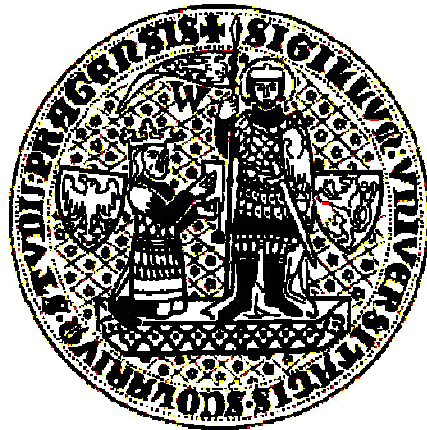**CHARLES UNIVERSITY IN PRAGUE**
Faculty of Science
Department of Zoology

Ph.D. Thesis
Ph.D. Study Program: Zoology

Nutritional biology of synanthropic mites (Acari: Acaridida)

Potravní biologie synantropních roztočů (Acari: Acaridida)

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Prague, 2012

DECLARATION

I hereby declare that this Ph.D. thesis is of my own composition and that it contains no material previously submitted for the award of any other degree. I have faithfully and exactly cited all the sources.

....
          Date   Tomáš Erban

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CONTENTS

Ph.D. THESIS TITLE

Nutritional Biology of Synanthropic Mites (Acari: Acaridida)

ABSTRACT

Several attempts to describe the nutritional biology of acaridid mites were undertaken, however full understanding of these processes remains incomplete. The objective of this Ph.D. thesis was to expand our knowledge concerning digestive physiology of stored product and house dust mites and to apply this knowledge to their nutritional biology. The research approach adopted in this Ph.D. thesis includes *in vitro* characterization of enzymatic activity in whole mite extracts (WME) and spent growth medium extracts (SGME), evaluation of the enzyme activities with respect to the gut physiological pH, enzyme inhibition experiments, *in vivo* localization of enzyme activities in the mite gut, determination of effects of nutrient or antifeedant additives in experimental diets on mite population growth and determination of the feeding preferences of synanthropic mites as assessed by *in vitro* and *in vivo* analyses. The gut contents of twelve species of synanthropic acaridid mites were determined to be within a pH range of 4 to 7 and showed a pH gradient from the anterior to the posterior midgut. The pH in digestive tract of synanthropic acaridid mites corresponds to the activity of proteases, α-glucosidases, α-amylases and bacteriolytic enzymes. The activity of these enzymes represents the major digestive activity in mites; however, different mite species vary in enzymatic activity. The house dust and stored product mites are capable of utilizing bacteria as a food source. They are also adapted to digest sucrose, starch-type substrates and proteins. *In vivo* enzymatic results showed that bacteria, starch and most of protein digestion were localized mainly in ventriculus and caeca; however, some enzyme activities were localized in posterior midgut. The enzymatic activity corresponds to the presence of food and the elevated enzymatic activity can affect the metabolic needs of mites. The findings also underline the importance of ecological interactions between mites and other microorganisms. Because the digestive enzymes present in mite feces are major human allergens, the medical and economic aspects of digestive enzymes are also discussed. The results section of the Ph.D thesis comprises five peer-reviewed articles.

Keywords: digestion, enzyme, inhibitor, allergen, mite

NÁZEV DISERTAČNÍ PRÁCE

Potravní biologie synantropních roztočů (Acari: Acaridida)

ABSTRAKT

V minulosti bylo provedeno několik pokusů o popsání potravní biologie akaroidních roztočů, nicméně plné porozumění těmto procesům pozbývá úplnosti. Cílem této disertační práce bylo rozšířit naše znalosti týkající se trávicí fyziologie skladištních a prachových roztočů a aplikovat tyto znalosti na jejich potravní biologii. Výzkumný přístup použitý v této disertační práci zahrnuje *in vitro* charakterizaci enzymové aktivity v celotělních extraktech (WME) a extraktech zbytkového růstového média (SGME), vyhodnocení enzymové aktivity s ohledem na fyziologické pH střeva, enzymatické inhibiční experimenty, *in vivo* lokalizaci enzymových aktivit ve střevě roztočů, určení efektů výživných nebo růst inhibujících aditiv v experimentálních dietách na populační růst roztočů a určení potravních preferencí synantropních roztočů na základě vyhodnocených *in vitro* a *in vivo* analýz. pH střeva dvanácti druhů synantropních akaroidních roztočů bylo determinováno v rozmezí pH 4 až 7 a ukázalo gradient v pH z předního do zadního střeva. pH v trávicím traktu synantropních akaroidních roztočů koresponduje aktivitám proteáz, α-glukosidáz, α-amyláz a bakteriolytických enzymů. Aktivita těchto enzymů reprezentuje hlavní trávicí aktivitu v roztočích, nicméně různé druhy roztočů se liší v enzymatické aktivitě. Prachoví a skladištní roztoči jsou schopni využívat bakterie jako potravní zdroj. Jsou také adaptovaní na trávení sacharózy, škrobových substrátů a proteinů. *In vivo* enzymové výsledky ukázaly, že trávení bakterií, škrobu a většiny proteinů bylo lokalizováno ve ventriculu a caecách, nicméně některé enzymové aktivity byly lokalizovány v zadním střevu. Enzymatická aktivita koresponduje s přítomnou potravou a zvýšená enzymová aktivita může ovlivňovat metabolické potřeby roztočů. Zjištění také zvýrazňují význam ekologických interakcí mezi roztoči a dalšími mikroorganismy. Protože trávicí enzymy reprezentují v exkrementech roztočů hlavní lidské alergeny, medicínské a ekonomické aspekty trávicích enzymů jsou také diskutovány. Výsledkovou část disertace tvoří pět publikací s impact-factorem a tedy recenzovaných metodou peer-review.

Klíčová slova: trávení, enzym, inhibitor, alergen, roztoč

AIMS OF THE Ph.D. THESIS

The goal of this Ph.D. thesis is to expand our knowledge concerning digestive enzymes of synanthropic acaridid mites and to apply this knowledge to their nutritional biology.

Specific Aims:

a) Determine the pH in the gut of stored product and house dust mites.

b) Characterize enzymatic activity in whole mite extracts (WME) and spent growth medium extracts (SGME).

c) Localize enzymatic activity in the mite midgut.

d) Determine the effects of nutrient and/or antifeedant additives in experimental diets on mite population growth.

e) Determine the feeding preferences of house dust and stored product mites as assessed by *in vitro* and *in vivo* analyses.

1.   GENERAL INTRODUCTION

1.1.   Foreword

The need for energy of animals reflects food resources in habitats and is crucial to sustain life. Although the nutritional biology and digestive enzymes of synanthropic acaridid mites have been studied for many decades, full understanding of these processes remains very incomplete. In particular, nutritional studies of enzymatic activities are lacking. The general introduction of this Ph.D. thesis summarizes recent knowledge concerning mite digestive enzymes and allergens and the digestive capability of mite gut. These findings are applied to the nutritional biology of synanthropic acaridid mites. The digestive enzymes in this Ph.D. thesis were selected based on food preferences of stored product and house dust mites. The house dust mites are found mostly in house dust where they can feed on the shed skin of humans and domestic animals. Stored product mites are usually found in grain or in various cereal products. In addition to the digestion of food, enzymes play an important role in the interaction between mites and microorganisms growing on decaying organic matter. The results and discussion section of the Ph.D. thesis comprises five peer-reviewed papers.

1.2.   Mites (Acari)

Mites (Acari) are the most diverse group of arachnids (Arachnida) with about 45,000 described species. Mites are typically very small in size (0.09-30 mm of compact body). Mites inhabit all types of habitats and they can be either free-living or parasitic. Free-living mites are more common. The free-living mites possess many physiological adaptations that enable them to act as predators, saprophages, fungivores, phytophages, microphages, coprophages and necrophages (Alberti & Coons 1999, Walter *et al.* 2006).

   The taxonomy of mites remains a matter of debate; however, the following is the most common classification scheme (Alberti & Coons 1999):

   Group: Parasitiformes (Anactinotrichida)
        Suborder: Opilionacarida (Notostigmata)
                Holothyrida (Tetrastigmata)
                Ixodida (Metastigmata)
                Gamasida (Mesostigmata)

   Group: Acariformes (Actinotrichida)
        Suborder: Actinedida (Trombidiformes *sensu lato*)
                Oribatida (Cryptostigmata)
                Acaridida (Astigmata)

1.3.   Synanthropic Acaridid Mites

Nearly 300 species of mites are associated with human habitats as household comensals, pests of stored products or parasites (Montealegre *et al.* 2002). Synanthropic acaridid mite species are usually classified into two artificial groups: (1) house dust mites (HDMs) and (2) stored product mites (SPMs). Both groups are of high medical and economic importance (Hughes 1976, Colloff 2009). Both groups are also known as ''domestic mites'' because many stored product mites from families Acaridae, Glycyphagidae, and Chortoglyphydae have also been found in house dust (Colloff & Spieksma 1992).

