, behavior and reproduction of a pest. Within the studies presented here the reproduction strategy of *Echinothrips americanus,* a widely-distributed potential greenhouse pest, was examined under different aspects. This thesis provides the first complete overview of the mating strategies of this species.

Morphology of reproductive system and mating behavior

The general structure of the male and female reproductive systems of *E. americanus* accord with other terebrantian species (Heming 1970a, b, Bode 1975, Schliephake and Klimt 1979, Moritz 1982, 1989, 1997, Dallai 1997, Kumm 2002). The female genitalia consist of the basal apparatus (1st and 2nd valvifers, basalvalvulae) and the shaft (1st and 2nd valvulae); the male genitalia comprise a phallobase, two parameres, a primitive aedeagus, a phallotheca, an endotheca and an aedeagus surrounded by paraphyses (Chapter 2).

During copulation the phallotheca and endotheca are evaginated and the male genitalia inserted into the female vagina. The associated mating behaviour (precopulation, copulation and postcopulation-phases) seems to be typical of Thysanoptera. Species differ only in the duration of the individual mating phases (Chapter 3, Terry and Schneider 1993, Milne et al. 2007, Rafter and Walter 2013). For the first time the chronological sequence is described of the internal processes. Initially, male accessory gland material is transferred to the female, followed by delivery of the spermatozoa, with both substances positioned at the entrance of the spermatheca. It is assumed, that the ejaculate migrates directly to the duct and spermatheca. At the end of the copulation the spherical sperm ball is visible within the spermatheca. The encapsulated sperm ball resembles those described in other species of Thripidae (Heming 1995). Bournier (1956) assumed that this type of sperm ball is a spermatophore in strict sense. Moreover, Teuber (2011) argued that the unequal distribution of secretion within the sperm ball suggested a spermatophore rather than a coagulation within the female reproductive tract. But the gradual releases of glandular secretion material, and the subsequent transfer of spermatozoa shown in this study, are reasons for these different distribution patterns. Therefore, the sequential transfer of secretion and spermatozoa, as well as the delivery near the spermathecal duct demonstrated here, supports Bode`s (1975) assumption of coagulation of the secretion within the spermatheca. The sperm ball seems to be a special form of “female-determined type 1 spermatophore” as described by Gerber

(1970), which is characterized by an ejection of secretions in a definite sequence before or after the spermatozoa transfer. The form of the delivered material is determined by the female genital tract, as was suspected by Heming (1995). A similar "spermathecal spermatophore" is known only in the psocids *Leptinotus patruelis* (Finlayson 1949) and *Trogium pulsatorium* (Klier 1956). Among Thysanoptera Tubulifera, only *Tiarothrips subramanii* is known to produce spermatophore-like objects (Ananthakrishnan 1990).

Spermatozoa of *E. americanus* measure about 75 µm in length. The size in other terebrantian species ranges from 20-200 µm, and in tubuliferan species from 70-130 µm, with some exceptions as for example, *Megathrips inermis* with 1,200 µm (Heming 1995, De Marzo 2005, Paccagnini et al. 2009).

The spermatozoa are produced in monocystic testes. In adult individuals only mature sperm are seen, and this seems to be a common feature common within the Terebrantia. In the suborder Tubulifera, species with polycystic testes have fully differentiated sperm (*Haplothrips verbasci*: Heming 1970b*, Bactrothrips brevitubus:* Kumm 2002), but sperm in different stages of development are also known (*Suocerathrips linguis*: Kumm 2002, *Gynaikothrips ficorum:* Moritz 2006). Interestingly, species within the same genus are described to differ in the temporal sequence of spermiogenesis: spermiogenesis in *Haplothrips verbasci* is completed prior to adult eclosion (Heming 1970b), and *Haplothrips setiger and H. simplex* continue spermiogenesis throughout their entire life (Paccagnini et al. 2007). Within parasitoid wasps, these two categories of sperm production are called prospermatogeny (spermiogenesis prior adult eclosion) and synspermatogeny (permanent spermiogenesis) with several intermediate forms (Boivin et al. 2005).

Until now, effect of prospermatogeny was unknown in Thysanoptera. Completion of spermiogenesis during adult life leads to a limited amount of sperm during their reproductive phase. Males can enhance their fitness only by increasing the number of their mating partners, thus availability of sperm could be a limiting factor in mating success. It is questionable whether it actually limits reproductive success.

Sperm limitation

Despite the potential limitations of prospermatogeny, *E. americanus* males are able to inseminate more than ten females (Chapter 3), which is similar to the situation known in *Frankliniella occidentalis* (Terry and Schneider 1993) and *Trichogramma evanescens* (Damiens and Boivin 2005). According to the local mate competition model, a female can be expected to produce just enough sons to ensure insemination of all her daughters, if all matings occur between the progeny of a single mate (Hamilton 1967). The known male sex ratio of 0.3 in *E. americanus* (Kumm 2002) implies that each male would copulate with 3.33 females. But each male is able to inseminate more than 10 females. Therefore, the prospermatogeny does not lead to a restriction in mating success. However, the driver for such a form of spermiogenesis is still unknown. Early studies associated the limited spermiogenesis with short-lived adults (Phillips 1970). But Boivin et al. (2005) suggested additional factors that might lead to development of prospermatogeny: (i) small size of the individuals, because of the energy costs for maintaining gamete production and testes tissue in proportion to size and (ii) mating opportunities occurring at the beginning of their life. Most species within the

Terebrantia fit this hypothesis, especially *E. americanus:* its body size measures about 1.3 mm (Morgan 1913), its testes size is about 70 x 20 µm (Chapter 2), and it has an adult lifespan of about 14 d (Krueger et al. 2015a). Additionally, female mating behaviour would also drive the development of prospermatogeny. Females do not re-mate (Chapter 3, Li et al. 2014) and they disperse after mating (Kumm 2002), thus the males´ opportunity to mate is brief. This behavior explains in part also the discrepancy in presumed ability of males to inseminate females (according to Hamiltons 1967 hypothesis), and the observed rate. Damiens and Boivin (2005) account for a similar discrepancy in *Trichogramma evanescens* by the following: (i) if many newly eclosed females are available simultaneously, males might run out of seminal fluid. *Drosophila melanogaster* run out of glandular material after 4 to 5 consecutive matings and fail to transfer sperm (Lefevre and Jonsson 1962). A similar phenomenon is described in *Drosophila silvestris* after two matings (Schwartz 1991). Glandular substances are linked to spermatozoa transfer and protection, as well as implicated in the induction of female refractoriness, reduction of attractiveness, sperm competition, regulation of egg development and induction of ovulation and oviposition (see Chen 1984, Gillot 1988, 2003). A strong decline in availability of seminal fluid with consecutive number of matings was shown but, presumably because of the refractory period of 24 h used in our study, no decline in spermatozoa transfer was observed. A modification of the experimental design might reveal such a connection. (ii) Some males might disperse and search for additional mating opportunities. Because of the practise of monandry in these species (Li et al. 2014), access to virgin females limits reproductive success, resulting in strong competition for mates (Hamilton 1967). Male dispersal may be a strategy to avoid this. (iii) Males might not have an equal capacity to acquire mates, with males of superior quality gaining the majority of mates. Preston (2001) shows that dominant males, which are the most successful in competition, run out of sperm and become ineffective. Presumably, subordinate males might use this niche. Such an alternative sneak-mating tactic by males is known for *Elaphrothrips tuberculatus* (Crespi 1988a). Possibly, this also occurs in *E. americanus* because mate choice by size was found that thus limited access to virgin females (Section 5.3).

Mate choice

Mate choice is not random within this species. Larger males copulate more frequently than smaller ones (Section 5.3). Assortative mating is one of the most common mating strategies in order to maximise individual fertility (Simmons 2001). In the only species of the Terebrantia analysed, *F. occidentalis,* male body size is also an indicator for copulation success (unpublished, mentioned in Terry and Schneider 1993), involving fighting activity for access to mating sites (Terry and Dyreson 1996). Crespi (1986 a,b, 1988 a,b) found a correlation between behavior and size of forelegs in adult male thrips of mycophagous species within the suborder Tubulifera. Guarding males have larger forelegs and protect territories of either reproductive females or oviposition sites, or both, and are more successful in mating before oviposition. In contrast, subordinate, non-guarding males successfully sneak-mate. Additionally, in *Hoplothrips karnyi* larger males are superior fighters (Crespi 1988b), and larger size and weight produce better performance in male-male competitions (Crespi 1989). Males of *E. americanus* guard and defend female pupae against other males (Chapter 6,

Supplementary Material S6). After eclosion, the female presumably mate with the successful defender and more competitive male. In terms of Fisherian model, the female opts for the indirect benefit to her offspring (Fisher 1915, 1930).
However, it was not possible to determine which sex actually discriminates on size. Females can be the choosier sex, allowing only bigger males to mate with them. Or bigger males are just more successful at guarding pupae, at competition with other males and/or in coercion of females, the later phenomenon being widespread among insects (Clutton-Brock and Parker 1995). *Thrips tabaci* females re-mate because of the higher energy loss involved in defence against male harassment (Li et al. 2015). *Limothrips* species practice an extreme form of male coercion: males rape female pupae by rupturing the integument with their outer genitalia (Bournier 1956, Holtmann 1962).

Male emitted pheromones-sex discrimination/ anti aphrodisiac
Besides mate choice on the base of physical characters, chemical cues might also play an important role (Conner et al. 1990, Clark et al. 1997). Chemical signals often vary with body size and were used as a proxy for condition by Bradbury and Vehrencamp (2011). Moth of the genus *Utetheisa* use the amount of male-emitted pheromone to judge male size, in turn an indicator of larval diet quality and value of the nuptial gift provided (Conner et al. 1990). Females of the cockroach *Nauphoeta cinerea* detect the amount and ratio of compounds of male pheromone to select superior mates (Clark et al. 1997). A similar mechanism is known for *Drosophila serrata* (Blows et al. 2004).
Males of many thripine species possess glandular structures on their sternites III/IV to VII/VIII, and in *Echinothrips americanus* there are multiple pore plates (Section 5.1, Morgan 1913, Krueger et al. 2015b). All authors have assumed an intraspecific function in reproduction for these glands, due to their absence in most females (Klocke 1926, Mound 2009) and their structural similarities to pheromone glands in other insects (Pelikan 1951, Bode 1978, Moritz 1997, 2006, Sudo and Tsutsumi 2002, 2003, Shitatani and Tsutsumi 2005, 2006, El-Ghariani and Kirk 2008, Olaniran et al. 2013). Until now, there has been no reliable support for this assumption.
However, the differences in shape, size and arrangement even within one genus might be an indication of a process in species recognition. Interspecific competition for communication channels and, for closely related species, selection for pre-mating reproductive isolation, are the main processes driving the evolution of pheromones involved in species recognition (Roelofs and Cardè 1974, Cardè and Baker 1984, Löfstedt 1993, Johannson and Jones 2007).
Signals used in species recognition are assumed to have evolved from signals with mate recognition or mate assessment function (Blows and Allan 1998). If such a shift in functionality from intraspecific to interspecific recognition has been occurred within thrips, it would explain why we were not able to detect a distinct function in *E. americanus*.
The long-range pheromones of moths (Svennson 1996) and mammals (Ptacek 2000) are presumably evolved under these mechanisms. Within sister taxa quality of pheromones often have dramatic differences in their chemical structure or composition (Roelofs 1995, Coyne and Orr 1997, Frey et al. 1998, Shine et al. 2002). In *Ips* bark beetles, the long-range pheromones are more dissimilar from one another in closely related species, than from more distantly

related species (Symonds and Elgar 2004a,b). Such a phenomenon would also explain the species-specific differences in sternal glands, for instance within *Limothrips* genus, where *L. cerealium* possess small sternal glands and their sister species *L. denticornis* does not (Mound 2009).

To investigate the function of this glandular area, several different approaches were used. We sought to identify the secreted substances by using GC-MS (Chapter 6), histochemical methods (Section 5.2), and by a behavioral assay (Section 5.3).

Although with GC-MS we were unable to find evidence of any pheromonal substances from the abdominal parts, the head-thorax region emits substances that function in reproduction.

Two dibasic esters (dimethyl glutarate, DBE-5 and dimethyl adipate DBE-6) were identified that seem to play an important role in the male mating strategy in *E. americanus*. This species has adopted a system of precopulatory (DBE-5) and postcopulatory (DBE-6) behavior that minimize sexual competition within this mating system that involves high local mate competition.

DBE-5 appears to act as a short-range male-recognition pheromone, similar to 7-methyltricosane in *Frankliniella occidentalis* (Thysanoptera: Thripidae) (Olaniran et al. 2013). Apart from *F. occidentalis,* male-produced contact compounds have been demonstrated only in a few species of Diptera (Carlson and Schlein 1991, Nelson et al. 1981, Lacaille et al. 2007), Blattodea (Takahashi and Fukui 1983) and ants (Smith et al. 2014). Females and males of *E. americanus* seem to be able to discriminate between the sexes, probably due to the absence or presence of DBE-5. As mentioned before, males have frequently been observed guarding female pupae and mating with them immediately after female eclosion (Chapter 6, Supplementary Material S6). *E. americanus* females mate only once, or re-mate with a very low frequency (Li et al. 2014, Krueger et al. 2015a). Availability of receptive females is the limiting factor in reproduction. Thus, this substance plays an important role in precopulatory mate guarding and monopolising of receptive females, a trait often found in species with high local mate competition (Simmons and Siva-Jothy 1998).

The second substance, DBE-6, was avoided by other *E. americanus* males, regardless of whether it was applied on prepared filter discs or on virgin females. This male-emitted substance was also detected on mated females. Therefore, these dibasic esters seem to have anti-aphrodisiac effects. They mask receptive females during copulation to limit take-overs from other males, and mark females with their mating status thus avoiding sexual competition (reviewed in Simmons 2001). Furthermore, the substances prevent females from being subject to further male harassment, which is known to reduce fertility and longevity in this species (Krueger et al. 2015a). Pheromones transferred from males to females help to signal the female mating status (Thomas 2011) and may work as anti-aphrodisiacs (Ayasse et al. 2001). This phenomenon is known in many arthropods (Happ 1969, Gilbert 1976, Tompkins and Hall 1981a,b, Kukuk 1985, Andersson et al. 2000, Schulz et al. 2008, Brent and Byers 2011), but is described here for the first time for a thysanopteran species.

Influence of mating on parents and their offspring - feedback system

Notwithstanding the diversity of male mating strategies in *E. americanus*, females are flexible in their response to mating and to male presence (Chapter 4). Males and females benefit from a single mating without further harassment. Repeated courtships and harassment by males

reduce longevity and fertility in both sexes, as is corroborated by studies on several other insects (Magurran and Seghers 1994, Rowe et al 1994, Cordts and Patridge 1996, Mappes et al. 1996, Clutton-Brock and Langley 1997, Watson et al. 1998, Kotiaho 2000, 2001, Dadda et al. 2005, Croft et al. 2006). Surprisingly, virgin males had the lowest longevity. A similar phenomenon is described by Cordts and Patridge (1996) where *D. melanogaster* males kept with females which could not mate died sooner than males kept with females which could mate. In contrast, Li et al. (2014) reported a higher longevity among virgin females. However, the extreme differences in longevity of her study and ours may distort a comparison.

Nevertheless, females are able to influence the sex ratio of their offspring. Females with just 24h male contact produce significant more male offspring. Sperm limitation of females was excluded as the reason, because sex ratio of offspring did not change with the time elapsed after the last mating (Chapter 4, Krueger et al. 2015a).

In terms of sex allocation theory, a female endeavours to maximise her fitness. Therefore, she will produce enough sons to fertilize all of her daughters (Hamilton 1967, Crespi 1993, see also Chapter 7-sperm limitation). Furthermore, she will increase the proportion of males under unfavourable environmental factors, such as a decline in food availability or change in temperature, to maximise the advantages of sexual reproduction (Wrensch 1993), or in our example, a low male density. This suggests that a female has control over the sex ratio. Haplo-diploid organisms may generally control the sex ratio by controlling sperm access to eggs and fertilization and have different sex allocation strategies in different environmental conditions (Clausen 1939, Charnov 1982, King 1987, Antolin 1993, Wrensch and Ebbert 1993, Godfray 1994, Hoddle et al. 2000, Kumm 2002).

As well as changes in the sex ratio, developmental time of progeny differed significantly between the treatments. Male and female developmental times were equal if their mothers were associated permanently with a male, but a significant decrease occurred within progeny of virgin or mated females. Differences in development time of progeny seem to be influenced more by maternal stress than by mating-status. In situations where the number of males in a population is low, mated females or virgin females have a higher probability of survival. They produce more offspring with a higher proportion of males under such conditions. Additionally, postembryonic development of these descendants is significantly faster. Therefore, this could produce a feedback-system, where mother-son mating occurs to ensure persistence or establishment of a new population. Preliminary trials show that a short overlap of mother and son generation is possible, despite the short longevity, and mother-son matings result in female offspring. Such a negative feedback system is known for several species (Borgia 1980). The assumption that arrhenotokous species represent a lower infestation risk should not be underestimated.

Comparison of different mating strategies in Terebrantia and Tubulifera

Altogether, a paradox in life style and mating strategy between Terebrantia and Tubulifera can be seen. The more stable environment and long-lived breeding sites of some fungus-feeding Tubulifera support the complex colonial and complex sub/eusocial behaviors (Evans 1977,

Terry 1997). Cooperation in carrying the young, overlap of at least two generations, and division of labor are known only from tubuliferan species (Wilson 1971, Andersson 1984, Crespi 1986a, b, 1988a,b,c,d, 1993, Crespi and Yanega 1995). Males and females seem to re-mate frequently (Ananthakrishnan 1990, Crespi 1986a,b, 1988a,b). This behavior is promoted by the morphological adaptations of frequent synspermatogeny of males and large sac-like spermathecae in females. Synspermatogeny, the possibility to produce sperm over their entire life, involves the requirement of larger amounts of sperm for multiple matings. Additionally, the longer lifespan of some tubuliferan species (Crespi et al. 2004) is likely to require a constant sperm supply over their whole lifetime.

Many flower-living Thripidae species are more closely adapted to rapid reproduction and distribution. Colonial lifestyles or division of labor are unknown among such thrips. Only functional and temporally limited aggregations are known among some Thripidae species, such as *Frankliniella occidentalis, F. schultzei, Thrips fuscipennis, T. major, T. atratus* or *Megalurothrips sjostedti*, which might aid in finding mates or food sources (Kirk 1985, Terry and Gardner 1990, Milne et al. 2002, Niassy et al. 2016). Males exhibit prospermatogeny, which constitutes an advantage in their short lifespan and the frequently performed protandry. Females have a small and simply constructed spermatheca. Most of the examined species mate only once or with a very low frequency (except *T. tabaci,* Li et al. 2015). Thelytokous species are known only within this suborder, regardless of whether it is obligate or microorganism-initiated. Additionally, some arrhenotokous species have developed thelytokous strains (e.g. *Thrips tabaci*: Kobayashi and Hasegawa 2012*, Aptinothrips rufus:* Van der Kooi and Schwander 2014).

While males of some Tubulifera play an important part in their complex reproductive behavior, terebrantian males seem to play just a minor role and their function is more or less suppressed.

Even if males were still needed for fertilization in several species, the example here of *E. americanus* shows that the species has evolved adaptive strategies to enhance their reproductive success. Therefore, the damage potential of this supposedly less harmful species (compared to *F. occidentalis* for instance) should not be underestimated. The biology of animals as a whole should always be investigated.

References

Ananthakrishnan TN, 1990. The reproductive biology of thrips. Indira Publishing House, Oak Park, Michigan, USA.

Andersson M, 1984. The evolution of eusociality. Ann Rev Ecol Syst 15, 165-189.

Andersson J, Borg-Karlson AK, Wiklund C, 2000. Sexual cooperation and conflict in butterflies: a male transferred anti-aphrodisiac reduces harassment of recently mated females. Proc Roy Soc B 267, 1271–1275.

Antolin MF, 1993. Genetics of biased sex ratios in subdivided populations: models, assumptions and evidence. Oxf Surv Evol Biol 9, 239-281.

Ayasse M, Paxton RJ, Tengö J, 2001. Mating behavior and chemical communication in the order Hymenoptera. Annu Rev Entomol 46, 31–78.

Blows MW, Allan RA, 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. Am Nat 152, 826-837.

Blows MW, Chenoweth SF, Hine E, 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Amer Nat 163, 329-340.

Bode W, 1975. Der Ovipositor und die weiblichen Geschlechtswege der Thripiden (Thysanoptera, Terebrantia). Z Morphol Ökol Tiere 81, 1-53.

Bode W, 1978. Ultrastructure of the sternal glands in *Thrips validus* Uzel (Thysanoptera, Terebrantia). Zoomorphologie 90, 53-65.

Boivin G, Jacob S, Damiens D, 2005. Spermatogeny as a life-history index in parasitoid wasps. Oecologia 143, 198-202.

Borgia G, 1980. Evolution of haplodiploidy: Models for inbred and outbred systems. Theor Popul Biol 17, 103-128.

Bournier A, 1956. Contribution à l´ étude de la parthénogèse des Thysanoptères et sa cytology. Arch Zool Exp 93, 220-317.

Bradbury JW, Vehrencamp SL, 2011. Principals of Animal communication. Sinauer Associates, Sunderland Massachusetts, USA.

Brent CS, Byers JA, 2011. Female attractiveness modulated by a male-derived antiaphrodisiac pheromone in a plant bug. Anim Behav 82, 937-943.

Cardè RT, Baker TC, 1984. Sexual communication with pheromones. In: Chemical Ecology of Insects. Ed. by Bell WJ, Cardè RT, Chapman and Hall, London, UK, 355-383.

Carlson DA, Schlein Y, 1991. Unusual polymethyl alkenes in tsetse flies acting as abstinon in *Glossina morsitans*. J Chem Ecol 17, 267-284.

Charnov EL, 1982. The theory of sex allocation, Princeton University Press, New York, USA.

Chen PS, 1984. The functional morphology and biochemistry of insect male accessory glands and their secretions. Ann Rev Entomol 29, 233-255.

Clark DC, DeBano SJ, Moore AJ, 1997. The influence of environmental quality on sexual selection in *Nauphoeta cinerea* (Dictyoptera: Blaberidae). Behav Ecol 8, 46-53.

Clausen C, 1939. The effect of host size upon the sex ratio of Hymenopterous parasites and its relation to methods or rearing and colonization. J NY Entomol Soc 47, 1-9.

Clutton-Brock T, Parker GA, 1995. Sexual coercion in animal societies. Anim Behav 49, 1345-1365.

Clutton-Brock T, Langley P, 1997. Persistent courtship reduces male and female longevity in captive tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). Behav Ecol 8, 392–395.

Conner WE, Roach B, Benedict E, Meinwald J, Eisner T, 1990. Courtship pheromone production and body size as correlates of larval diet in males of the arctiid moth, *Utetheisa-ornatrix*. J Chem Ecol 16, 543-552.

Cordts R, Patridge L, 1996. Courtship reduces longevity of male *Drosophila melanogaster*. Anim Behav 52, 269-278.

Coyne JA, Orr HA, 1997. Patterns of speciation in *Drosophila* revisited. Evolution 51, 295-303.

Crespi BJ, 1986a. Territoriality and fighting in a colonial thrips, *Hoplothrips pedicularis*, and sexual dimorphism in Thysanoptera. Ecol Entomol 11, 119-130.

Crespi BJ, 1986b. Size assessment and alternative fighting tactics in *Elaphorthrips tuberculatus* (Insecta: Thysanoptera). Anim Behav 34, 1324-1335.

Crespi BJ, 1988a. Alternative mating tactics in thrips: effects of sex ratio variation and body size. Am Midl Nat 122, 142-150.

Crespi BJ, 1988b. Risks and benefits from lethal male fighting in colonial *Hoplothrips karnyi* (Insecta: Thysanoptera). Behav Ecol Sociobiol 22, 293-301.

Crespi BJ, 1988c. Sex-ratio selection in bivoltine thrips. I. Conditional sex ratio manipulation and fitness variation. Evolution 42, 1199-1211.

Crespi BJ, 1988d. Adaption, compromise and constraint: the development, morphometrics, and behavioural basis of a fighter-flier polymorphism in male *Hoplothrips karnyi* (Insecta: Thysanoptera). Behav Ecol Sociobiol 23, 93-104.

Crespi BJ, 1989. Sexual selection and assortative mating in subdivided populations of the thrips *Elaphrothrips tuberculatus* (Insecta: Thysanoptera). Ethology 83, 265-278.

Crespi BJ, 1993. Sex Allocation Ratio Selection in Thysanoptera. In: Evolution and diversity of sex ratio in insects and mites. Ed. by Wrensch DL and Ebbert MA, Chapman & Hall, New York, USA, 214–234.

Crespi BJ, Yanega D, 1995. The definition of eusociality. Behav Ecol 6, 109-115.

Crespi BJ, Morris DC, Mound LA, 2004. Evolution of ecological and behavioural diversity. Australian acacia thrips as model organisms- Australian Biological Resource Study and Australian National Insect Collection, CSIRO, Canberra, Australia.

Croft DP, Morrell LJ, Wade AS, Piyapong C, Ioannou CC, Dyer JRG, Chapman BB, Wong Y, Krause J, 2006. Predation risk as a driving force for sexual segregation: a cross-population comparison. Am Nat 167, 867-878.

Dadda M, Pilastro A, Bisazza A, 2005. Male sexual harassment and female schooling behaviour in the eastern mosquitofish. Anim Behav 70, 463-471.

Dallai R, Del Bene G, Lupetti P, 1997. Fine structure of the male reproductive organs of the Western Flower Thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Int J Insect Morphol & Embryol 26, 97-112.

Damiens D, Boivin G, 2005. Male reproductive strategy in *Trichogramma evanescens*: sperm production and allocation to females. Physiol Entomol 30, 241-247.

De Marzo L, 2005. Lunghezza degli spermatozoi in *Megathrips inermis* Priesner (Thysanoptera. Phlaeothripidae). Boll Zool Agrar Bachic Ser II 37, 179-183.

El-Ghariani IM, Kirk WDJ, 2008. The structure of the male sternal glands of the western flower thrips, *Frankliniella occidentalis* (Pergande). Acta Phytopathol Entomol Hung 43, 257-266.

Evans HE, 1977. Extrinsic and intrinsic factors in the evolution of insect sociality. Bioscience 27, 613-616.

Finlayson LH, 1949. The life-history and anatomy of *Leptinotarsus patruelis* Pearman (Psocoptera-Atropidae). Proc zool Soc Lond 119, 301-323.

Fisher, R., 1915. The evolution of sexual preferences. Eugen Rev 7, 184-192.

Fisher, R., 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford, UK.

Frey MA, Lonsdale DJ, Snell TW, 1998. The influence of contact chemical signals on mate recognition in a harpacticoid copepod. Phil Trans R Soc B 353, 745-751.

Gerber GH, 1970. Evolution on the methods of spermatophore formation in pterygotan insects. Can Entomol 102, 358-362.

Gilbert L, 1976. Postmating female odor in *Heliconius* butterflies: a male-contributed antiaphrodisiac? Science 193, 419–420.

Gillot C, 1988. Accessory Sex Glands. In: Arthropoda-Insecta Reproductive Biology of Invertebrates, Vol 3. Ed. by Adiyodi KG, Adiyodi RD, Wiley, New York, USA, 356-471.

Gillot C, 2003. Male accessory gland secretions: Modulators of female reproductive physiology and behaviour. Ann Rev Entomol 48, 163-184.

Godfray HCJ, 1994. Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, New York, USA.