It is believed that these synanthropic acaridid mites are derived from an ancestral fungivorous mite that originally inhabited the soil and migrated into human habitats from the nests of birds and mammals during the Neolithic revolution (OConnor 1979, OConnor 1982). It is also believed that the acaridid mites originated within the Oribatida (Norton 1998, Dabert *et al.* 2010) and that the sheep scab mite *Psoroptes ovis* is an ancestor of *Dermatophagoides* spp. (Hamilton *et al.* 2003). However, the molecular analysis of two molecular markers, ribosomal 18S RNA (18S) and the nuclear elongation factor 1 alpha (*ef1*) gene, did not support such a close relationship between Acaridida and Oribatida, and instead placed Acaridida outside monophyletic Oribatida (Domes *et al.* 2007). More recent phylogenetic analysis of Acariformes using sequence data from the nuclear small subunit rRNA gene (18S rDNA) and the mitochondrial cytochrome c oxidase subunit I (COI, amino acids) supported origin of Acaridida (Astigmata) within Oribatida (Dabert *et al.* 2010). Two orders within monophyletic Acariformes were recognized: Sarcoptiformes, consisting of Endeostigmata and Oribatida + Astigmata, and Trombidiformes. The data revealed the origin of Astigmata within Oribatida with the desmonomatan superfamily Crotonioidea as the source of astigmatan radiation and the sexual family Hermanniidae as the sister group, which generally supports previous morphological hypotheses (Dabert *et al.* 2010). Such an origin of acadidid mites would mean that their digestive adaptations are similar to those of the oribatid mites of the soil and of mites found in vertebrate nests. Whatever the evolutionary origin of these mites, it is possible to apply some aspects of the research done on Oribatida or other mite or arthropod species or even other animals to the stored product and house dust Acaridida.

1.4.   Digestive Adaptations of Acaridid Mites

Many digestive adaptations are based on the structure of the mite feeding "apparatus" and digestive enzymes (Schuster 1956, Luxton 1972, Akimov 1985, Kaneko 1988). Mites, like other animals, are equipped with numerous enzymes to digest food. House dust and stored

product mites have developed a diversity of enzymes, including carbohdrases, proteases, phosphatases and lipases/esterases needed to digest the different types of food found in their habitats. The presence and activity of digestive enzymes is an important determinant of the feeding ability of mites (Luxton 1972, Akimov & Barabanova 1976b, Akimov & Barabanova 1978, Akimov 1985, Siepel & de Ruiter-Dijkman 1993, Robinson *et al.* 1997). The digestive enzymes of house dust and stored product mites are extensively studied because they are medically important proteins, and because many of them are very important allergens (Tovey *et al.* 1981, Robinson *et al.* 1997, Fernandez-Caldas & Calvo 2005, Thomas *et al.* 2010). Therefore, understanding mite nutritional biology and digestion is important both for understanding the adaptations mites have evolved in order to survive in the human environment as well as for understanding the medical importance of mites. In the following sections are described essential enzymes that participate in mites' digestion of proteins, carbohydrates (saccharides), bacteria and fungi. The impact of gut physicochemical properties and mite-symbiotic interactions in utilization of food sources is also mentioned.

1.4.1. Gut Morphology and Food Processing

The gut of synanthropic acaridid mites is divided to the following parts: (i) foregut – pharynx and esophagus; (ii) midgut – ventriculus, paired caeca, colon, intercolon and postcolon; and (iii) hindgut – anal atrium (Sobotnik *et al.* 2008a, Wu *et al.* 2009). It is suggested that caeca and vetriculus play a key role in food digestion because they compose a large portion of the gut (Wu *et al.* 2009). Some species also have a postcolonic diverticula, sometimes called Malphighian tubules, in their gut (Hughes 1950, Sobotnik *et al.* 2008a, Wu *et al.* 2009). The transmission electron microscopy (TEM) showed that the postcolonic diverticula of *A. siro* can host bacterial symbionts (Sobotnik *et al.* 2008a).

Mites pulverize food using cheliceres. Food then enters the pharynx and passes through the esophagus into the ventriculus (Sobotnik *et al.* 2008a). The food is concentrated into the middle of the ventriculus and becomes enveloped by a peritrophic membrane (Wharton & Brody 1972), which includes chitin fibers (Sobotnik *et al.* 2008b). The ventricular and caecal lumen contains fine particles probably of mucoid nature and digestive enzymes. Endogenous enzymes are produced by secretory cells in the ventriculus and caeca (Hughes 1950, Kuo & Nesbitt 1970). The enzymes that are enclosed by or attached to the peritrophic membrane are concentrated into food bolus, resulting in higher enzymatic activity in the feces than in the whole body (Stewart *et al.* 1992a, Stewart *et al.* 1994b). The food bolus then passes to the colon and intercolon. The next part of the midgut is the postcolon, a long, wide chamber that

is separated from the intercolon by an inconspicuous constriction. Finally, the food bolus enters the anal atrium and is defecated (Sobotnik *et al.* 2008a).

1.4.2. Role of pH in Digestion

The pH of the gut contents is considered the most important factors that affects digestive processes. The pH in the gut is important for the activity of digestive enzymes and there is a close relationship between gut pH and the pH optima of digestive enzymes (Terra *et al.* 1996, Funke *et al.* 2008). Such a close relationship explains the event that enzymes contain basic and acid groups which charge is changing with the pH of their environment. In addition, the velocity of enzymatic reaction is affected by many other condions, i.e. temperature and ionic strength of the solution (Copeland 2000).

The study of digestive enzymes should be performed with respect to the gut physiological pH (Terra & Ferreira 1994, Regel *et al.* 1998, Funke *et al.* 2008). The pH of the midgut lumen is actively regulated and varies with phylogeny and feeding ecology (Harrison 2001). The gut physicochemical properties are optimized with respect to the food source (Zimmer & Brune 2005). The pH in the gut can also influence the solubility of food components, the dissociation or coagulation of ingested proteins, and the presence of gut microflora (Funke *et al.* 2008).

Several attempts to determine the acidobasic conditions of acaridid mites to understand their digestive physiology were undertaken. For determination of the physiological pH in mites were used pH indicators such as litmus, phenol red, universal indicator or neutral red (Hughes 1950, Akimov & Barabanova 1976b, Akimov & Barabanova 1978, Akimov 1985). Hughes (1950) determined the pH in the ventriculus and caeca of *Acarus siro* (syn. *Tyroglyphus farinae*) to be between 5.0 and 6.0, in the colon above 7.0 and in the postcolon below 8.0 (Hughes 1950). Overall in 16 mites was pH determined to be in the range of 5.4 to 6.3 in the caeca and ventriculus, 5.9 to 7.4 in the colon, and 6.8 to 8.0 in the postcolon (Akimov & Barabanova 1976b, Akimov & Barabanova 1978, Akimov 1985).

1.4.3. Chemical Breakdown of Key Food Components

1.4.3.1.   Digestion of Proteins

Digestion of proteins is catalyzed by proteases. In animals, proteins serve as a source of aminoacids that are primarily utilized for protein biosynthesis. Proteins serve also as important source of nitrogen. When susbtrates for catabolic reactions are unavailable,

aminoacids can be converted to glucose, a main source of energy, through gluconeogenesis (Mallette *et al.* 1969, Exton *et al.* 1970, Voet & Voet 2003, Bilsborough & Mann 2006).

Protease is any enzyme that catalyzes the hydrolytic cleavage of peptide (amide) bonds of proteins and peptides. Proteases are classified to two groups endopeptidases and exopeptidases and are further dividied to groups based on character of their active site (Beynon & Bond 2001). Endopeptidases (EC 3.4.21-24) split proteins into smaller peptides by targeting peptide bonds near the center of the molecule and may cause esterolysis, and they exhibit various degrees of amino acid specificity (Berrens 1968). The exopeptidases (EC 3.4.11-19), aminopeptidases (EC 3.4.11) and carboxypeptidases (EC 3.4.16-18) act only near the ends of polypeptide chains. Described mite endopeptidases belong to one of the following three groups: (i) serine proteases (EC 3.4.21), with the presence of a serine residue in the active site involved in catalysis; (ii) cysteine proteases (EC 3.4.22), utilizing a Cys residue for their catalytic activity; and (iii) aspartate proteases (EC 3.4.23), utilizing Asp residue for catalysis (Beynon & Bond 2001).