Hamilton WD, 1967. Extraordinary Sex Ratios. Science 156,477-488.

Happ GM, 1969. Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor* L.. Nature 222, 180–181.

Heming B, 1970a. Postembryonic development of the female reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc Publs Entomol Soc Amer 7, 197-234.

Heming B, 1970b. Postembryonic development of the male reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). Misc Publs Entomol Soc Amer 7, 237-272.

Heming B, 1995. History of the Germ Line in Male and Female Thrips. In: Thrips Biology and Management. Ed. by Parker BL, Skinner M, Lewis T, Plenum Press, New York, USA, 505-535.

Hoddle MS, Robinson L, Drescher K, Jones J, 2000. Developmental and reproductive biology of a predatory *Franklinothrips n. sp*. (Thysanoptera: Aeolothripidae). Biol Control 18, 27-38.

Holtmann H, 1962. Untersuchungen zur Biologie der Getreide-Thysanopteran (Teil 1). Z angew Entomol 51, 1-41.

Johannson BJ, Jones TM, 2007. The role of chemical communication in mate choice. Biol Rev 82, 265-289.

King B, 1987. Offspring sex ratios in parasitoid wasps. Q Rev Biol 62, 367-396.

Kirk W, 1985. Aggregation and mating of thrips in flowers of *Calystegia sepium*. Ecol Entomol 10, 433-440.

Klier E, 1956. Zur Konstructsionsmorphologie des männlichen Geschlechtsapparates der Psocopteren. Zool Jb Anal 75, 207-286.

Klocke F, 1926. Beiträge zur Anatomie und Histologie der Thysanopteren. Z wiss Zool Abt A 128, 1-36.

Kobayashi K, Hasegawa E, 2012. Discrimination of reproductive forms of *Thrips tabaci* (Thysanoptera: Thripidae) by PCR with sequence specific primers. J Econ Entomol 105, 555-559.

Kotiaho JS, 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. Behav Ecol Sociobiol 48, 188-194.

Kotiaho JS, 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. Biol Rev 76, 365-376.

Kukuk P, 1985. Evidence for an antiaphrodisiac in the sweat bee *Lasioglossum (Dialictus) zephyrum*. Science 227, 656–657.

Kumm S, 2002. Reproduction, progenesis and embryogenesis of thrips (Thysanoptera, Insecta). PhD thesis. Martin-Luther University Halle-Wittenberg, Halle, Germany.

Krueger S, Mound LA, Moritz GB, 2015a. Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* MORGAN 1913 (Thysanoptera). J Appl Entomol, doi: 10.1111/jen.12280

Krueger S, Subramanian S, Niassy S, Moritz G, 2015b. Sternal gland structures in males of bean flower thrips, *Megalurothrips sjostedti,* and Poinsettia thrips, *Echinothrips americanus*, in comparison with those of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Arth Struct & Dev 44, 455-467.

Lacaille F, Hiroi M, Twele R, Inoshita T, Umemoto D, Maniere G, Marion-Poll F, Ozaki M, Francke W, Cobb M, Everaerts C, Tamimura F, Ferveur JF, 2007. An inhibitory sex pheromone tastes bitter for *Drosophila* males. PLOS ONE 2:e661

Lefevre G, Jonsson UB, 1962. Sperm transfer, storage and displacement, and utilization in *Drosophila melanogaster*. Genetics 47, 1719-1736.

Li X-W, Jiang H-X, Zhang X-C, Shelton AM, Feng J-N, 2014. Post-mating interactions and their effects on fitness of female and male *Echinothrips americanus* (Thysanoptera: Thripidae), a new insect pest in China. PLOS ONE, doi: 10.1371/journal.pone.0087725

Li X-W, Fail J, Shelton AM, 2015. Female multiple matings and male harassment and their effects on fitness of arrhenotokous *Thrips tabaci* (Thysanoptera: Thripinae).Behav Ecol Sociobiol, 69, 1585-1595.

Löfstedt C, 1993. Moth pheromone genetics and evolution. Phil Trans R Soc B 340, 167-177.

Magurran AE, Seghers BH, 1994. A cost of sexual harassment in the guppy, *Poecilia reticulata.* Proc R. Soc B 258, 89-92.

Mappes J, Alatalo RV, Kotiaho J, Parri S, 1996. Viability costs of condition-dependent sexual male display in a drumming wolf spider. Proc R Soc B 263, 785-789.

Milne M, Walter GH, Milne JR, 2002. Mating aggregations and mating success in the flower thrips, *Frankliniella schultzei* (Thysanoptera: Thripidae), and a possible role for pheromones. J Insect Behav 15, 351-368.

Milne M, Walter GH, Milne JR, 2007. Mating Behavior and Species Status of Host-Associated Populations of the Polyphagous Thrips, *Frankliniella schultzei*. J Insect Behav 20, 331-346.

Morgan AC, 1913. New genera and species of Thysanoptera, with notes on distribution and food plants. Proc US Nat Mus 46, 1-56.

Moritz G, 1982. Zur Morphologie und Anatomie des Fransenflüglers *Aeolothrips intermedius* Bagnall. 3. Mitteilung: Das Abdomen. Zool Jb Anat 108, 239-340.

Moritz G, 1989. Die Ontogenese der Thysanoptera (Insecta) unter besonderer Berücksichtigung des Fransenflüglers *Hercinothrips femoralis* (O.M. Reuter 1891) (Thysanoptera, Thripidae, Panchaetothripinae). IV. Mitteilung: Imago, Abdomen. Zool Jb Anat 119, 157-217.

Moritz G, 1997. Structure, growth and development. In: Thrips as Crop Pests. Ed. by Lewis T, CAB International Oxon, New York, USA, 15-63.

Moritz G, 2006. Thripse - Fransenflügler, Thysanoptera., Westarp-Verlag, Hohenwarsleben, Germany.

Mound LA, 2009. Sternal pore plates (glandular areas) of male Thripidae (Thysanoptera). Zootaxa 2129, 29-46.

Nelson DR, Dillwith JW, Blomquist GJ, 1981. Cuticular hydrocarbons of the house fly, *Musca domestica*. Insect Biochem 11, 187-197.

Niassy S, Ekesi S, Maniania NK, Moritz GB, Kogel WJ de, Subramanian S, 2016. Active aggregation among sexes in bean flower thrips (*Megalurothrips sjostedti*) on cowpea (*Vigna unguiculata*). Entomol Exp Appl 158, 17-24.

Olaniran OA, Sudhakar AVS, Drijfhout FP, Dublon IAN, Hall DR, Hamilton JGC, Kirk WDJ, 2013. A male-predominant cuticular hydrocarbon, 7-methyltricosane, is used as a contact pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem Ecol 39, 559-568.

Paccagnini E, Mencarelli C, Mercati D, Afzelius BA, Dallai R, 2007. Ultrastructural Analysis of the Aberrant Axoneme Morphogenesis in Thrips (Thysanoptera, Insecta). Cell Motil Cytoskeleton 64, 645-661.

Paccagnini E, Lupetti P, Afzelius BA, Dallai R, 2009. New findings on sperm Ultrastructure in thrips (Thysanoptera, Insecta). Arth Struct & Dev 38, 70-83.

Pelikan J, 1951. On carnation thrips, *Taeniothrips dianthi* Priesner. Folia Entomol 14, 5-38.

Phillips DM, 1970. Insect sperm: their structure and morphogenesis. J Cell Biol 44, 243-277.

Preston BT, Stevenson IR, Pemberton JM, Wilson K, 2001. Dominant rams lose out by sperm depletion. Nature 409, 681-682.

Ptacek MB, 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. Behav Process 51, 111-134.

Rafter MA, Walter GH, 2013. Mate Recognition in the South African Citrus Thrips *Scirtothrips aurantii* (Faure) and Cross-Mating Tests with Populations from Australia and South Africa. J Insect Behav 26, 780-795.

Roelofs WL, 1995. The chemistry of sex attraction. In: Chemical Ecology: the Chemistry of Biotic Interaction. Ed. by Eisner T, Meinwald J, National Academy Press, Washington DC, USA, 103-117.

Roelofs WL, Cardè RT, 1974. Sex pheromones in the reproductive isolation of lepidopterous species. In: Pheromones. Ed. by Birch MC, North Holland Publishing Company, Amsterdam, Netherlands, 96-114.

Rowe L, Arnqvist G, Sih A, Krupa J, 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. Trends Ecol Evol 9, 289-293.

Schulz S, Estrada C, Yildizhan S, Boppè M, Gilbert LE, 2008. An antiaphrodisiac in *Heliconius melpomene* butterflies. J Chem Ecol 34, 82-93.

Schwartz JM, 1991. Effect of sexual experience on male mating success in *Drosophila silvestris*. Anim Behav 42, 1017-1019.

Shine R, Reed RN, Shetty S, Lemaster M, Mason RT, 2002. Reproductive isolating mechanisms between two sympatric sibling species of sea snakes. Evolution 56, 1655-1662.

Shitatani S, Tsutsumi T, 2005. Ultrastructure of sternal glands in *Tenothrips frici* (Uzel) (Thysanoptera: Terebrantia). Proc Arthropod Embryol Soc Jpn 40, 35-39.

Shitatani S, Tsutsumi T, 2006. Ultrastructure of sternal glands in *Hydatothrips* (*Hydatothrips*) *abdominalis* (Kurosawa) and *H.* (*Neohydatothrips*) *gracilicornis* (Williams) (Thysanoptera: Terebrantia). Proc Arthropod Embryol Soc Jpn 41, 59-65.

Simmons LW, 2001. Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton, USA.

Simmons LW, Siva-Jothy MT, 1998. Sperm competition in insects: mechanisms and the potential for selection. In: Sperm competition and sexual selection. Ed. by Birkhead TR, Møller AP. Academic Press, San Diego, USA, 341-434.

Smith AA, Vanderpool W, Millar JG, Hanks LM, Suarez AV, 2014. Conserved male-specific cuticular hydrocarbon patterns in the trap-jaw ant *Odontomachus brunneus*. Chemoecology 24, 29-34.

Sudo M, Tsutsumi T, 2002. Ultrastructure of sternal glands in two thripine thrips and one plaeothripinae thrips (Thysanoptera: Insecta). Proc Arthropod Embryol Soc Jpn 37, 35-41.

Sudo M, Tsutsumi T, 2003. Ultrastructure of the primordia of sternal glands in the second-instar pupal stage of *Frankliniella intonsa* (Thysanoptera: Insecta). Proc Arthropod Embryol Soc Jpn 38, 27-29.

Svensson M, 1996. Sexual selection in moth: the role of chemical communication. Biol Rev Camb Philos Soc 71, 113-135.

Symonds MRE, Elgar MA, 2004a. The mode of pheromone evolution: evidence from bark beetles. Proc R Soc B 271, 839-846.

Symonds MRE, Elgar MA, 2004b. Species overlap, speciation and the evolution of aggregation pheromones in bark beetles. Ecol Lett 7, 202-212.

Takahashi S, Fukui M, 1983. Studies on the mating behavior of the cockroach *Nauphoeta cinerea* (Oliver) (Dictyoptera: Blaberidae). III. Isolation and identification of intermale recognition pheromone. Appl Ent Zool 18, 351-356.

Terry I, 1997. Host Selection, Communication and Reproductive Behaviour. In: Thrips as Crop Pests. Ed. by Lewis T, CAB International Oxon, New York, USA, 65-118.

Terry I, Gardner D, 1990 Male mating swarms in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). J Insect Behav 3, 133-141.

Terry I, Schneider M, 1993. Copulatory behaviour and mating frequency of the western flower thrips, *Frankliniella occidentalis* (Insecta, Thysanoptera). J Pure Appl Zool 4, 339-354.

Terry I, Dyreson E, 1996. Behavior of *Frankliniella occidentalis* (Thysanoptera: Thripidae) within aggregations, and morphometric correlates of fighting. Ann Entomol Soc Am 89, 589-602.

Teuber J, 2011. Spermienultrastruktur von arrhenotoken und thelytoken Thysanoptera-Arten (Insecta). Diploma-thesis. Martin-Luther University Halle-Wittenberg, Halle, Germany.

Thomas ML, 2011. Detection of female mating status using chemical signals and cues. Biol Rev 86, 1-13.

Tompkins L, Hall JC, 1981a. *Drosophila* males produce a pheromone which inhibits courtship. Z Naturforsch C 36, 694-696.

Tompkins L, Hall JC, 1981b. The different effects on courtship of volatile compounds from mated and virgin *Drosophila* females. J Insect Physiol 27, 17–21.

Van der Kooi C, Schwander T, 2014. Evolution of asexuality via different mechanisms in grass thrips (Thysanoptera: Aptinothrips). Evolution 68, 1883-1893.

Watson PJ, Arnqvist G, Stallmann RR, 1998. Sexual conflict and the energetic costs of mating and mate choice in water striders. Am Nat 151, 46-58.

Wilson CR, 1971. The insect societies. Harvard University Press, Cambridge, Massachusetts, USA.

Wrensch DL, 1993. Evolutionary flexibility through haploid males or how chance favors the prepared genome. In: Evolution and diversity of sex ratio in insects and mites. Ed. by Wrensch DL, Ebbert MA, Chapman and Hall, New York, USA, 118–149.

Wrensch DL, Ebbert MA, 1993. Evolution and diversity of sex ratio in insects and mites. Chapman and Hall, New York, USA.

CHAPTER 8

SUMMARY

Despite the damage inflicted on agricultural products by some thrips species, our knowledge of their biology remains incomplete. Any useful and effective control method necessitates a comprehensive understanding of the biology of a species, especially of its reproduction. However, no previous studies address the relationships between morphology, mating behavior, mating strategies, and their possible adaptations to different environmental conditions. This thesis reports on various aspects of reproductive behaviour, from the morphology of reproductive organs to their function and examines their reproductive strategies. A special focus is placed on the species *Echinothrips americanus,* a widely-distributed, potential greenhouse pest in temperate climates.

Morphology

The morphology of the inner and outer genitalia of the target species, *Echinothrips americanus,* was analyzed with the help of light- and electron microscopy. The main structures of males and females accord with those of other terebrantian species. Additionally, representative species of a possible sister group, the Hemiptera (*Pyrrhocoris apterus, Campyloneura virgula, Arboridia ribauti*), were also examined in order to compare the genital morphology and use of the genital tract.

Reproductive behavior

Mating behavior of *E. americanus* encompasses the typical thysanopteran phases of precopulation, copulation and postcopulation. After an initial head to head or head to abdomen/thorax contact, receptive females lower their abdomen, at which stage the male mounts female´s back. The male twists his abdomen underneath that of the female and insert his phallus (with parameres) into the vagina of the female, where the phallotheca and endotheca are evaginated; the paraphyses are located near the entrance of the spermathecal duct. Within the first minutes after insertion, only accessory gland material is transferred. After 3 minutes the amount of spermatozoa increases. A filled spermatheca is seen some 7 minutes after the start of copulation. Females are monandric and reject any further mating attempt with the same or a different male. Males mate with several females, and are able to inseminate ten females without a decline in the transferred amount of spermatozoa, but with a significant decrease in quantity of accessory gland material transferred.

Offspring sex ratio and development

The attribute of having mated, and even the presence of the opposite sex, have a significant effect on the lives of both sexes. Females permanently reared with a male had a lower probability of survival, a lower fecundity and a lower male sex ratio among their offspring compared to mated, but individually reared females. The increased male sex ratio of offspring in mated, but individually reared mothers was not caused by sperm limitation of the just once

mated mothers. These females were able to produce female offspring over their entire lifetime. Virgin females produced only male offspring.
In addition, the presence of a male had a significant effect on the developmental time of a mother´s offspring. Developmental time of offspring of a mated, but individually reared or virgin mother is significantly shorter, compared to offspring of permanently associated mothers.
In response to conditions of a lower male population density, *Echinothrips americanus* appears to have evolved a feedback system, responding with increased female fecundity, a more (or exclusively) male-biased sex ratio, and a faster development of offspring. This means that newly hatched males effect mother-son matings, fertilizing their mothers, and thereby ensuring persistence or establishment of a new population. Therefore, the generally assumed lower infestation risk of arrhenotokous species, compared to thelytokous species, needs to be carefully reconsidered.

Sternal glands
Three general forms of sternal glands are described, from three economically important species: *Frankliniella occidentalis, Megalurothrips sjostedti* and *Echinothrips americanus*. The most frequent type is the c-shaped gland arrangement around a common extracellular cavity with an oval pore plate providing the extrusion area (*F. occidentalis)*. Some species are exceptional, with multiple pore plates on each sternite. *E. americanus* has up to 100 small pore plates that are observable under light microscope. Each of these small areas is supplied with its own gland cell and extracellular cavity, and is thus independent pore plate (multiple pore plate-type). The morphological structure of this type is described here for the first time. In contrast, an extreme reduction is observed in *M. sjostedti*, where just a small pore is present with a few associated cells.
In addition to the structural differences in the sternal glands of *F. occidentalis* and *E. americanus,* their histochemistry differs. *E. americanus* has large amounts of lipids in the reservoir and gland cells, and proteins and mucins in the reservoir. In contrast in *F. occidentalis* mainly protein structures were detected in the gland cells. The mating status of both species plays just a minor role determining the histochemical substances presence in the sternal glands.
To elucidate the possible function of sternal glands in assortative mating in *E. americanus*, individuals were measured and analyzed in relation to their copulation success. A mechanical obstruction of the pore plates should give indications of a possible sex pheromonal function. Whereas a large body size, especially pronotum length, distance between 1st and 2nd coxa and total sternite area, have a significant positive correlation with copulation success, but the shape of sternal glands does not. Also the mechanical obstruction had no significant effect on copulation success in terms of a possible secreted mate choice pheromone, nor as an anti-aphrodisiac.

Male emitted pheromones
Males of *E. americanus* produce pheromonal substances. Using GC-MS we surprisingly identified two dibasic esters in head-thorax samples, but not in samples from the abdomen. Both esters play a typical role in reproduction. Dimethyl glutarate (DBE-5) appears to act as a

short range male recognition pheromone. It induced abdomen wagging and raising in males and females, a typical behavior when encountering a male. Dimethyl adipate (DBE-6) produces an avoidance reaction by males. It can be detected also on mated females, but not on virgin females. Both substances applied to virgin females caused avoidance by males. The ecological significance of both substances was discussed.

This study provides comprehensive information about the reproduction strategy, and represents useful information concerning how populations of Thripidae-species are able to increase rapidly and adaptability to environmental-, as well as population-dependant factors.

We recommend that further studies be conducted focusing on similar integrated analyses of morphology, behavior and developmental processes to understand biology. This could lead to development of individual and more effective management and control of these invasive species.

ZUSAMMENFASSUNG

Trotz der Schadwirkung einiger Thrips-Arten an landwirtschaftlichen Produkten, ist unser Wissen über die Biologie dieser Tiere noch immer lückenhaft. Eine sinnvolle und effektive Bekämpfung erfordert ganzheitliches Wissen über die Tiere, insbesondere über deren Reproduktion. Bisher gibt es keine Studie, welche auf den Zusammenhang der morphologischen Grundlagen, des Kopulationsverhaltens, der Reproduktionsstrategie und deren Anpassungsfähigkeit an verschiedene Umweltbedingungen abzielt. Diese Arbeit befasst sich mit den unterschiedlichen Aspekten des Reproduktionsverhaltens und zeigt die daraus resultierenden Reproduktionsstrategien auf. Der besondere Fokus liegt dabei auf der Art *Echinothrips americanus*, eine weltweit verbreitete Art, vor allem in Gewächshäusern, mit möglichem Schadpotential.

Morphologie

Die Morphologie der inneren und äußeren Genitalorgane von *E. americanus* wurde mittels licht- und elektronenmikroskopischer Methoden untersucht. Die Strukturen der Männchen und Weibchen entsprechen dabei denen anderer terebranten Arten. Zusätzlich wurden Vertreter der möglichen Schwestergruppe Hemiptera (*Pyrrhocoris apterus, Campyloneura virgula, Arboridia ribauti*) morphologisch untersucht und hinsichtlich Ähnlichkeiten und Veränderungen des Genitaltraktes miteinander verglichen.

Reproduktionsverhalten

Das Kopulationsverhalten besteht aus den für Thysanopteren typischen Phasen von Prekopulation, Kopulation und Postkopulation. Nach einer initialen Phase des Kontakts von Kopf-an-Kopf oder Kopf-an-Abdomen/Thorax, verringert das rezeptive Weibchen die Höhe seines Abdomens, während das Männchen aufsteigt. Das Männchen krümmt sein Abdomen unter das des Weibchens und führt den Phallus mitsamt der Parameren in die Vagina des Weibchens ein, wo Phallotheca und Endotheca ausgestülpt werden. Die Paraphysen kommen am Übergang zum Spermathekengang zu liegen. In den ersten Minuten nach der Insertion wird nur akzessorisches Drüsensekret übertragen. Nach 3 min nimmt die Menge der übertragenen Spermien zu. Eine gefüllte Spermathek ist erstmals 7 min nach Kopulationsbeginn zu erkennen. Begattete Weibchen verhalten sich monandrisch und lehnen jede weitere Kopulation mit dem gleichen, wie auch mit anderen Männchen ab. Männchen verpaaren sich mit mehreren Weibchen und sind in der Lage 10 Weibchen zu inseminieren, ohne Einbußen in der übertragenden Menge an Spermien, aber mit abnehmender Menge an Drüsensekret.

Geschlechterverhältnis und Entwicklung der Nachkommen

Kopulation und die Anwesenheit des anderen Geschlechts hat einen signifikanten Einfluss auf beide Geschlechter. Weibchen, welche permanent mit einem Männchen gehalten wurden, hatten eine geringere Überlebenswahrscheinlichkeit, eine geringere Fekundität, sowie bei den Nachkommen ein geringeres Geschlechterverhältnis der Männchen, im Vergleich zu Weibchen, welche zwar verpaart, aber individuell gehalten wurden. Der höhere Anteil der Männchen bei den zuletzt genannten Weibchen kann dabei nicht mit einer möglichen

Spermienlimitierung der Weibchen, aufgrund der einmaligen Möglichkeit zur Kopulation, erklärt werden. Diese Weibchen waren in der Lage über ihre gesamte Lebenszeit weibliche Nachkommen zu produzieren.
Außerdem hat die Anwesenheit der Männchen einen signifikanten Einfluss auf die Entwicklungszeit der Nachkommen. Nachkommen von verpaarten, individuell gehaltenen oder jungfräulichen Müttern entwickeln sich schneller im Vergleich mit Nachkommen verpaarter und permanent assoziierter Mütter.
In Testbedingungen mit einer geringen Männchendichte zeigt *E. americanus* ein Feedback-System, bei dem Weibchen eine höhere Überlebensrate und eine höhere Fekundität haben, während ihre Nachkommen einen größeren Männchenanteil hervorbringen und eine schnellere Entwicklung aufweisen. Somit können neu geschlüpfte männliche Nachkommen, die Mutter begatten und die Erhaltung bzw. die Etablierung einer Population sichern. In Folge dieser Tatsache sollte die bisher allgemein angenommene Behauptung über das geringere Befallspotential von arrhenotoken Arten gegenüber thelytoken Arten überdacht werden.

Sternaldrüsen

Es werden drei Differenzierungsformen der Sternaldrüsen bei den ökonomisch bedeutenden Arten *Frankliniella occidentalis, Megalurothrips sjostedti* und *E. americanus* beschrieben. Die häufigste Form der Sternaldrüsen zeichnet sich durch c-förmig angeordnete Drüsenzellen über einer extrazellulären Kavität und einer oval geformten Extrusionsfläche (*F. occidentalis*) aus. Einige Arten fallen durch eine Vervielfältigung der Porenplatten pro Segment auf. Bei *E. americanus* können bis zu 100 lichtmikroskopisch sichtbare „Poren“ pro Segment auftreten. Es konnte erstmals gezeigt werden, dass jede dieser „Poren“ eine eigenständige Porenplatte mit dazugehöriger Drüsenzelle, extrazellulärer Kavität und rundlicher Extrusionsfläche ist (multipler Porenplatten-Typus). Im Gegensatz zu der hier erfolgten Erhöhung an Drüsen- und Austrittfläche, kann auch eine Reduzierung stattfinden: bei *M. sjostedti* ist nur eine kleine, einzelne Pore pro Segment vertreten, welche mit einigen Drüsenzellen assoziiert ist.
Neben den strukturellen Unterschieden der Sternaldrüsen bei *F. occidentalis* und *E. americanus*, unterscheidet sich auch die Histochemie beider Drüsentypen. *E. americanus* verfügt über große Mengen an Lipiden im Reservoir und den Drüsenzellen. Proteine und muköse Substanzen zeigen sich ausschließlich innerhalb der Drüsenzellen. In *F. occidentalis* finden sich hauptsächlich Proteinsubstanzen innerhalb der Drüsenzellen. Der Kopulationsstatus hat bei beiden Arten nur einen minimalen Einfluss auf die gebildeten Substanzen.
Um eine mögliche Rolle der Sternaldrüsen im Rahmen des assortative matings bei *E. americanus* nachzuweisen, wurden die Individuen vermessen und ein möglicher Zusammenhang mit deren Kopulationserfolg analysiert. Außerdem diente eine mechanische Störung der Porenplatten zum Nachweis möglicher Funktionen als Sexpheromon.
Während eine höhere Körpergröße, insbesondere Länge des Pronotums, Abstand zwischen ersten und zweiten Coxae, sowie die Sternitoberfläche, einen positiven Effekt auf den Kopulationserfolg haben, spielt die Ausprägung der Sternaldrüsen keine Rolle. Auch zeigte die mechanische Störung der Porenplatten keinen Effekt auf den Kopulationserfolg, im Sinne eines möglicherweise abgegebenen mate-assessment Pheromones oder eines Anti-Aphrodisiakums.

Pheromone der Männchen

Dennoch verfügen die Männchen von *E. americanus* über Pheromone. Mithilfe von GC-MS-Analysen wurden zwei dibasische Ester in den Kopf-Thorax-Proben, nicht jedoch in Abdomenproben, identifiziert. Die Substanzen übernehmen eine Rolle in der Reproduktion. Dimethylglutarat (DBE-5) scheint als Männchen-Erkennungspheromon auf kurzen Distanzen zu fungieren. Es induziert Abdomenbewegungen in Männchen und Weibchen, welche typisch für das Verhalten bei dem Aufeinandertreffen mit einem Männchen sind. Dimethyladipat (DBE-6) wird von Männchen gemieden. Es kann außerdem auf verpaarten, nicht aber auf jungfräulichen Weibchen nachgewiesen werden. Werden beide Substanzen jeweils auf jungfräuliche Weibchen aufgetragen, werden diese von Männchen ignoriert. Die ökologische Bedeutung beider Substanzen wird diskutiert.

Diese Arbeit zeigt ganzheitliche Ergebnisse über die Reproduktionsstrategie innerhalb der Thysanopteren auf und verdeutlicht, wie vor allem Arten der Thripidae die Fähigkeit zur schnellen Reproduktion und Anpassungsfähigkeit an unterschiedliche Bedingungen realisieren können. Weitere Studien sollten den Fokus auf solche umfassenden Analysen des Zusammenhangs von Morphologie, Verhalten und Entwicklungsprozesse legen, vor allem bei potentiellen Schaderregern. Nur mit dem Verständnis für die Biologie der Tiere ist eine individuelle und effektive Strategie zur Bekämpfung solcher Arten und deren Pathogene möglich.