Trypsin serine proteases (EC 3.4.21.4) preferentially cleave protein chains with arginine (Arg) and lysine (Lys) residues (Weiner *et al.* 1985). Trypsin-like serine proteases were detected in whole mite extracts and in feces rich fraction spent growth medium extract (SGME), suggesting their digestive potential (Stewart *et al.* 1992a, Stewart *et al.* 1992c, Ando *et al.* 1993, Stewart *et al.* 1994a, Stewart *et al.* 1994b, Ortego *et al.* 2000, Sanchez-Ramos *et al.* 2004). The molecular weight of house dust and stored product mite trypsins ranges from 28 kDa to 30 kDa (Table 1). In addition, Der f3, a trypsin allergen, was shown to be localized in the postcolon and in fecal pellets using monoclonal antibody against recombinant Der f3 (Zhan *et al.* 2010).

Chymotrypsins (EC 3.4.21.1) are serine endopeptidases that preferentially cleave protein chains on the carboxyl side of aromatic amino acids (Beynon & Bond 2001). The mite chymotrypsins are considered to be digestive enzymes, because their activity was identified in mite feces (Stewart *et al.* 1992a, Ortego *et al.* 2000, Sanchez-Ramos *et al.* 2004). Mite chymotrypsins are also considered important mite allergens similarly to mite trypsins. Chymotrypsin-like activity exhibited group 6 and group 9 mite-derived allergens (Table 1). Whereas group 6 are typical chymotrypsins, Der p9 is a collagenase, which is enzymatically similar to chymotrypsin and cathepsin G (King *et al.* 1996). This similarity of Der p9 to cathepsin G may be connected to antibacterial features of this allergen (Shafer *et al.* 2002).

The most familiar mite proteases are allergens of group 1 (Table 1). These proteases are termolabile glycoproteins that are structurally homologous to Cathepsins B and H, papain and

actidine (Khlgatyan & Perova 1995). The activity of these 24 to 39 kDa glycoproteins has traditionally been attributed to cysteine protease activity. More recently, it was established that Der p1 contains both cysteine and serine components and exhibits dual pH optima at pH 6 and pH 8 (Brown *et al.* 2003). These allergens are localized in the digestive tract and feces of pyroglyphid mites (Tovey *et al.* 1981, Tovey & Baldo 1990); the greatest concentration is observed in the ventriculus and caeca (Thomas *et al.* 1991). The possible digestive function of group I mite allergens may be related to the replacement of serine proteases by cysteine proteases. This event was described in insects (Oppert *et al.* 2005). Ortego *et al.* (2000) detected in *Tyrophagus putrescentiae* a 3-fold greater cathepsin B activity in the feces than in mite bodies. In comparison, the activity of serine proteases was 50-fold higher in SGME. These indicate that the digestive function of cysteine proteases may be minor in comparison to serine proteases (Ortego *et al.* 2000).

In some insects, aspartate proteases are involved in digestion and are related to the replacement of serine proteases (Terra & Ferreira 1994). The digestive function of aspartate proteases in mites has not been confirmed (Ortego *et al.* 2000, Nisbet & Billingsley 2000, Sanchez-Ramos *et al.* 2004).

Aminopeptidases hydrolyze single amino acids from the N-terminus of peptide chains. The activity of leucine aminopeptidase was observed in the *Dermatophagoides* species and in *Tyrophagus putrescentiae* (Stewart *et al.* 1992a, Ortego *et al.* 2000). Leucine and valine aminopeptidases were detected in the extracts of *Acarus siro* and *Psoroptes ovis* (Nisbet & Billingsley 2000). Insect glycoproteins located in the midgut membrane are very similar to the enzyme aminopeptidase N (EC 3.4.11.2) and serve as a receptor for δ-endotoxins of insecticidal *Bacillus thuringiensis* (Knight *et al.* 1994, Budatha *et al.* 2007).

Carboxypeptidases act at the free C-terminus of polypeptides and liberate single amino acid residues. Carboxypeptidases A and B were 50-fold more active in feces extracts than in the mite homogenates of *T. putrescentiae,* indicating a digestive function (Ortego *et al.* 2000). In addition, carboxypeptidases A and B have been detected in *D. pteronyssinus* and *D. farinae* (Stewart *et al.* 1991).

Collagen, keratin and elastin are thought to be an important food source for pyroglyphid mites (van Bronswijk & Sinha 1974). In addition, Bowman (1981) suggested that collagenous material could be digested by *Acarus siro*, *Lepidoglyphus destructor*, *Glycyphagus domesticus*, *Rhizoglyphus callae* and *R. robini* mites (Bowman 1981). However, these substrates are not a suitable source of nutrition for astigmatid mites (Bowman 1981). Ortego *et al*. (2000) did not find elastase activity in *Tyrophagus putrescentiae* (Ortego *et al.* 2000).

Digestion of low-cysteine, epidermal cytoskeletal α-keratin probably does not need specialized enzymes and the keratinolysis can be achived by trypsins and chymotrypsins. However the house dust mites are equipped also by cysteine proteases having the ability to hydrolyze keratin. Moreover, the keratinous substrates could be utilized through symbiotic interactions of the mites and microorganisms (Colloff 2009).

1.4.3.2.   Digestion of Starch, Maltose and Sucrose

The most abundant class of organic compounds found in stored plant products, especially in cereals, are carbohydrates (saccharides). Glycosidases are enzymes that catalyze the hydrolysis of a bond joining the sugar of a glycoside to an alcohol or to another sugar unit. Starch and sucrose are the primary digestible carbohydrates (Nichols *et al.* 2003). Starch is degraded by the combined action of α-amylases (EC 3.2.1.1), α-glucosidases (EC 3.2.1.20) and α-dextrinases (EC 3.2.1.10; EC 2.4.1.2). The products of α-amylase are maltotriose and maltose from amylose. Amylopectin is hydrolyzed to maltose, glucose and a lesser amount of dextrin. Sucrose is degraded by invertase (beta-fructofuranosidase) enzymes (EC 3.2.1.26) (Voet & Voet 2003).

The enzymatic activities of α-amylases and α-D-glucosidases have been measured in many species of mites (Matsumoto 1965, Akimov & Barabanova 1978, Bowman & Childs 1982, Bowman 1984, Akimov 1985, Morgan & Arlian 2006). Additional amylase isoforms have been observed in the WME and SGME of mites (Stewart *et al.* 1992a, Stewart *et al.* 1998). In addition, α-amylases are group 4 mite allergens and their molecular weight ranges from 56 to 60 kDa (Table 1). *D. pteronyssinus* and *E. maynei* α-amylases are physicochemically and sequence similar to mammalian α-amylase. The pH optimum was 6.4 (Lake *et al.* 1991b, Mills *et al.* 1999).

1.4.3.3.   Digestion of Bacteria

Bacteria are not considered to be a mite food source (Luxton 1972, Sinha & Harasymek 1974, Siepel & de Ruiter-Dijkman 1993). Symbiotic mite-bacterial interactions are hypothesized to exist, especially the breakdown of hard digestible polysaccharides by microflora in the gut (Smrz *et al.* 1991). Sinha & Harasymek (1974) observed the survival and reproduction of *Glycyphagus domesticus* and *Acarus siro* using six species of bacteria as a food source. Mortality was relatively high, but the mites were able to survive on pure bacteria for more than seven days; this was a longer survival than when mites were not provided food. Although the mortality on a diet of pure bacteria was relatively high, mites were able survive for more than 50 days. A difference in tolerance to various species of bacteria was confirmed (Sinha &

Harasymek 1974). Bacterial cell walls are small in size (usually 1 to 3 µm in diameter) they are ingested without mechanical damage by chelicerae, unlike the ingestion of larger fungal mycelium and spores (Schuster 1956, Kaneko 1988, Sobotnik *et al.* 2008a). Thus, to utilize nutrients from bacteria, bacterial cell walls must be digested.

Diverse bacterial colonies were observed in the parasitic sheep scab mite *Psoroptes ovis* (Acari: Psoroptidae), a taxonomically similar species to *Dermatophagoides* sp. (Hogg & Lehane 1999). It is suggested that *Psoroptes ovis* digestive enzymes are supplemented with bacteria as a direct and indirect source of nutrition (Nisbet & Billingsley 2000, Hamilton *et al.* 2003). Some bacteria were also found in *D. pteronyssinus* and *D. farinae* (Oh *et al.* 1986, Valerio *et al.* 2005). Recently, Hubert *et al.* (2011) described bacterial communities in *A. siro*, *L. destructor*, *T. putrescentiae* and *D. farinae*. Among the commonly detected bacteria were *Bacillus* spp. and *Staphylococcus* spp. (Hubert *et al.* 2012) which were also detected in house dust (Horak *et al.* 1996). These findings suggest that mites ingest bacteria. However, to utilize bacteria as a food source the gut of mites must be adapted by appropriate enzymes and physicochemical properties.

The most famous antibacterial agents are enzymes lysozymes. Lysozymes are thought to digest bacterial cell walls. Lysozymes (1,4-β-N-acetylmuramidase; EC 3.2.1.17) are enzymes that break down peptidoglycan (murein), a component of the bacterial cell wall, by hydrolyzing the $\beta(1 \rightarrow 4)$ glycosidic bond from N-acetylmuramic acid (NAM) to N-acetylglucosamine (NAG) (Chipman & Sharon 1969). Lysozymes are widely distributed and are present in many animals (Callewaert & Michiels 2010). The primary function of this enzyme is to protect against attack by pathogenic bacteria, as a part of the nonspecific immune system (Jolles & Jolles 1984). However, in many invertebrates and vertebrates, lysozymes have a digestive function (Callewaert & Michiels 2010). These digestive lysozymes are resistant to proteolytic degradation (Lemos *et al.* 1993). Furthermore, lysozymes exhibit weak non-specific esterase and proteolytic activity. These activities are topographically distinct form the lysozyme lytic site (Jolles & Jolles 1983, Oliver & Stadtman 1983). The digestive function of lysozymes has been studied in haematophagous soft tick *Ornithodoros moubata* (Grunclova *et al.* 2003). In mites, lysozyme activities were detected in the WME and SGME of stored product and house dust mites (Childs & Bowman 1981, Stewart *et al.* 1992b, Stewart *et al.* 1998). The lysozymes of *D. farinae* *D. pteronyssinus* have a molecular weight of 10 kDa to 13 kDa and are optimally active in a pH of 6.2 (Stewart *et al.* 1991, Stewart *et al.* 1992b). There are many lysozyme isoforms present in *L. destructor* (Stewart *et al*. 1998). The possibility that the 14 kDa "lysozyme-like" (group 2 mite allergens,

see table 1) protein is lysozyme (Stewart *et al.* 1992b) was negated and the "lysozyme-like" proteins are now assigned as NPC2 proteins (Ichikawa *et al.* 2005). Mathaba *et al.* (2002) purified from the SGME of *D. pteronyssinus* a 14.5-kDa (size in BLAST) protein with antibacterial activity and suggested prokaryotic origin of the protein (Mathaba *et al.* 2002).

Although the bacterial cell wall is highly resistant to disruption by the proteases (Voet & Voet 2003), some proteases have evolved the ability to digest these walls. In the larvae of *Musca domestica* (Diptera: Cyclorrhapha), the central region of the midgut can digest bacteria. These larvae grow in decaying organic material and digest material under acidic pH using digestive lysozymes and cathepsin D-like (aspartate) proteases (Espinoza-Fuentes *et al.* 1987, Lemos & Terra 1991a, Lemos & Terra 1991b). Similarities were found among cyclorrhaphan larvae, *Anastrepha fraterclus* (Lemos & Terra 1991b), and *Drosophila melanogaster* (Kylsten *et al.* 1992, Regel *et al.* 1998). Whereas the acid conditions in the ventriculus and caeca are favorable for digesting bacteria, the digestive function of aspartate proteases in mites has not been confirmed (Ortego *et al.* 2000, Nisbet & Billingsley 2000, Sanchez-Ramos *et al.* 2004).

1.4.3.4.   Digestion Fungal Contents

It is well known that acaridid and oribatid mites are associated with microorganisms, such as fungi (Siepel & de Ruiter-Dijkman 1993, Hubert *et al.* 2003, Hubert *et al.* 2004, Smrz & Catska 2010). The stored product mites have fungal vectors and can undergo selective transfer (Sinha 1979, Armitage & George 1986, Hubert *et al.* 2003, Hubert *et al.* 2004). Sinha and Harasymek (1974) studied the survival and reproduction of stored product mites *G. domesticus* and *A. siro* using 19 species of fungi as a food source. In each case the mites survived longer (more than 7 days) on pure fungi than without food (Sinha & Harasymek 1974). Recently it was demonstrated that stored product mites *T. putrescentiae*, *A. siro* and *L. destructor* are able to feed on various species of *Fusarium* fungi. *T. putrescentiae* was able to feed and reproduce on all ten *Fusarium* strains tested (Nesvorna *et al.* 2012).

Oribatid mites can influence decomposition by feeding on microorganisms to affect their metabolic activity (Siepel & Maaskamp 1994). The digestion of fungal cell walls was observed in *T. putrescentiae* (Smrz & Catska 1989, Nesvorna *et al.* 2012). *Dermatophagoides pteronyssinus* has been associated with fungi *Aspergillus penilloides* (de Saint Georges-Gridelet 1987). Hubert & Sustr (2001) observed that fungal cell walls remain undigested and spores can retain their viability after passage through the gut (Hubert & Sustr 2001).

The most energy beneficial substances of both cell walls and cell contents in fungi include: trehalose, chitin, glycogen, lipids, proteins and sugar alcohols (Bowman & Free 2006,van Leeuwen *et al.* 2012). Fungi can perform extracellular digestion by secreting enzymes into the environment and absorbing the nutrients thus produced (Romani *et al.* 2006).

The major constituents of the fungal cell wall are glucan, chitin, and proteins. Chitin is a tough and rigid glucosamine homopolymer that functions as a protective structure in the exoskeleton of fungi and arthropods (Lee *et al.* 2010). Chitinolytic enzymes include chitinase (EC 3.2.1.14), which catalyze the hydrolysis of internal bonds in chitin and β-acetyl-D-glucosaminidase (chitobiase, EC 3.2.1.52), thus liberating N-acetylglucosamine from the non-reduced end of oligosaccharides (Terra & Ferreira 1994). Another chitinolytic enzyme is lysozyme (see section 2.3.3.3.) (Skujins *et al.* 1973). In addition, some chitinases act as lysozyme and in some cases they can be utilized for defense against pathogens as well as lysozymes (Minic *et al.* 1998).

Chitinases are enzymes that hydrolyze the β(1,4)-N-acetyl-D-glucosamine bonds within chitin, an essential fungal cell wall component. Chitinases are enzymes required for the growth and morphogenesis of fungi and arthropods, including mites (Alberti & Coons 1999, Adams 2004, Rao *et al.* 2005, Hurtado-Guerrero & van Aalten 2007). In general, chitinase functions in arthropods in the molting process. Nevertheless, this enzyme is localized to the midgut in some species (Terra *et al.* 1996). The utilization of chitin from fungal cell walls has been studied (Smrz & Catska 1989, Siepel & de Ruiter-Dijkman 1993). Chitinolytic activity was detected in the WME of acaridid mites (Bowman & Childs 1982) with an optimal pH from 5 to 7.5 (Akimov & Barabanova 1976a, Akimov & Barabanova 1976b, Akimov & Barabanova 1978, Akimov 1985). More recently, the chitinase isoforms Der f15 and Der f18 (Table 1) have been purified from *Dermatophagoides farinae*. Both chitinases are allergenic (McCall *et al.* 2001, Weber *et al.* 2003). Localization of Der f15 using a monoclonal antibody showed intracellular distribution in the mite midgut, suggesting a digestive rather than a molting-related function (McCall *et al.* 2001). Der f18 is similar to Der f15, which is localized in the digestive system of *D. farinae* but not to the feces (Weber *et al.* 2003).

Fungi store excess energy as glycogen. Glycogen is a starch polymer of glucose and has analogous structure to plant starch. Whereas glycogen is composed of a branched chain of glucose units, starch is a long, straight chain of glucose units (Voet & Voet 2003). The combined action of amylases and α-glucosidases digest glycogen similarly to starch in mites (see section 2.3.3.2). The amylase-mediated digestion of glycogen from spores and mycelia from fungi has been reported in mites Bowman & Childs (1982).

Trehalose (α,α-trehalose) is a non-reducing and widely distributed disaccharide and has been isolated from several species of bacteria, fungi, invertebrates and plants. Trehalose functions as a source of energy and protects against the effects of freezing or dehydration (Richards *et al.* 2002, Iturriaga *et al.* 2009). Trehalose is very stable (in comparison to sucrose and maltose) (Richards *et al.* 2002) and the enzyme that splits trehalose into two glucose units is trehalase (EC 3.2.1.28) (Dahlqvisz 1962). Threhalase is one of the most widespread carbohydrases in insects (Terra & Ferreira 1994) The function of trehalose and trehalase have been studied extensively and appear to be species-dependent (Richards *et al.* 2002).