ACKNOWLEDGMENT

I would like to thank Prof. Dr. habil. Gerald Moritz for the opportunity to work on this interesting topic. He awakened my enthusiasm for the fascinating and diverse microcosm of thrips biology. He is thanked for the enduring support, encouragement and the confidence placed in me to work independently in this topic. Sincere thanks are also extended for providing opportunities to participate actively in various international conferences.

Furthermore, I owe thanks to the entire working group in Developmental Biology, foremost to PD Dr. habil. Gunther Tschuch for continuous technical support and active discussions. Julia Chuttke, Renate Kranz, Dr. Ingeborg Schütz, and Angelika Steller are thanked for creating a pleasant working atmosphere and providing support in laboratory-related inquiries.

Marcus Jilge, I particularly want to thank for his tireless support in the lab. He never hesitated in checking our ongoing experiments on weekends and public holidays or to spend time examining the extensive Excel-spreadsheets.

For thematic and technical discussions, I thank Prof. Tanja Schwander Ph.D. (University of Lausanne, Department of Ecology and Evolution), Dr. Katrin Bittrich (University of Halle, Institute of Psychology) and Dr. Christiane Stahr. PD Dr. habil. Wolf-Rüdiger Große, I thank you for the support during the mentoring-program and Doz. Dr. habil. Werner Witsack for providing me the *Arboridia* specimens and introducing me to the world of cicadas.

Furthermore, I thank Dr. Alice Wells for her helpful comments on the English translation, critical notes and comments to misleadingly worded passages.

A special thank to my husband, Daniel Krüger, who has supported me in all the trials and stresses of this work. I also thank my family who have always supported me fully and paved the way to enable of my study of biology.

For financial support, I am grateful to the Landesgraduiertenförderung Sachsen-Anhalt, the German Academic Exchange Service, the German Society for General and Applied Entomology, the Laurence-Mound-Fellowship and the Advancement of Women of the University of Halle.

DANKSAGUNG

Bei Prof. Dr. habil. Gerald Moritz möchte ich mich herzlichst für die Möglichkeit bedanken an diesem interessanten Thema arbeiten zu können. Er weckte in mir die Begeisterung für den faszinierenden und vielfältigen Mikrokosmos der Thripse. Vielen Dank für die stete Unterstützung, Förderung und das entgegengebrachte Vertrauen mich selbstständig in diesem Themengebiet austoben zu dürfen. Großen Dank möchte ich auch für die Unterstützung aussprechen, meine Arbeit bereits auf verschiedenen internationalen Konferenzen vorstellen zu können.

Desweiteren gilt mein Dank der gesamten Arbeitsgruppe Entwicklungsbiologie, allen voran PD Dr. habil. Gunther Tschuch für die stete technische Betreuung und Diskussionsbereitschaft. Julia Chuttke, Renate Kranz, Dr. Ingeborg Schütz und Angelika Steller danke ich für die angenehme Arbeitsatmosphäre und Unterstützung in labortechnischen Fragen.

Marcus Jilge möchte ich ganz besonders danken für die unermüdliche Unterstützung im Labor. Dabei schreckte er auch nicht vor Kontrollen der Versuchstiere am Wochenende und Feiertagen oder ellenlangen Excel-Tabellen zurück.

Für thematische und fachliche Diskussionen danke ich Prof. Tanja Schwander Ph.D. (University of Lausanne, Department of Ecology and Evolution), Dr. Katrin Bittrich (Universität Halle, Institut für Psychologie) und Dr. Christiane Stahr. PD Dr. habil. Wolf-Rüdiger Große danke ich für die Unterstützung im Mentoring Programm und Doz. Dr. habil. Werner Witsack für die Bereitstellung der *Arboridia*- Exemplare und die Einführung in die Welt der Zikaden.

Darüber hinaus danke ich Dr. Alice Wells für ihre Hilfe bei der englischen Übersetzung, sowie kritischen Anmerkungen und Kommentaren zu missverständlich formulierten Passagen.

Dank gebührt auch meinem Mann, Daniel Krüger, der mir in Höhen und Tiefen der Arbeit stets zur Seite statt. Meiner Familie, die mich immer uneingeschränkt unterstützt haben und mir vor allem mit der Ermöglichung des Studiums den Weg geebnet haben, möchte ich ebenfalls danken.

Für die finanzielle Unterstützung meiner Arbeit möchte ich der Landesgraduiertenförderung Sachsen-Anhalt, dem Deutschen-Akademischen-Austauschdienst, der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, dem Laurence-Mound-Fellowship und der Frauenförderung der Martin-Luther-Universität Halle-Wittenberg danken.

CHAPTER 10

SUPPLEMENTARY MATERIAL

DIRECTORY

Used solution, chemicals, methods of animal breeding, time schedules and additional informations are given in the Supplementary Material on the attached CD-ROM. It also provides a pdf version of the whole thesis and pdf- files of the published manuscripts and prior studies. The numbering of the single chapters here is in accordance with the chapter-numbers given in the thesis.

pdf thesis Stephanie Krüger

Published manuscripts and prior studies

- 2012_Formation of sperm bundles in *Pterostichus nigrita*
- 2012_Transfer und postkopulatorisches Schicksal der Spermiozeugmen
- 2013_Structure of the male reproductive accessory glands of *Pterostichus nigrita*
- 2015_Male pheromones influence the mating behaviour
- 2015_Offspring sex ratio and development are determined by copulation activity
- 2015_Sternal gland structures in males of bean flower thrips

Chapter S2 General morphology

- Animal breeding
- Methods for ITS-RFLP
- Scanning electron microscopic for investigation of outer reproductive organs
- Light microscopic for investigation of outer reproductive organs
- Light microscopy of spermatozoa
- Transmission electron microscope/ preparation of semithin sections
- Results
  - Molecular evidence for correct species determination in *Arboridia ribauti*
- References

Chapter S3 Reproductive behavior

- Results
  - Figure of virgin and mated male *E. americanus* with collapsed accessory gland
  - Detailed results of measurements

Section S5.1 Sternal gland structures

- Transmission electron microscopy
- Toluidine blue staining
- Grid-staining
- Dapi-Staining

CURICULUM VITAE

Stephanie Krüger, née. Schneider
Diplom-biologist
born on December, 23, 1986
in Leipzig

Occupational development

since 11/2012	**scientific staff member** **Developmental Biology** Martin-Luther-University Halle /Wittenberg
04/2011 -10/2012	**postgraduate student** Developmental Biology „Biodiversity of reproduction strategies in plant sucking paraneopteran insects" Martin-Luther-University Halle-Wittenberg

Education

10/2007-10/2010	**Martin-Luther-University Halle-Wittenberg, Germany** **Study of Biology**; advanced studies diploma thesis: "Transfer and postcopulatory fate of spermiozeugmata in *Pterostichus nigrita*" Original title: „**Transfer und postkopulatorisches Schicksal der Spermiozeugmen bei *Pterostichus nigrita*"**
10/2005-09/2007	**Ernst-Moritz-Arndt University Greifswald**, **Germany** Study of Biology; prediploma
08/1997-07/2005	**F. - A. - Brockhaus- Gymnasium Leipzig** A-level

Present publications

Krueger S, Mound LA, Moritz GB, 2015. Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* MORGAN 1913 (Thysanoptera: Thripidae). Journal of Applied Entomology, DOI: 10.1111/jen.12280

Krueger S, Subramanian S, Saliou N, Moritz GB, 2015. Sternal gland structures in males of bean flower thrips, *Megalurothrips sjostedti* and Poinsettia thrips *Echinothrips americanus* in comparison with those of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Arthropod Structure and Development 44, 455-467.

Krueger S, Ferenz HJ, Randall M, Hodgson AN, 2013. Structure of the male reproductive accessory glands of *Pterostichus nigrita* (Coleoptera: Carabidae), their role in spermatophore formation. Invertebrate Reproduction & Development 58, 75-88.

Hodgson AN, Ferenz HJ, Schneider S, 2012. Formation of sperm bundles in *Pterostichus nigrita* (Coleoptera: Carabidae). Invertebrate Reproduction & Development 57, 120-131.

Schneider S, Ferenz HJ, 2012. Transfer und postkopulatorisches Schicksal der Spermiozeugmen bei *Pterostichus nigrita* PAYKULL, 1790 (Coleoptera: Carabidae) Mitteilungen der DGaaE 18, 107-110.

Conference attendance and contributions

05/2015 Xth International Symposium on Thysanoptera and Tospoviruses, Pacific Groove, California (USA), Talk: "Offspring sex ratio and duration of ontogenesis are determined by copulation activity in *Echinothrips americanus* (Thysanoptera: Thripidae) "

03/2015 DGaaE Frankfurt, talk: "Morphology of area porosae and histology of gland cells in males of economic important thrips species (Thysanoptera: Thripidae)"

09/2014 4th Symposium on Palaearctic Thysanoptera, Vienna (A), talk: "Sternal glands (area porosae) in important thrips species – structure, function and distribution"

08/2014 European congress of Entomology, York (GB) talk: "Sternal glands in Legume flower thrips, *Megalurothrips sjostedti* and some other economically important thrips species (Thripidae)"

08/2013 Microscopic conference Regensburg, conference attendance

04/2013 DGaaE Göttingen, poster: "Morphology and ultrastructure of the spermatheca (Receptaculum seminis) in *Pyrrhocoris apterus* L. (Hemiptera: Heteroptera) before copulation and during sperm storage."

05/2011 DGaaE Berlin, talk: "Schicksal der Spermiozeugmen bei *Pterostichus nigrita*"

Additional talks

03/2015 University of Lausanne (CH), Department of Ecology and Evolution, Prof. Dr. Tanja Schwander "Offspring sex ratio and duration of ontogenesis are determined by copulation activity in *Echinothrips americanus* (Thysanoptera)"

Funding and scholarships

05/2015	DGaaE funding conference attendance
03/2015	Laurence Mound Scholarship
2015-2013	promotion of women of MLU Halle- Wittenberg
10/2012	DAAD short term scholarship
	4 week research visit at Rhodes University Grahamstown, RZA
	Prof. A. Hodgson, Department of Zoology & Entomology
04/2011-10/2012	postgraduate scholarship of Saxony Anhalt

Memberships

German Society of general and applied entomology (DGaaE)
German Society for Electron Microscopy
European Microscopic Society

Advanced training

- job application coaching for women (16 h)
- career strategies for young women scientists (16 h)
- time- , self- and stress management (16 h)
- project management / external funding (16 h)
- series of events : “external funding” (3 h)
- presentation training (16 h)
- individual coaching: reflecting your own path (1 h)
- leadership for scientists (16 h)
- didactics in higher education I & II (each 16 h)

Teaching

WS 2015/16	practical course developmental biology -development of insects
	study course developmental - and human biology-senses
SoSe 2015	project module for master students-Insect reproduction
	project module for bachelor students-development of insects II
WS 2014/15	practical course developmental biology-development of insects
	study course developmental-and human biology -senses
SoSe 2014	project module for master students-Insect reproduction
WS 2013/14	practical course developmental biology-development of insects
	study course developmental-and human biology-senses
SoSe 2013	project module for master studentsInsect Reproduction
WS 2012/13	practical course developmental biology-development of insects
	study course developmental - and human biology-senses
SoSe 2012	project module for master students-Insect reproduction

Halle, 10th February 2016

EIGENSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich ehrenwörtlich, dass ich die vorliegende Dissertation selbstständig angefertigt und keine anderen als die angegebenen Quellen oder Hilfsmittel verwendet habe.

Diese Dissertation wurde bisher weder in dieser noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegt.

Ich erkläre weiterhin, dass ich außer meinem Diplom in Biologie an der Universität Halle keine akademischen Grade erworben oder zu erwerben versucht habe.

Statement of authorship

Hereby, I declare on my honor, that this document has been composed by myself and without additional references or tools than that given.

This complete work or in a similar form has not been submitted for any other degree.

Further, I certify that I have no degree or have tried to acquire, except my diploma in Biology at the University of Halle.

Stephanie Krüger Halle, 10th February 2016

SUPPLEMENTARY MATERIAL

CHAPTER 2

GENERAL MORPHOLOGY

Animal breeding

Echinothrips americanus was maintained at the University of Halle, Germany. Animals were provided from the Netherlands. They were reared on *Phaseolus vulgaris* and *Gossypium spec.* as host plants. Culture was kept at 23 ± 1 °C, 60 ± 10 % RH and photoperiod of 16:8 h L:D at 5000 Lux during photophase.

Individuals of *Campyloneura virgula* were collected at Halle, Germany (N51.487069°, E11.960644°) on elders from May to August in 2011 to 2014.

Individuals of *Pyrrhocoris apterus* were collected at Halle, Germany (N51.483544°, E11.978375°) on linden trees. Maintaining of individuals was carried on at 23 °C, 70 ± 10 % RH and L:D 16:8 at 20.000 Lux during photophase in the lab. They were fed ad libidum on pelled sunflower and linden seeds. Water was provided with the help of moistened cotton.

Arboridia ribauti were collected at Halle, Germany (N51.495599°, E11.925935°) on hornbeam hedges from July to August 2013.

Methods for ITS-RFLP

Determination of species within the *Arboridia*-genus is normally conducted via the morphology of the male external genitalia. Because of the thelytoky in the used population, no males were available in the region of Halle (Germany). Therefore molecular identification methods after Moritz et al. (2000, 2001) were applied. As control, individuals from Göttingen, which were determined by H. Nickel (University of Göttingen, Germany), were used.

100-Fly-method extraction buffer	0.1 M TRIS	2.5 ml
pH 9.2	0.1 M Sodium chloride	2.5 ml
	0.05 M EDTA	5 ml
	0.2 M Saccharose	5 ml
	0.5 % SDS	2.5 ml
	Distilled water	ad. 50 ml
10 x TBE	900 mM TRIS	109 g
pH 8.2	900 mM Boric acid	55.5 g
	25 mM EDTA	9.0 g
	Distilled water	ad. 1000 ml

6 x Loading buffer	30 % Glycerin	6 ml
pH 8.2	0.25 % Bromophenol blue	0.05 g
	0.25 % Xylene cyanol	0.05 g
	Distilled water	ad. 20 ml

The DNA-isolation was performed with the 100-Fly method after Roberts (1998). Afterwards the PCR is conducted with IT 1-f/IT1-R and 28SS/28SA-primers (Tab. 1).

Table 1 Sequence of used primers for ITS-RFLP

Primer pair	Sequence
IT 1-F/ IT 1-R	TGT GAA CTG CAG GAC ACA TGA / GGT AAT CTC ACC TGA ACT GAG GTC
28SS/ 28SA	GAC CCG TCT TGA AMC AMG GA TCG GAR GGA ACC AGC TAC TA

The DNA amplification was conducted after the following PCR approaches and programmes (Tab. 2):

PCR approach with the IT 1-F/IT1-R primer pair (50 µl):

4.0 µl	template DNA
5.0 µl	10 x buffer for PCR (Eppendorf©, Germany)
4.0 µl	25 mM MgCl
0.4 µl	25 mM dNTPs (Eppendorf©, Germany)
1.5 µl	primer (forward)
1.5 µl	primer (reverse)
33.4 µl	double distilled water
0.2 µl	Taq Polymerase (Eppendorf©, Germany)

Table 2a Schedule of PCR-program for ITS-RFLP with the IT1-F/IT1-R primer pair

Step	Temperature	Duration	
Initial denaturation	95 °C	3 min	
Denaturation	95 °C	45 s	31 cycles
Annealing	60 °C	45 s	
Extension	72 °C	3 min	
final extension	72 °C	5 min	
End	4 °C		

PCR approach with the 28SS/28SA primer pair (50 µl):

2.0 µl	template DNA
5.0 µl	10 x buffer for PCR (Eppendorf©, Germany)
4.0 µl	25 mM MgCl
0.4 µl	25 mM dNTPs (Eppendorf©, Germany)
1.5 µl	primer (forward)

1.5 µl	primer (reverse)
3.1 µl	double distilled water
0.5 µl	Taq Polymerase (Eppendorf©, Germany)

Table 2b Schedule of PCR-program for ITS-RFLP with the 28SS/28SA primer pair

Step	Temperature	Duration	
Initial denaturation	95 °C	5 min	
Denaturation	95 °C	30 s	35 cycles
Annealing	54 °C	40 s	
Extension	72 °C	45 s	
Final extension	72 °C	6 min	
End	4 °C		

Afterwards PCR- products were prepared for digestion with the following restriction enzymes: Rsa I, Hae III, Msp I, Hinf I and Alu I.

restriction approach (10 µl):	7.0 µl	template
	1.0 µl	10 x buffer
	0.1 µl	BSA
	1.5 µl	double distilled water
	0.5 µl	enzyme

Digestion was performed at 37 °C for 2 h. Reaction was stopped by adding 2 µl loading-buffer. To rip up the DNA-fragments, agarose gel-electrophoresis was used. On a 2 % agarose gel with 1 x TBE buffer, 10 µl of each digestion-product were applied . Electrophoresis was conducted for 4 h at 70 V.

In order to analyse the inner and outer reproductive organs, light- and electron microscopic techniques were applied:

Scanning electron microscopy for investigation of outer reproductive organs

Stretching solution	Ethanol 60 %	50 ml
	Glycerol	5 ml
	Glacial acetic acid	5 ml

Normally, the outer genitalia are retracted in a genital chamber. With a human hair, a loop was formed and tightened around the abdomen. Individuals with evaginated external genitalia were fixed in stretching solution overnight at 70 °C. After dehydration in a graded ethanol series, specimens were finished in hexamethyldisilazane (Tab. 3). Later, these were mounted on aluminium stubs with double-sided adhesive pads. Gold coating was performed with a Balzers SCD 004 sputter coater (Bingen, Germany) for 200 s at 20 mA, visualized with a scanning electron microscope Hitachi SEM S-2400 (Tokyo, Japan) at 18 kV and documented on ILFORD FP 4 (Knutsford, Great Britain) roll film. Scanned images were contrast and brightness adjusted with the help of Photoshop CS5 (Adobe, San Jose, California, USA).

Table 3 Schedule of standard SEM fixation and dehydration method

SEM fixation schedule	Concentration	Time
AGA		overnight, 70 °C
Ethanol	70 %	10 min
Ethanol	80 %	10 min
Ethanol	90 %	10 min
Ethanol	100 %	10 min
HMDS	100 %	5 min

Furthermore, for investigation of the chitin structure of the spermathecae of the firebugs, spermathecae were dissected out of the genital tract, macerated for 25 min in 1 % KOH-solution (at 65 °C), washed in Ringer-solution and prepared for SEM as described above.

Light microscopy for investigation of inner reproductive organs

Ringer`s solution	Sodium chloride	9.82 g
	Magnesium chloride	0.73 g
	Sodium dihydrogen phosphate	0.67 g
	Potassium chloride	0.48 g
	Calcium chloride	0.31 g
	Sodium hydrogen carbonate	0.18 g
	Distilled water	1000 ml

Ringer`s solution for Pyrrhocoris after Martin 1966	Sodium chloride	1.87 g
	Potassium chloride	0.024 g
	Sodium hydrogen carbonate	0.05 g
	Calcium chloride	0.05 g
	Distilled water	250 ml
Carnoy	Absolute ethanol	30 ml
	Chloroform	15 ml
	Glacial acetic acid	5 ml
Saturated picric acid	Picric acid	3 g
	Distilled water	200 ml
Bouin	Saturated picric acid	15 ml
	Formaldehyde (37 %)	5 ml
	Glacial acetic acid	1 ml
Haematoxylin after Mayer	Haematoxylin	1 g
	Distilled water	1000 ml
	Potassium alaun	50 g
	Sodium iodate	0.2 g
	Chloral hydrate	5 g
	Citric Acid	0.1 g
Eosin	Eosin	1 g
	Distilled Water	1000 ml
Dapi stock solution	Dapi	2 mg
	Distilled water	10 ml
Dapi working solution	Dapi stock solution	1 µl
	Distilled water	1 ml
10 x PBS pH 7.4	Sodium chloride	8 g
	Potassium chloride	0.2 g
	Sodium phosphate dibasic *2 H_20	1.15 g
	Potassium phosphate monobasic	0.2 g
	Distilled water	ad. 100 ml
20 % Propyl gallate	Propyl gallate	2 g
	Formamide	10 ml
Anti-fade medium	10 x PBS	3 ml

20 % Propyl gallate	0.3 ml
Glycerol	27 ml

Individuals of thrips were fixed in Carnoy`s solution for 1 h and dehydrated in a graded ethanol series. Specimen of *Campyloneura virgula, Arboridia ribauti* and *Pyrrhocoris apterus* were fixed 24 h in Bouin solution. After several washing steps with 70 % ethanol, specimens were dehydrated in a graded ethanol series (Tab. 4). Afterwards, samples were embedded in Surgipath Paraplast® (Leica, Wetzlar, Germany) via isopropanol and cut in 6 µ sections with a Leica SM 2000R microtome (Leica, Wetzlar, Germany). Samples were stained in Haematoxylin–Eosin (Tab. 5, 6) or Dapi (Tab. 5, 7) and observed with a Leitz DMRBE (Leica, Wetzlar, Germany) fitted with a Canon EOS 600D (Canon, Tokyo, Japan). Images were contrast and brightness adjusted with the help of Photoshop CS5 (Adobe, San Jose, California, USA).

Table 4 Fixation and embedding for light microscopic observation

Fixation and embedding	Concentration	Time
Carnoy/ Bouin		1 h/24 h
Ethanol	70 %	1 h
Ethanol	80 %	1 h
Ethanol	90 %	1 h
Ethanol	100 %	1 h
Isopropanol	100 %	3 x 2 h
Isopropanol: Paraplast	1:1	24 h
Paraplast		3 x 24 h

Table 5 Schedule of rehydration after paraplast embedding

Rehydration	Concentration	Time
Xylene	100 %	5 min
Isopropanol	100 %	3 min
Ethanol	96 %	3 min
Ethanol	80 %	3 min
Ethanol	60 %	3 min
Distilled Water	100 %	> 3 min

Table 6 Schedule of Haematoxylin-eosin staining for light microscope samples

Haematoxylin-eosin staining	Concentration	Time
Haematoxylin		5 min
Tab water		10 min
Distilled water		1 min
Eosin		4 min
Distilled water		1 min
Ethanol	96 %	1 min
Isopropanol	100 %	2 x 1 min
Xylene	100 %	2 min
Canada balsam		

Table 7 Schedule of DAPI staining for light microscope samples

Dapi staining schedule	Time
Distilled water	3 min
Dapi working solution	15 min
Distilled water	2 x 3 min
Anti-fade medium	

In addition, dissected spermathecae of *P. apterus* were embedded, as mentioned above (Tab. 4). They were cut into half with the help of the microtome. Afterwards paraplast was removed (Tab. 8) and samples were mounted on aluminium stubs and prepared for SEM as mentioned above.

Table 8 Schedule of paraplast removing

Removal of paraplast schedule	Concentration	Time
Xylene	100 %	2 x 30 min, 40 °C
Xylene	100 %	2 h, RT
Xylene	100 %	overnight
Ethanol	90 %	20 min
HDMS	100 %	10 min
drying		overnight

Light microscopy of spermatozoa

Male specimens were dissected with fine tweezers (No. 5, Dumont, Montignez, Switzerland) (P. *apterus*)*,* or minutien pins (*E. americanus*) in Ringer`s solution. Isolated testis were squashed on Polysine™ slides (G. Menzel GmbH, Braunschweig, Germany) and covered with a cover slip. Observation takes place with a Leitz DMRBE (Leica, Wetzlar, Germany) microscope in Differential Interference Contrast-mode. Measurements have been performed with the software Automontage (Syncroscopy, Cambridge, UK).

Transmission electron microscope/ preparation of semithin sections

4 % Paraformaldehyde	Paraformaldehyde	2 g
	Distilled water	10 ml
	Soerensen buffer	40 ml
0.1 M Soerensen buffer	(A) Sodium phosphate monobasic * H_20	27.6 g
pH 7.2	Distilled water	1000 ml
	(B) Sodium phosphate dibasic * $2H_20$	35.61 g
	Distilled water	1000 ml

	Solution A (0.2 M)	14.0 ml
	Solution B (0.2 M)	36.0ml
	Distilled Water	ad. 100 ml
2 % Osmium tetroxide	Osmium tetroxide	100 mg
	0.1 M Soerensen buffer	5 ml
1 % Safranin-solution	Safranin	1 g
	50 % Ethanol	100 ml

Additionally, semithin sections of *E. americanus* and were consulted for a more detailed analysis. Samples of *E. americanus* originate from analysis of the sternal glands, chapter 5. For analysis of the spermatheca of the firebug *P. apterus* semithin sections were conducted after standard methods (Tab. 9). Semithin sections (500 nm) were cut with an Ultracut R microtome (Leica, Germany) and stained with 1 % safranin. All sections were examined under a light microscope (Leitz DMRBE, Leica, Germany) fitted with a Canon EOS 600D digital camera. Finally, all images were processed using Photoshop CS5 (Adobe, Ireland).

Table 9 Schedule of TEM fixation and embedding

TEM fixation schedule	Concentration	Time
Paraformaldehyde	4 %	3 h (RT)+ 1 h (4 °C)
Soerensens buffer		4 x 20 min
Osmiumtetroxide	2 %	2 h
Bidistilled water		4 x 20 min
Ethanol	30 %	2 x 10 min
Ethanol	50 %	2 x 10 min
Ethanol	70 %	2 x 10 min
Ethanol	80 %	2 x 10 min
Ethanol	95 %	2 x 10 min
Ethanol	100 %	2 x 10 min
Propylenoxide	100 %	2 x 10 min
Araldite: propylenoxide	1:1	2 x overnight
Araldite	100 %	2 d (65 °C)

Results

Because of the absence of males in our *Arboridia ribauti* population, we conducted ITS-RFLP identification on clearly determined species from Göttingen and from our population. Specimens from Göttingen were kindly determined and provided by H. Nickel.

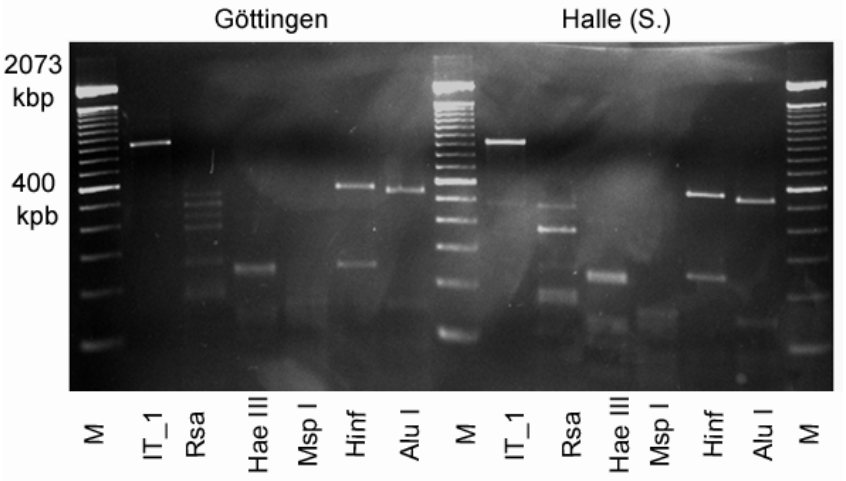


Figure 1a Gel pattern of ITS-RFLP enzyme digestion of *Arboridia ribauti* individuals from two different populations (primer: IT 1-F/IT 1-R).