Trehalose provides an energy source for flight in a variety of insects, and hydrolysis of trehalose is likely to be a specific adaptation to flight (Clegg & Evans 1961, Richards *et al.* 2002). The role of trehalase in chitin synthesis during molting has also been reported (Tatun *et al.* 2008). In past decades, the digestive function of trehalase has been described. Apical and basal trehalases have been found in the insect midgut. The apical midgut trehalase is a true digestive enzyme, whereas the basal trehalase probably plays a role in the midgut utilization of haemolymph trehalose (Terra & Ferreira 1994). In recent years, trehalases have been purified from several insect species and are divided into soluble (Tre-1) and membrane-bound (Tre-2) proteins. The function of Tre-1 and Tre-2 has been investigated in *Spodoptera exigua*. Tre-1 plays a major role in expression of the chitin synthase gene A and chitin synthesis in the cuticle. Tre-2 has an important role in the expression of chitin synthase gene B and chitin synthesis in the midgut (Chen *et al.* 2010).

Trehalase has been detected in the WME of oribatid mites (Siepel & de Ruiter-Dijkman 1993). However, mite trehalases have not been biochemically characterized. The presence of trehalase is suggested as an adaptation to the digestion of fungi in oribatid and acaridid mites (Luxton 1972, Siepel & de Ruiter-Dijkman 1993).

1.4.3.5.   Digestion of Lipids

Lipids serve as a source of energy for animals and are present in seeds and in the fat of animals and fungi as well as in cell membranes (Voet & Voet 2003). Esterases digest lipids (EC 3.1.-.-), and lipases are a subclass of esterases. Esterases and lipases both hydrolyse ester bonds. Whereas the lipases display high activity towards the substrate in an aggregated state, the activity of esterases is typically highest toward the soluble state of the substrate (Fojan *et al.* 2000).

Alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) activity has been detected in acaridid mites (Bowman 1984, Stewart *et al.* 1992a). Lipases were detected in the

SGME of house dust mites and are thought to be digestive (Stewart *et al.* 1991, Stewart *et al.* 1992a). A comparative study showed that extracts of *A. siro* and *P. ovis* contain acid and alkaline phosphatase, C4 and C8 esterases and lipase. *A. siro* has low C14 lipase activity in comparison to C4 esterase and C8 esterase lipase (Nisbet & Billingsley 2000). Lipids are considered important digestible part of skin scales for house dust mites. However skin scales from people with atopic dermatitis have lower total lipid concentration than those of non-atopics and people with psoriasis (Colloff 2009).

1.4.3.6.   Enzymatic Degradation of Cellulose

Polysaccharide cellulose is widely distributed in few bacteria, protists, fungi, invertebrate animals, and plants. It is the most abundant compound in plant cell walls, contributing to approximately 20–40% of its dry weight (Watanabe & Tokuda 2010).

Cellulose is a linear polymer that consists of β-1,4-linked D-glucopyranosyl units. It is synthesized in every higher-order plant. Cellulose differs from starch and glycogen and is extremely hard to digest due to its structure. The enzymes that digest cellulose are cellulytic enzymes, of which three classes are recognized: endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.74 and 3.2.1.91), and β-glucosidases (EC 3.2.1.21) (Watanabe & Tokuda 2010).

Cellulolytic activity was detected in several stored product mites, whereas most of the mites lacked cellulase activity (Bowman & Childs 1982, Bowman 1984). Nevertheless, to date, cellulase has not been isolated or characterized. The optimal pH for cellulolytic activity in the WME of acaridid mites varies from pH 5 to 8 (Akimov & Barabanova 1976a, Akimov 1985). Bowman and Childs (1982) suggested that the cellulolytic activity is a result of microorganism exoenzymes in the mite gut (Bowman & Childs 1982).

1.4.3.7.   Digestive Gut Microflora

The mite midgut and body fat contain associated microflora that produce exogenous enzymes (Smrz 2003, Smrz & Catska 2010). Members of the intestinal microbial community have the ability to decompose cellulose, chitin and lignin (Stefaniak & Seniczak 1976, Smrz 2003)). Chitinase and trehalase activity in mites assist in the mite digestive process, as do bacterial exoenzymes and the symbiotic relationship between bacteria and mites (Smrz *et al.* 1991, Smrz & Trelova 1995, Smrz 1998, Smrz & Catska 2010). The type of species and pattern of feeding and digestion is important for the relationship between the mites and internal/extraintestinal bacteria. The amount of bacteria in mesenchymal tissue of *T. putrescentiae* was correlated with chitinase activity in mite homogenate. *Serratia marcescens* exhibit strong chitinolytic and trehalolytic activity in *T. putrescentiae* (Smrz 2003). In *A. siro*,

the transmission microscopy (TEM) observation of the gut showed that the postcolonic diverticula contain symbiotic bacteria (Sobotnik *et al.* 2008a). The recently described internal bacterial communities in *A. siro*, *L. destructor*, *T. putrescentiae* and *D. farinae* supported the idea that the mites ingest bacteria. Among the common bacteria in these mites are *Bacillus* spp. and *Staphylococcus* spp. Beside parenchymal and reproductive tissues, the bacteria were located in the gut, suggesting that the bacteria are ingested with food and further processed by the mite gut (Hubert *et al*. 2012). However, to utilize bacteria as a food source the gut of mites must be adapted by appropriate enzymes and physicochemical properties.

Also intracellular bacteria that do not serve as direct food source play often important physiological role in arthropods, from these were in mites identified clones related to Bartonellaceae. Also the clones of high similarity to *Xenorhabdus cabanillasii* were found in *L. destructor* and *D. farinae*. In addition, members of Sphingobacteriales cloned from *D. farinae* and *A. siro* clustered with the sequences of "*Candidatus Cardinium hertigii*" (Hubert *et al*. 2012).

1.5.   Alergenic Proteins in Acaridid Mites

Currently, there are 58 known allergens from seven species of synanthropic acaridid mites. These allergens are classified into 24 groups and exhibit enzymatic and antimicrobial activity with a potential digestive function belonging to groups 1, 3, 4, 6, 9, 15, 18 and 19 (Table 1). Based on localization, the allergens are utilized for the digestion of groups 1, 3, 4 and 6; the digestive properties of groups 9 and 19 are speculative. Groups 15 and 18 have been localized to the midgut but not to the feces of mites (McCall *et al.* 2001, Weber *et al.* 2003). The list of stored product and house dust mite allergens including their description such as molecular weight and biochemical name is in Table 1. (for details see http://www.allergen.org). The allergens were recently reviewed by Collof (2009).

Table 1. Classes of mite allergens as denoted by the International Union of Immunological Societies (IUIS) list of allergens to date 09-2012 (for details see http://www.allergen.org). LEGEND: Aca sir – *Acarus siro*; Blo tro – *Blomia tropicalis*; Der far – *Dermatophagoides farinae*; Der mic – *Dermatophagoides microceras*; Der pte – *Dermatophagoides pteronyssynus*; Eur may – *Euroglyphus maynei*; Gly dom – *Glycyphagus domesticus*; Lep des – *Lepidoglyphus destructor*; Tyr put – *Tyrophagus putrescentiae*.

Gr.	Molecular weight (kDa)	Biochemical name	Species								
			Aca sir	*Blo tro*	*Der*			*Eur may*	*Gly dom*	*Lep Des*	*Tyr put*
					far	*mic*	*pte*				
1	25	cysteine protease		Blo t1	Der f1	Der m1	Der p1	Eur m1			
2	14-18	NPC2 family		Blo t2	Der f2		Der p2	Eur m2	Gly d2	Lep d2	Tyr p2
3	30	trypsin		Blo t 3	Der f 3		Der p3	Eur m3			
4	60	amylase		Blo t4			Der p4	Eur m4			
5	14	unknown		Blo t5			Der p5			Lep d5	
6	25	chymotrypsin		Blo t6	Der f6		Der p6				
7	22-28	unknown			Der f7		Der p7			Lep d7	
8	26	glutathione-S-transferase					Der p8				
9	24	collagenolytic serine protease					Der p9				
10	36	tropomyosin		Blo t10	Der f10		Der p10			Lep d10	Tyr p10
11	98	paramyosin		Blo t 11	Der f 11		Der p11				
12	14	unknown		Blo t12							
13	15	fatty acid-binding protein	Aca s13	Blo t13	Der f13					Lep d13	Tyr p13
14	80-100	apolipophorin			Der f14		Der p14	Eur m14			
15	98	chitinase			Der f15						
16	53	gelsolin/villin			Der f16						
17	53	calcium binding protein			Der f17						
18	60	chitinase			Der f18						
19	7	antimicrobial peptide homologue		Blo t19							
20	unknown	arginine kinase					Der p20				
21	unknown	unknown		Blo t21			Der p21				
22	unknown	unknown			Der f22						
23	14	unknown					Der p23				
24	18	troponin C									Tyr p24
Total			**1**	**12**	**14**	**1**	**15**	**5**	**1**	**5**	**4**