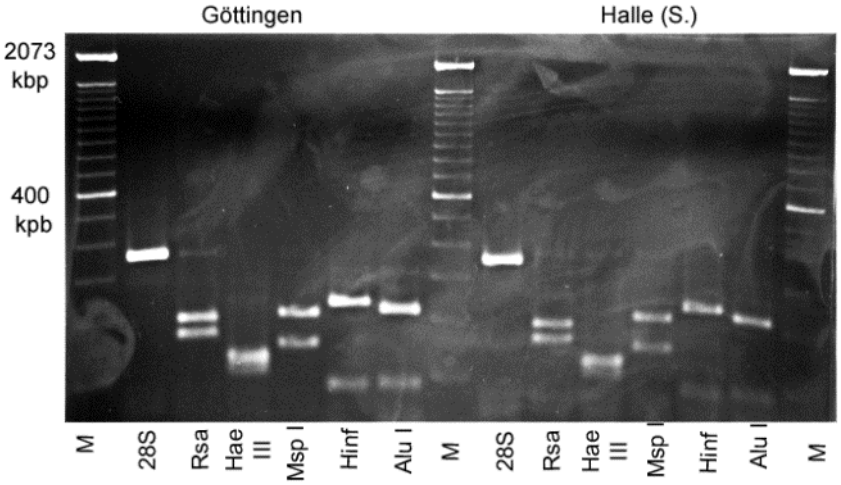


Figure 1b Gel pattern of ITS-RFLP enzyme digestion of *Arboridia ribauti* individuals from two different populations (primer: 28SS/28SA).

Because of the similar gel patterns we concluded, that both populations belong to the similar species: *Arboridia ribauti*.

References

Bock C, 1987. A quick and simple method for preparing soft insect tissues for scanning electron microscopy using carnoy and hexamethyldisilazane. Beitr. elektronenmikr. Direktabb. Oberfl. 20, 209-214.

Moritz G, Delker C, Paulsen M, Mound LA, Burgermeister W, 2000. Modern methods in thrips-identification and information (Insecta, Thysanoptera). Bulletin OEPP/EPPO (Paris) 30, 591-593.

Moritz G, Morris DC, Mound LA, 2001. Thrips*ID*-Pest thrips of the world. An interactive identification and information system,. CSIRO Publishing, Collingwood, Australia. online available: http://thripsnet.zoologie.uni-halle.de/key-server-neu/data/09070302-040a-4006-8c08-0e0105060801/media/Html/index.html

Roberts D B, 1998. Drosophila, a practical approach. University Press, New York, USA.

SUPPLEMENTARY MATERIAL

CHAPTER 3

REPRODUCTIVE BEHAVOIR OF *ECHINOTHRIPS AMERICANUS* (THYSANOPTERA: THRIPIDAE)

Results

Collapsed accessory gland

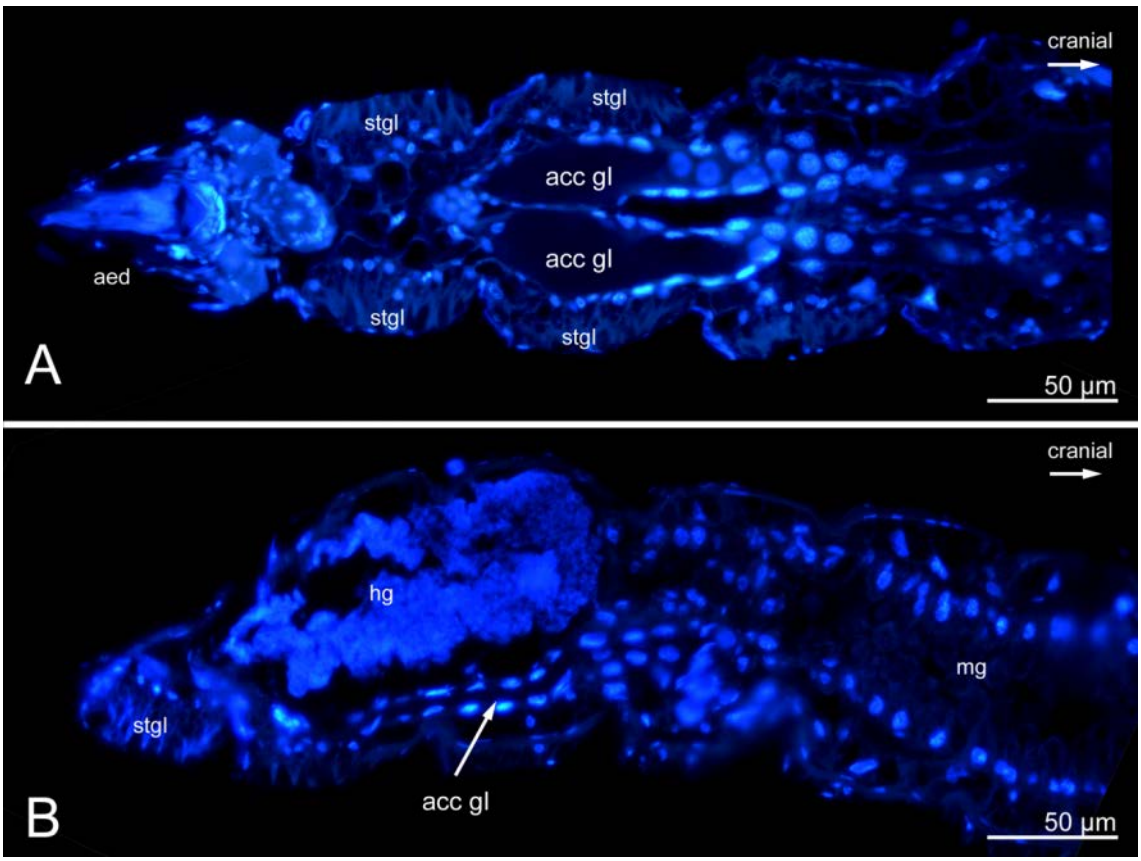


Figure 1 Male *Echinothrips americanus,*
A 5d old virgin male, DAPI stained, frontal,
B 10 d old and 10x mated male, DAPI, sagittal,
acc gl-accessory gland, aed-aedeagus, mg-midgut, hg-hindgut, stgl-sternal gland

Measurement of testis and grey value

Table 1 Measurement of testis and grey value of male *Echinothrips americanus* depending on age and number of consecutive matings

	0 d virgin	5 d virgin	5 d, 5 x mating	10 d virgin	10 d, 10 x mating
n	20	14	16	20	9
Wide testis in µm	16.33 ± 1.63	17.99 ± 3.22	16.39 ± 3.5	14.96 ± 1.98	11.41 ± 2.68
Length testis in µm	58.60 ± 9.53	48.5 ± 7.54	45.34 ± 8.33	50.74 ± 10.58	37.95 ± 9.98
Area testis in µm²	825.79 ± 150.89	761.38 ± 111.70	611.50 ± 154.68	605.38 ± 118.4	345.83 ± 107.31
Relative grey value	86.66 ± 11.13	101.84 ± 11.31	91.06 ± 12.11	92.36 ± 19.08	72.90 ± 12.75
Index of grey value	-1.33 ± 0.11	0.018 ±0.11	-0.89 ± 0.12	-0.74 ± 0.19	-0.27 ± 0.13

Table 2 Measurement of spermathecae and grey value of female *Echinothrips americanus* depending on age and number of consecutive matings of male

	1-2 x mating	3-4 x mating	5-6 x mating	7-8 x mating	9-10 x mating
n	15	6	14	8	13
Wide sperm ball in µm	17.21 ± 2.74	16.1 ± 3.03	15.44 ± 1.13	13.56 ± 2.27	15.72 ± 1.86
Length sperm ball in µm	16.97 ± 3.03	15.32 ± 2.29	15.52 ± 0.80	13.29 ± 1.69	14.7 ± 1.19
Area sperm ball in µm²	243.23 ± 67.9	196.93 ± 63.04	196.44 ± 21.79	150.72 ± 36.53	185.19 ± 37.74
Relative grey value sperm ball	95.59 ± 8.7	61.51 ± 47.57	60.92 ± 33.99	74.69 ± 30.35	104.58 ± 31.81

SUPPLEMENTARY MATERIAL

SECTION 5.1

STERNAL GLAND STRUCTURES

Transmission electron microscopy

0.1 M Soerensen buffer pH 7.2	(A) Sodium phosphate monobasic * H_20	27.6 g
	Distilled water	1000 ml
	(B) Sodium phosphate dibasic * $2H_20$	35.61 g
	Distilled water	1000 ml
	Solution A (0.2 M)	14.0 ml
	Solution B (0.2 M)	36.0 ml
	Distilled Water	ad. 100 ml
10 % Paraformaldehyde	Paraformaldehyde	2 g
	Bidistilled water	20 ml
	1N Sodium hydroxide	few drops
Paraformaldehyde-glutaraldehyde fixative	0.2 M Soerensen buffer	50 ml
	10 % Paraformaldehyde in water	20 ml
	25 % Glutaraldehyde	10 ml
	Distilled water	ad. 100 ml
2 % Osmium tetroxide	Osmium tetroxide	100 mg
	0.1 M Soerensen buffer	5 ml

Samples of whole individuals were handled as described below (Tab. 1). Semithin sections (500 nm) and ultrathin sections (50-70 nm) were cut with an Ultracut R microtome (Leica, Germany). Semithin sections were stained with toluidine blue (see below) and examined under a light microscope (Leitz DMRBE, Leica, Germany) fitted with a Canon EOS 600D digital camera. Finally, all images were processed using Photoshop CS5 (Adobe, Ireland). Ultrathin sections were placed on coated grids and stained after 24h drying (Tab. 2). Observations were obtained with a JEOL TEM 1010 (at 80 kV) fitted with a Megaview II camera.

Table 1 Schedule of TEM- fixation and embedding

TEM embedding schedule	Concentration	Time
Paraformaldehyde-Glutaraldehyde fixative (4 °C)		2 h
Soerensen buffer (4 °C)		4 x 20 min, then overnight
Osmium tetroxide	2 %	1 h
Bidistilled water		4 x 20 min
Ethanol	30 %	2 x 20 min
Ethanol	50 %	2 x 20 min
Ethanol	70 %	2 x 20 min
Ethanol	80 %	2 x 20 min
Ethanol	95 %	2 x 20 min
Ethanol	100 %	2 x 20 min
Propylene oxide (PO)	100 %	2 x 10 min
Araldite: PO	1:1	2 x overnight
Araldite (65°C)	100 %	2 d

Toluidine blue staining

1 % Toluidine blue solution	Sodium tetraborate	1 g
	Toluidine blue	1 g
	Distilled water	100 ml

Specimens were prepared for TEM observation (see above). Sections were made with a Leica Ultracut R. Sections were stained with 1 % toluidine blue on a heating plate for 30 s and washed with distilled water afterwards.

Grid staining

2 % Uranyl acetate	Uranyl acetate	1 g
	50 % ethanol	50 ml
2 % Lead citrate	Lead citrate	1 g
	Bidistilled water	42 ml
	1N sodium hydroxide	8 ml

Grids were staining with 2 % uranyl acetate for 10 min in a darkened petri dish. Afterwards grids were washed 3 x 1 min with bidistilled water. Grids were dried on a filter paper and stained 10 min in 2 % lead citrate in a petri dish with 5-6 sodium hydroxide granules to reduce humidity.

Dapi-Staining

Dapi stock solution	Dapi	2 mg
	Distilled water	10 ml
Dapi working solution	Dapi stock solution	1 µl
	Distilled water	1 ml
10 x PBS	Sodium chloride	8 g
pH 7.4	Potassium chloride	0.2 g
	Sodium phosphate dibasic * 2 H_20	1.15 g
	Potassium phosphate monobasic	0.2 g
	Distilled water	ad. 100 ml
20 % Propyl gallate	Propyl gallate	2 g
	Formamide	10 ml
Anti-fade medium	10 X PBS	3 ml
	20 % Propyl gallate	0.3 ml
	glycerol	27 ml

Specimens were treated as described in scheme Chapter S2 (light microscopy). Afterwards rehydrated sections were stained after the scheme in Table 2 and mounted with anti-fade medium.

Table 2 Schedule of DAPI-staining

Dapi staining schedule	Time
Distilled water	3 min
Dapi working solution	15 min
Distilled water	2 x 3 min
Anti-fade medium	

SUPPLEMENTARY MATERIAL

SECTION 5.2

HISTOCHEMICAL STUDY

Specimen for Coomassie-, Periodic acid-Schiff`s-reagent- and Aldehyde fuchsine-alcian blue-staining were Carnoy- fixed. After fixation specimen were dehydrated via a graded ethanol series and embedded in paraplast via isopropanol (see Chapter S2). Sections (6 µm) were made with a Leica SM 200R microtome. Fixation technique for lipid-staining is mentioned in the text.