1.6.   Feeding Guilds

The classification of feeding guilds is important to understand the role of species within the biotope (Siepel & de Ruiter-Dijkman 1993). Due to the relationship between acaridid and oribatid mites (Norton 1998), we can apply knowledge of certain aspects of the nutritive biology of oribatid mites to acaridid species. Oribatid mites are a group of mites which live in the soil. In addition, they are considered the most important decompositors in mesofauna (Alberti & Coons 1999). It has been stated that the habits, nutrition, and large numbers of oribatid mites in soil must have an important effect on the environment. The major and minor feeding habits for oribatid mites have been defined (Luxton 1972):

A. Major feeding habits
1) Macrophytophages – feed strictly on higher plant material
    a) Xylophages – feed on woody tissue
    b) Phyllophages – feed on leaf tissue
2) Microphytophages – feed strictly on microflora
    a) Mycophages – feed on fungi and yeasts
    b) Bacteriophages – feed on bacteria
    c) Phycophages – feed on algae
3) Panphytophages – combine all or some of the sub-headings above and are similar to macrophytophages and microphytophages
B. Casual and incidental feeding habits
4) Zoophages – feed on living animal material
5) Necrophages – feed on carrion
6) Coprophages – feed on fecal material

It is thought that the nutritional capability of oribatid mites is relatively extensive. Two decades after Luxton´s classification later, Siepel & de Ruiter-Dijkman (1993) defined the feeding guilds of oribatid mites based on the carbohydrase activity and the dependence on cellulose, chitin and trehalose as food sources in 49 species of oribatid mites and one member of acaridid mites (Siepel & de Ruiter-Dijkman 1993). An analogous study was performed by Berg *et al.* (2004) on 20 Collembola species. Seven feeding guilds have been distinguished for soil arthropods (Berg *et al.* 2004). Hubert *et al.* (1999) measured the activities of α-amylases, laminarinase, xylanase, cellulase and trehalase in homogenates of the panphytophagous oribatid mite *Schlerobates laevigatus.* The results were used to determine the food preferences of the mite. (Hubert *et al.* 1999). In addition, several previous studies

have analyzed carbohydrase activity in oribatid mites (Zinkler 1971, Luxton 1972, Luxton 1979).

 In the study of Siepel & de Ruiter-Dijkman (1993), the enzymatic activity was used to delineate five major (guilds No. 1 to 5) and two minor (guilds No. 6 and 7) feeding guilds. In addition, they defined two basic groups; the members of the first group (guilds No. 1, 2, 3, 5 and 7) are able to digest both the cell wall and cell contents and the members of the second group (guilds No. 4 and 6) digest only the cell contents (Siepel & de Ruiter-Dijkman 1993). The following feeding guilds have been defined by Siepel & de Ruiter-Dijkman (1993):

1) Herbivorous grazers – have cellulase activity only; feed on both cell contents and the cell walls of green plants

2) Fungivorous grazers – have chitinase and trehalase activity; feed on fungi and dead mycelium and are able to digest both cell contents and the cell walls of fungi

3) Herbo-fungivorous grazers – are able to digest all main food components of both green plants and fungi

4) Fungivorous browsers – digest only trehalose

5) Opportunistic herbo-fungivores – are able to digest cellulose in litter and cell walls of living green plants and trehalose in fungi

6) Herbivorous browsers – lack of cellulase, chitinase and trehalase activities; these could be predators

7) Omnivores – an unexpected guild based to be distinguished based on carbohydrase activities

Siepel & de Ruiter-Dijkman (1993) mentioned that carbohydrase activities, especially those for the digestion of differentiating plant and fungal components, appear to provide a solid basis for the feeding guild definition. It is thought that the quantitative data of enzyme activities is the best characterization of guilds (Siepel & de Ruiter-Dijkman 1993, Siepel & Maaskamp 1994, Berg *et al.* 2004).

1.7.   Evolutionary Origin of Stored Product and House Dust Mites

The members of families Acaridae, Glycyphagidae, Chortoglyphydae, Carpoglyphydae comprise the most abundant and diverse group in stored product habitats. Pyroglyphidae are the most common mites in house dust (Hughes 1976, OConnor 1979). Stored product and house dust mites are synanthropic species, whose origin lies somewhere within Oribatida (Norton 1998, Dabert *et al.* 2010) (see also section 1.3.), and are ancestrally related to

fungivorous mites. These mites originally inhabited the soil and penetrated into human habitats through the nests of birds and mammals. Therefore, their digestive adaptations are similar to those of oribatid mites and the soil and vertebrate nests are thought to be their habitat. OConnor showed that the habitat preference of naturally occurring species may indicate the evolutionary origins of synanthropic acaridid mites (OConnor 1979, OConnor 1982, OConnor 1984). OConnor also concluded that the mites evolved tolerance by feeding under variable environmental conditions. Some mites reduced independence on hypopus for survival and many species often form deuteronymphs (OConnor 1979, OConnor 1982). The stored product and house dust mites are associated with specific resources and may be grouped into four categories (OConnor 1979, OConnor 1982, OConnor 1984). The species genera of Acaridida that relate to this Ph.D. thesis are listed below:

a) mites that are associated with specific resources, such as fruit or meat, which are not widely distributed in space or time

- *Carpoglyphus* (Carpoglyphidae) – infests rotting fruit in the field and other materials with high sugar content
- *Aeroglyphus* (Glycyphagidae) – is associated with a variety of specialized habitats, such as bat roosts, bird nests and nests of social insects

b) mites that are associated widespread field resources

- *Tyrophagus* (Acaridae) – widely distributed in a variety of natural habitats and is abundant in grassland soil and litter
- *Glycyphagus* (Glycyphagidae) – occupies the widest habitat range; species reside in grassland, tree foliage, caves, bat roosts, rodent nests, stored products and house dust

c) mites associated with the nests of mammals, especially rodents

- *Acarus* and *Aleuroglyphus* (Acaridae) – inhabit a wide variety of mammals nests, including the roosts of bats
- *Lepidoglyphus* (Glycyphagidae) –commonly encountered in stored products
- *Chortoglyphus*

d) mites associated with nests of birds

- *Dermatophagoides* (Pyroglyphidae) – inhabit house dust or stored products and are clearly derived from species inhabiting the nests of birds

Similarly to OConnor (1979, 1982, 1984), concluded Colloff (2009) that the major mite taxa of homes are also found in nonsynanthropic habitats. Pyroglyphidae live in birds nests, Acaridae in soil and plant litter and are also associated with insects and small mammals, and Glycyphagidae live in mammal nests. No species is specific to human habitation, however houses have similarities with other habitats that make them suitable for mites (Colloff 2009).

2.   RESULTS – PEER-REVIEWED ARTICLES

This Ph.D. thesis is based on the following articles. The full texts of pertinent publications are attached as supplementary material.

Erban, T., and J. Hubert. 2008. Digestive function of lysozyme in synanthropic acaridid mites enables utilization of bacteria as a food source. *Experimental and Applied Acarology* 44: 199-212.

Erban, T., M. Erbanova, M. Nesvorna, and J. Hubert. 2009. The importance of starch and sucrose digestion in nutritive biology of synanthropic acaridid mites: alpha-amylases and alpha-glucosidases are suitable targets for inhibitor-based strategies of mite control. *Archives of Insect Biochemistry and Physiology* 71: 139-158.

Erban, T., and J. Hubert. 2010. Determination of pH in regions of the midguts of acaridid mites. *Journal of Insect Science* 10.42.

Erban, T., and J. Hubert. 2010. Comparative analyses of proteolytic activities in seven species of synanthropic acaridid mites. *Archives of Insect Biochemistry and Physiology* 75: 187-206.

Erban, T., and J. Hubert. 2011. Visualization of protein digestion in the midgut of the acarid mite *Lepidoglyphus destructor*. *Archives of Insect Biochemistry and Physiology* 78: 74-86.

3.   GENERAL DISCUSSION

The primary enzymatic activities thought to be involved in the digestion of stored product and house dust mites have been characterized in up to 14 species of synanthropic acaridid mites. The research approach adopted in this Ph.D. thesis was based on *in vitro* characterization of enzymatic activity in whole mite extracts (WME) and spent growth medium extracts (SGME), evaluation of the enzyme activities with respect to the gut physiological pH, inhibition experiments, *in vivo* localization of enzyme activities in the mite gut, determination of effects of nutrient additives in experimental diets on mite population growth and determination of the feeding preferences of synanthropic mites as assessed by *in vitro* and *in vivo* analyses. The main findings are discussed below.