Coomassie- staining for proteins

Coomassie blue staining solution	Coomassie-Blue R250	40 mg
	Acetic ethanol	200 ml
Acetic ethanol	Glacial acetic acid	100 ml
	Absolute ethanol	300 ml

Table 1 Schedule of Coomassie staining

Coomassie staining procedure	Concentration	Time
Coomassie-solution	0.2 %	1 min
Acetic-ethanol	1:3	10 min
Distilled water		1.5 min
Haematoxylin		1.5 min
Tab water		5 min
Ethanol	96 %	1 min
Isopropanol	100 %	2 x 1 min
Xylene	100 %	2 min
Canada balsam		

Periodic acid-Schiff`s-reagent – haematoxylin staining for carbohydrates (PASH)

Periodic acid solution	Periodic acid	0.5 g
	Distilled water	100 ml
Schiff`s reagent	Basic fuchsine	1 g
	Distilled water	200 ml
	Potassium metabisulfite	2 g
	Hydrochloric acid (37 %)	2 ml

Table 2 Scheme of PASH staining

PASH staining procedure	Concentration	Time
Periodic acid	0.5 %	10 min
Tab water		5 min
Distilled water		1 min
Schiffs reagent		3 min
Tab water		5 min
Haematoxylin		1 min
Tab water		5 min
Ethanol	96 %	5 min
Isopropanol	100 %	2 x 1 min
Xylene	100 %	1 min
Canada balsam		

Aldehyde fuchsine-alcian blue-staining for mucins

Alcian blue staining solution	Alcian Blue	1 g
	Glacial acetic acid	3 ml
	Distilled Water	97 ml
Aldehyde fuchsine solution	Basic fuchsine	1 g
	Paraldehyde	2 ml
	Hydrochloric acid (37 %)	1 ml
	Ethanol	60 ml
	Distilled water	40 ml

Table 3 Schedule of AF-AB staining

Aldehyde fuchsine-alcian blue staining procedure	Concentration	Time
Ethanol	60 %	1 min
Aldehyde fuchsine solution		10 min
Ethanol	70 %	1 min
Distilled Water		1 min
Alcian blue solution		30 min
Distilled Water		1 min
Ethanol	96 %	1 min
Isopropanol	100 %	2 x 1 min
Xylene	100 %	2 min
Canada balsam		

Oilred-O staining for lipids

Oilred-O staining stock solution	Oilred-O	1 g
	Isopropanol	1000 ml
Dextrin-solution	Dextrin	1 g
	Distilled Water	1000 ml

Oilred-O staining working solution	Oilred stock solution	100 ml
	Dextrin solution	150 ml

Table 4 Schedule of Oilred-O staining

Oilred-O staining procedure	Concentration	Time
Ethanol	60 %	1 min
Oilred-O working solution		20 min
Ethanol	60 %	1 min
Distilled water		4 x 1 min
Haematoxylin		1 min
Tab water		10 min
Distilled water		1 min
Roti®-Mount Aqua (Carl Roth)		

Nile blue sulphate method for acidic and neutral lipids

Formol calcium	Formaldehyde (40%)	10 ml
	Calcium chloride	1 g
	Distilled Water	90 ml
Nile blue sulphate solution	Nile blue sulphate	2 g
	Distilled water	200 ml
	sulphuric acid 1 %	10 ml
Methyl green solution	Methyl green	1 g
	Distilled water	100 ml
Glycerine jelly	Gelatine	10 g
	Distilled Water	60 ml
	Glycerine	70 ml
	Phenol	1 ml

Table 6 Schedule of Nile blue sulphate staining

Nile blue sulphate staining procedure	Concentration	Time
Formol calcium		1 h
Nile blue sulphate (60°C)		30 min
Acetic acid	1 %	1 min
Methyl green		10 min
Distilled water		2 x 1 min
Glycerine jelly		

Cryosectioning

Embedding procedure for cryosectioning was occurred as followed: Lens-tissue-embedding technique was used to ensure correct orientation for sagittal sectioning. A piece (1 cm x 1cm) of lens-tissue paper (Ross- Optical Lens Tissue, USA) was moistened with a drop of Tissue-Tek (Tissue Tek, Sakura, Japan) and placed in a cryomould (Tissue Tek, Sakura, Japan, 25 mm x 20 mm x 5 mm). Specimens were placed and orientated within this drop under a stereomicroscope. Afterwards they were chilled down, and cryomould was filled up with embedding media, and frozen. Cryosections (6-8 µm) were cut with a Leica CM1900, chamber temperature of -25°C, object temperature of -30°C. Knife angle was adjusted to 8°. Sections were attached on SuperFrost Plus-slides (Menzel, Heidelberg, Germany).

SUPPLEMENTARY MATERIAL

SECTION 5.3

BODY SIZE AND MATING SUCCESS

Whole mounts

AGA	Ethanol 60 %	10 ml
	Glycerol	1 ml
	Acetic acid	1 ml

Table 1 Schedule of whole mounts, adjusted after Moritz et al. (2004)

Whole mounts procedure	Concentration	Time
AGA		1 h
Ethanol	60 %	24 h
NaOH (45 °C)	5 %	7-8 h
Distilled water		20 min
Ethanol	70 %	1 h
Ethanol	80 %	20 min
Ethanol	95 %	10 min
Ethanol	100 %	2 x 5 min
Terpineol	100 %	30 min
Canada balsam		

After fixation and stretching in AGA, specimen were washed in 60 % ethanol and cleared in NaOH at 45 °C. This was followed by dehydration in a graded ethanol series (Tab. 1). As clove oil terpineol was used to ensure clearing. Afterwards, a drop of Canada balsam was placed onto centre of cover slip and the thrips were added ventral side uppermost. Legs and wings were spread; antennae were straightened with a fine needle. Another drop of Canada balsam was placed on microscope slide. Slide was inverted and placed on the cover slip. A gentle pressure was performed to get a flattest sample as possible to ensure optimal measurement results.

Table 2 Measurement and level of significance of head and thorax characteristics of male *Echinothrips americanus*

Part of the body	Mating success	n	Mean ± SD	df	t	p
Compound eye length	mating	10	52.77 ±6.67	19	-1.831	0.083
	no mating	11	56.93 ± 4.75			
Number of brown ommatidia	mating	9	7.56 ± 1.01	14	1.691	0.113
	no mating	7	6.71 ± 0.95			
Antennae length	mating	8	284.50 ± 24.06	15	0.820	0.425
	no mating	9	277.476 ± 8.79			
Length antennae segment 6 to 8	mating	10	95.21 ± 5.03	17	0.275	0.786
	no mating	9	94.656 ± 3.51			
Forefemora width	mating	10	33.56 ± 2.31	20	0.537	0.597
	no mating	12	33.08 ± 1.86			
Forefemora length	mating	10	94.92 ± 12.66	20	0.921	0.368
	no mating	12	90.57 ± 9.48			
Foretibia width	mating	10	32.76 ± 1.59	20	-0.259	0.798
	no mating	12	32.97 ± 2.18			
Foretibia length	mating	10	95.78 ± 9.77	18	-0.190	0.852
	no mating	10	96.58 ± 9.08			
Pronotum width	mating	9	162.05 ± 12.34	18	0.509	0.617
	no mating	10	159.39 ± 11.01			
Pronotum length	mating	9	95.84 ± 8.36	16	2.763	0.014
	no mating	9	85.689 ± 7.18			
Distance 1^{st} coxa to 2^{nd} coxa	mating	9	123.93 ± 20.56	16	3.100	0.007
	no mating	9	95.955 ± 17.62			
Distance 2^{nd} coxa to 3^{rd} coxa	mating	9	110.83 ± 7.34	16	1.904	0.075
	no mating	9	102.66 ± 10.58			
Mesosternum width	mating	9	221.76 ± 14.38	17	0.336	0.741
	no mating	10	219.15 ± 18.91			
Mesosternum length	mating	9	64.87 ± 5.04	16	0.647	0.527
	no mating	9	62.41 ± 10.24			
Metasternum width	mating	9	202.87 ± 13.79	17	0.229	0.821
	no mating	10	201.170 ± 17.92			
Metasternum length	mating	9	111.39 ± 5.86	17	1.639	0.120
	no mating	10	103.68 ± 12.94			

Table 3 Measurement and level of significance of abdominal characteristics of male *Echinothrips americanus*

Part of the body	Mating success	n	Mean ± SD		df	t	p
Sternite III area	mating	9	10972.03 ± 1460.95	µm²	19	1.396	0.179
	no mating	12	9862.24 ± 2015.66	µm²			
Sternite IV area	mating	9	11717.49 ± 1252.25	µm²	19	1.709	0.104
	no mating	12	10572.12 ± 1688.27	µm²			
Sternite V area	mating	9	11794.32 ± 1085.06	µm²	19	2.463	0.023
	no mating	12	10502.26 ± 1259.97	µm²			
Sternite VI area	mating	9	10866.49 ± 1266.71	µm²	19	2.231	0.038
	no mating	12	9764.15 ±1001.33	µm²			
Sternite VII area	mating	9	9336.54 ± 1098.39	µm²	19	1.570	0.133
	no mating	12	8392.56 ±1527.67	µm²			
Sternite VIII area	mating	9	7811.51 ± 778.63	µm²	19	1.805	0.087
	no mating	12	7013.15 ± 1138.98	µm²			
Sternite III size pore plates	mating	9	11.97 ± 1.79	µm²	19	0.409	0.687
	no mating	12	11.63 ± 1.95	µm²			
Sternite IV size pore plates	mating	9	11.41 ± 1.47	µm²	19	-0.295	0.771
	no mating	12	11.64 ± 1.98	µm²			
Sternite V size pore plates	mating	9	12.09 ± 1.22	µm²	19	-0.043	0.966
	no mating	12	12.11 ± 0.93	µm²			
Sternite VI size pore plates	mating	9	11.55 ± 2.06	µm²	19	-0.447	0.660
	no mating	12	11.93 ± 1.87	µm²			
Sternite VII size pore plates	mating	9	11.10 ± 2.30	µm²	19	-0.491	0.629
	no mating	12	11.55 ± 1.92	µm²			
Sternite VIII size pore plates	mating	9	10.95 ± 1.22	µm²	19	-1.30	0.209
	no mating	12	12.02 ± 2.23	µm²			
Sternite III number pore plates	mating	9	52.67 ± 9.53		19	1.462	0.160
	no mating	12	44.42 ± 14.73				
Sternite IV number pore plates	mating	9	69.11 ± 7.86		19	1.652	0.115
	no mating	12	58.67 ± 17.62				
Sternite V number pore plates	mating	9	81.33 ± 8.29		19	1.339	0.196
	no mating	12	75.83 ± 9.99				
Sternite VI number pore plates	mating	9	89.11 ± 9.59		19	1.818	0.085
	no mating	12	79.81 ± 12.66				

Sternite VII number pore plates	mating	9	85.00 ± 9.41		19	1.723	0.101
	no mating	12	72.00 ± 21.01				
Sternite VIII number pore plates	mating	9	76.00 ± 8.86		19	0.768	0.452
	no mating	12	72.00 ± 13.56				
Sternite III summed area pore plates	mating	9	610.44 ± 161.11	µm²	19	1.651	0.115
	no mating	12	490.23 ± 167.98	µm²			
Sternite IV summed area pore plates	mating	9	762.62 ± 116.19	µm²	19	1.489	0.153
	no mating	12	651.11 ± 200.07	µm²			
Sternite V summed area pore plates	mating	9	963.88 ± 177.53	µm²	19	1.052	0.306
	no mating	12	892.81 ± 132.66	µm²			
Sternite VI summed area pore plates	mating	9	1015.04 ± 253.37	µm²	19	0.849	0.407
	no mating	12	930.53 ± 203.42	µm²			
Sternite VII summed area pore plates	mating	9	918.36 ± 212.22	µm²	19	0.990	0.335
	no mating	12	812.08 ± 263.79	µm²			
Sternite VIII summed area pore plates	mating	9	803.27 ± 66.85	µm²	19	-0.583	0.567
	no mating	12	848.42 ± 223.79	µm²			
Total sternite area (mean) (Sum sternite areas)	mating	9	62498.36 ± 6170.75	µm²	19	2.286	0.034
	no mating	12	56106.49 ± 6463.60	µm²			
Sum pore areas (mean)	mating	9	5073.62 ± 679.66	µm²	19	1.435	0.168
	no mating	12	4625.18 ± 729.26	µm²			
Proportion pore area (mean)	mating	9	8.12 ± 0.69	%	19	-0.369	0.716
	no mating	12	8.23 ± 0.77	%			

SUPPLEMENTARY MATERIAL

CHAPTER 6

MALE PHEROMONES INFLUENCING MATING SUCCESS OF *ECHINOTHRIPS AMERICANUS* (INSECTA, THYSANOPTERA, THRIPIDAE)

Males can be observed frequently when guarding female pupae on the bottom side of their host plant *Phaseolus vulgaris*. Thereby, they sit near to the pupae until their adult moult.

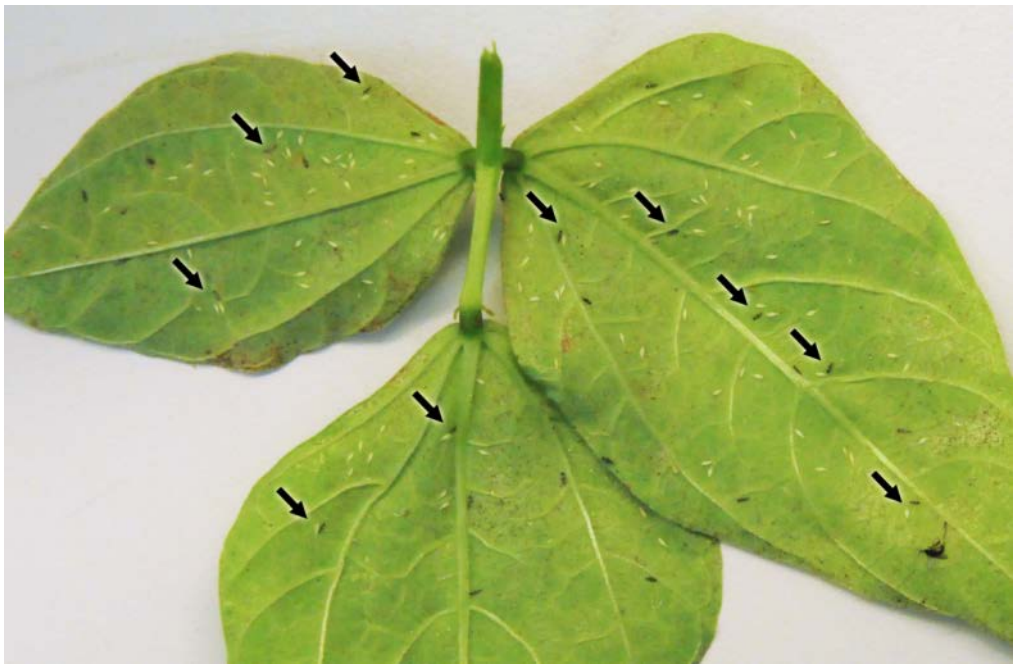


Figure 1 *Echinothrips americanus* population on *Phaseolus vulgaris*, arrows indicate the guarding males

Figure 2 *Echinothrips americanus* male in between female pupae and propupae