3.1.   Mite Gut pH

Studies of digestive enzymes should take into account the physiological gut pH. Previous studies have determined the acidobasic conditions in the mite gut range from pH 5.4 to 6 in the ventriculus and caeca, from pH 5.9 to 7.4 in the colon and from pH 6.5 to 8 in the postcolon (Hughes 1950, Wharton & Brody 1972, Akimov & Barabanova 1976a, Akimov & Barabanova 1978, Akimov 1985). Regardless, some discrepancies were found between the *in vitro* measured enzymatic activities and the *in vivo* results in available publications. Earlier results were based on pH determinations using a very limited number of indicators. In addition, the pH was determined using acidobasic indicators to an accuracy of 0.1 pH units. This is impossible, because each pH indicator determines the pH at an accuracy of pH ± 1. Thus, we suspected that the mite gut pH was not determined correctly in previous studies. Hence, the gut pH was determined in twelve species of mites. A wide spectrum of 18 indicators was used to determine the physiological pH in the gut compartments of twelve species of synanthropic mites (Acari: Acaridida). The pH indicators were selected according to the earlier studies and the pH indicators were grouped. Such a wide spectrum of pH indicators was necessary for the precise evaluation of the data (Erban & Hubert 2010b). These results were used in further analyses of mite digestive enzymes.

In the study of Erban and Hubert (2010b), the gut contents of acaridid mites were determined to be within a pH range of 4 to 7 and showed a pH gradient from the anterior to the posterior portion. The anterior gut (ventriculus and caeca) of most species has a pH ranging from 4.5 to 5, or slightly more alkaline, whereas the hindgut (intercolon/colon) has a pH of 5 to 6. The pH of the rectum (postcolon) is the most alkaline part of the gut and the pH ranges between 5.5 and 7. Significant differences were found only in *D. farinae* and

D. pteronyssinus, which had a more acidic anterior gut (pH of 4 to 5) and colon (pH of 5) with postcolon (pH of below 6) (Erban & Hubert 2010b). House dust mites were unique due to their acidobasic conditions in gut. The acidic buffering in gut of *Dermatophagoides* spp. corresponds to their preferred food source in comparison to the mites that predominate in stored products (Erban & Hubert 2010b). Finally, it is necessary to mention that digestive enzymes should have an optimal pH in the range of 4 to 7. This is crucial for maintaining the digestive enzymatic activity in the feces, which are commonly used as the source of digestive enzymes for mites (Erban & Hubert 2010b).

The gut pH also plays an important role in bacterial colonization (Funke *et al.* 2008). Bacterial colonization is limited to the mite midgut; however the midgut conditions are not friendly for symbiotic bacteria due to the presence of mildly acid pH and many antibacterial proteins, such as lysozyme-like proteins and some proteases.

3.2.   Bacteriolytic Enzymes – Bacteria as a Food Source

The study confirmed the presence of lysozyme-like (bacteriolytic) activity on *Micrococcus lysodeikticus* cells in the WME and SGME of 14 species of synanthropic acaridid mites. The highest activity of digestive bacteriolytic activity was found in *Lepidoglyphus destructor*, *Chortoglyphus arcuatus* and *D. farinae*. The optimal pH of bacteriolytic activity was 4.5 in SGME for the majority of the species tested and the absence of bacteriolytic activity at pH levels above 7.0 suggests a digestive rather than defensive function of the enzymes (Erban & Hubert 2008). These enzymatic properties of bacteriolytic activity were consistent with the pH of the ventriculus and caeca (Erban & Hubert 2010b). Eight species showed a higher rate of population growth on a *M. lysodeikticus* diet than on a control diet. Interesingly, the differences between *D. farinae* and *D. pteronyssinus* in standardized rate of population increase on bacteria-enriched diet and bacteriolytic activity in SGME showed the various degree of bacteriophagy of these two species (Erban & Hubert 2008). The positive correlation between bacteriolytic activity in SGME and standardized rate of population increase, and the absence of a correlation between bacteriolytic activity in WME and standardized rate of population increase supports the digestive fuction of the examined bacteriolytic activity in mites. A positive correlation of bacteriolytic activity between SGME and WME would indicate that lysozyme is produced in large amounts in the ventriculus and ceacal cells and passes through the gut into the excrement without proteolytic degradation. However, no such positive correlation between the means of bacteriolytic activity in WME and SGME was found. The observed negative correlation could be explained by the coexistence of both

defensive (non-digestive) lysozyme-like and digestive activities, or by the existence of an enzyme recycling mechanism in the gut (Erban & Hubert 2008). In addition, the digestion of bacteria was examined *in vivo* using fluorescein-labeled *M. lysodeikticus* cells. Fluorescein released peritrophic membrane of food bolus after hydrolysis of *M. lysodeikticus* cell walls. Digestion began in ventriculus and continued during the passage of a food bolus through the gut. Fluorescein was absorbed by midgut cells and penetrated the parenchymal tissues (Erban & Hubert 2008). These results indicated various adaptations of stored product and house dust mites to utilizaction of bacteria as a food source. These results indicate that mites are able to feed on the decomposing tissues and utilize bacteria growing there (Erban & Hubert 2008).

Because studies have determined that proteolytic enzymes participate in the digestion of bacteria in insect guts (Espinoza-Fuentes *et al.* 1987, Lemos & Terra 1991a, Lemos & Terra 1991b), it is possible that such mode of digestion occurs in mites. The cysteine proteases, formerly group 1 mite allergens, could be candidates.

3.3.   Digestion of Starch, Maltose and Sucrose (Glycosidases)

In general, carbohydrates (saccharides) are the most abundant class of organic compounds found in stored plant products, especially in cereals. We studied starch and sucrose because they are the main digestible carbohydrates in nature. Previously, the enzymatic activities of α-amylases and α-D-glucosidases were observed in homogenates of many species of mites. However, differences in α-amylase and α-glucosidase activity among synanthropic mite species, which is indicative of adaptation to starch digestion, were not remarkable in the studies.

The differences in α-amylases and α-glucosidases activity among nine species of synanthropic mites have been studied (Erban *et al.* 2009a). These results indicated various adaptations to starch digestion, however this was not remarkable in the previous studies (Erban *et al.* 2009a). We also suggested that the fungi-feeding mites having amylolytic activity are able to utilize glycogen as a stored polysaccharide of microscopic fungi rather than very rigid structural polysaccharide chitin (Erban *et al.* 2009a). The optimal pH for the digestion of starch, maltose and sucrose in the WME and SGME of nine species of synanthropic acaridid mites ranged from pH 4 to 6.75, with maximal activity at pH 5 (Erban *et al.* 2009a). These results are in agreement with previously reported data using mites (Hughes 1950, Matsumoto 1965, Akimov & Barabanova 1976b, Akimov & Barabanova 1978, Lake *et al.* 1991a, Lake *et al.* 1991b, Sanchez-Monge *et al.* 1996) and correspond to the acidobasic conditions in the anterior midgut (Erban & Hubert 2010b) and the equivalent

release of glucose from four types of starch, amylopectin, dextrin, maltose and sucrose by hydrolysis of WME and SGME indicated that the enzymes digesting these substrates are produced in the anterior midgut. The species with higher starch hydrolytic activities in the SGME are more tolerant to acarbose, which inhibits starch digestion. No significant correlations found among WME and IC_{50} and rc_{50} on acarbose indicated that a high production of α-amylase and/or the absence of recycling mechanisms leads to a high concentration of α-amylase in the feces. In addition, the high specific hydrolytic activities of α-amylases and maltase demonstrated the importance of their synergetic activity in producing glucose from the starch (Erban *et al.* 2009a).

The stored product mites *A. siro*, *A. ovatus* and *T. lini* were associated with the starch-type substrates and maltose, with a higher enzymatic activity observed in the whole mite extracts. *Lepidoglyphus destructor* was associated with the same substrates but had higher activity in feces. *D. farinae*, *C. arcuatus* and *C. redickorzevi* were associated with sucrose. *T. putrescentiae* and *C. lactis* had low or intermediate enzymatic activity as determined by the tested substrates. Biotests on starch additive diets showed accelerated growth of species associated with the starch-type substrates. On other hand, the biotest did not confirm that sucrose is a suitable addition to diets for population growth; however, surprisingly, it was partially suppressed. Therefore, we concluded that the sucrose additive in the diets was available for microbial growth, and high microbial growth could suppress mite growth (Erban *et al.* 2009a).

Starch digestion was localized by *in vivo* observation of starch azure digestion and the primary component of starch digestion was in the ventriculus and caeca; however, digestion also occurs in the colon and postcolon. This is consistent with the wide pH range for optimal starch and maltase digestion (4 to 6.75). Only *D. farinae* showed very poor activity towards starch azure as determined by *in vivo* observations. (Erban *et al.* 2009a).

3.4.   Digestion of Proteins (Proteases)

Proteases in domestic mites have been intensively studied since they are compounds of high allergenic hazard. Even though many studies have been performed, comparative studies are very rare. The enzymatic activities from the literature had some discrepancies in the evaluation of the data. For example, Ortego *et al*. (2000) analyzed the proteolytic activities with a highest activity measured at pH 10 in the feces (Ortego *et al.* 2000). Monteallegre *et al.* (2002) measured the activity and the inhibition of serine proteases in the mite bodies at pH 7.8 and 7.4 (Montealegre *et al.* 2002). The results of digestive function were not in accord with

the mite gut pH (Erban & Hubert 2010b). General proteolytic activities of *A. siro*, *A. ovatus*, *T. putrescentiae*, *T. lini*, *C. lactis*, *L. destructor* and *D. farinae* exhibited non-specific proteolytic activity in buffers from pH 2 to 12; three peaks of highest activity at pH 3, 5 to 6 and 10 were distinguished. The high protease activity close to pH 5 and 6 is considered to be digestive (Erban & Hubert 2010a).

The comparative analysis of proteases in homogenates of seven mite species showed very low activity towards chromogenic trypsin substrate BApNA (N_α-Benzoyl-D,L-arginine p-nitroanilide hydrochloride) at pH 5 and 6, indicating that trypsin-like proteases are likely to be of limited functional importance (Erban & Hubert 2010a). In addition, there is a possible effect of cysteine proteases on BApNA, indicating a lower trypsin activity (Erban & Hubert 2010a). The *in vivo* localization of trypsin-like activity was tested in the midgut of *L. destructor* using chromogenic and fluorescence trypsin substrates BApNA and BAAMC (N_α-Benzoyl-L-arginine-7-amido-4-methylcoumarin hydrochloride). While, the trypsin-like activity generated by hydrolysis of the BApNA substrate was not observed *in vivo*, the fluorescence substrate analog BAAMC allowed the visualization of trypsin-like activity in food boli in the posterior midgut. This suggests that trypsins are low active in the mite gut and are activated in the posctocolon (Erban & Hubert 2011). This corresponded to the event that Der f3, a trypsin allergen, was shown to be localized in the postcolon and in feces using monoclonal antibody (Zhan *et al.* 2010).

The optimal activity towards AAPpNA (N-Succinyl-L-alanyl-L-alanyl-L-phenylalanine p-nitroanilide), a chymotrypsin substrate, at pH 6 suggests that chymotrypsins play an important role as a digestive enzyme in mites (Erban & Hubert 2010a). The presence of Der p9 allergens was predicted in the other seven species of synanthropic acaridid mites because high proteolytic activity was observed towards chromogenic substrate MAAPMpNA (N-Methoxysuccinyl-L-alanyl-L-alanyl-L-prolyl-L-methionine p-nitroanilide), which is designed for cathepsin G. In addition, this is supported by the fact that the proteolytic activity in seven species of mites was similar using azocol as a substrate in comparison to MAAPMpNA (Erban & Hubert 2010a). And in addition, the activity on SAAPFpNA (N-Succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine p-nitroanilide) can be influenced by both chymotrypsin-like and cathepsin G-like activities (Erban & Hubert 2010a). The high level of activity of chymotrypsin-like enzymes at a pH corresponding to the mite gut pH led to the conclusion that proteases exhibit chymotrypsin-like activity and may be the primary digestive proteases in mites (Erban & Hubert 2010a). It is also hypothesized that the cathepsin G enzymatic features may be connected to antibacterial activity in domestic mites (Shafer *et al.* 2002). The

visualized chymotrypsin-like activity in *Lepidoglyphus destructor* indicated that chymotrypsin-like activity occurs in the whole midgut and begins in ventriculus and caeca; this was proved using AAPAMC (N-Succinyl-L-alanyl-L-alanyl-L-phenylalanine-7-amido-4-methylcoumarin hydrochloride), AAPpNA, and SAAPFpNA substrates. This result confirms (Erban & Hubert 2011) the results of *in vitro* analyses that chymotrypsin-like enzymes are digestive (Erban & Hubert 2010a). The possible cathepsin G-like activity was indicated in *L. destructor* using chromogenic substrate MAAPMpNA; the enzymatic activity was apparent in the ventriculus and in the caeca and inside of the food bolus in the colon and the postcolon (Erban & Hubert 2011). The *in vivo* visualized enzymatic activity (Erban & Hubert 2011) supported the previusly *in vitro* analysis that the chymotrypsin-like activity represents important part of protease action in the mites (Erban & Hubert 2010a).

Relativelly low proteolytic activity towards ZRRpNA at a pH corresponding to the mite gut pH in the seven species of mites tested suggests a low cathepsin B activity. However, the effect of TCEP (Tris(2-carboxyethyl)phosphine hydrochloride), an activator of cysteine proteases, showed a negative effect on general protease activity at pH 5 and 6. The increased cysteine protease activity using TCEP does not compensate decrease of general proteolytic activity caused by disruption of disulfide bridges of other proteases that may be thus less active. And because the inhibition effect of TCEP was lower at pH 6 than at pH 5, it is suggested that cysteine proteases are more active at pH 6 (Erban & Hubert 2010a). The cysteine protease-like activity was visualized in *L. destructor* using the ZRRAMC (Benzyloxycarbonyl-L-arginine-L-arginyl-7-amido-4-methylcoumarin hydrochloride) and ZRRpNA (Benzyloxycarbonyl-L-arginine-L-arginyl 4-nitroanilide) substrates. The presence of *in vivo* hydrolysis of both ZRRAMC and ZRRpNA in the whole gut indicated that digestion of this substrate begins in foregut. However, localization of the cysteine proteases is problemic using the avialable cysteine substrates ZRRpNA and ZRRAMC, because of their specifity not only to cathepsin B, but also to trypsin (Erban & Hubert 2010a, Erban & Hubert 2011). The low cysteine protease activity is in agreement with the results obtained by Ortego *et al.* (2000), whereas serine protease activity was much higher in the feces than the cysteine protease activity when comparing the WME and SGME (Ortego *et al.* 2000). This indicated an unusual cysteine protease activity in mites (Ortego *et al.* 2000). Nevertheless, the efficiency of proteolytic degradation in mites is based on the interplay of serine and cysteine proteases.

The enzymatic activities of the small peaks of general protease activity at pH 3 suggest the presence of aspartate proteases. This protease activity was proposed to be is probably limited

to acid lysozomes (Erban & Hubert 2010a). Aspartate protease activity has previously been found in *A. farris* (Sanchez-Ramos *et al.* 2004), *A. siro* (Nisbet & Billingsley 2000), and *T. putrescentiae* (Ortego *et al.* 2000), but in these prior studies was suggested that aspartate proteases in acaridid mites are non-digestive. However, cathepsin D-like activity was identified by feeding of the *L. destructor* on the fluorescent substrate AGPPPAMC (N-Acetyl-Arg-Gly-Phe-Phe-Pro-7-amido-4-trifluoromethylcoumarin). The fluorescence liberated from AGPPPAMC was observed in the whole midgut of *L. destructor* suggesting possible aspartate protease activity (Erban & Hubert 2011). This situation can be similar to that cathepsin D-like aspartate proteases may be involved in extracellular digestion after degeneration of the ''digestive cells'' or following exocytosis in *Psoroptes cuniculi* (Nisbet & Billingsley 2000) and is possible for synanthropic acaridid mite *L. destructor* (Erban & Hubert 2011). The degeneration of the ''digestive cells'' was observed also in *A. siro* (Sobotnik *et al.* 2008a).

Optimal and high aminopeptidase activity corresponding to the mite gut pH was observed in seven species of acaridid mites (Erban & Hubert 2010a). This was observed using ArgpNA (L-arginyl 4-nitroanilide) as a substrate, which lacks activity towards Der pI (Brown *et al.* 2003). *In vivo* was localized aminopeptidase-like activity using ArgAMC (L-Arginine-7-amido-4-methylcoumarin hydrochloride) and ArgpNA. These substrates may be targeted also by cathepsin H-like activity. The event that both substrates were best localized in the ventriculus and caeca suggest that digestion begins in these compartments (Erban & Hubert 2011). Aminopeptidases serve as targets for Bt toxins. The toxic effect of *Bacillus thuringiensis* var. *tenebrionis* using Cry3A toxin, for which the aminopeptidases can serve as receptor (Knight *et al.* 1994, Budatha *et al.* 2007), was tested on the mites *Acarus siro*, *Tyrophagus putrescentiae*, *Dermatophagoides farinae* and *Lepidoglyphus destructor* using a feeding test. The results confirmed that it may be possible to control mites using Bt toxins (Erban *et al.* 2009b) but also support suggestion that the gut of mites is equipped by the aminopeptidases.

Proteolytic activity towards the substrates azocol (collagenase substrate), keratin azure (keratinase substrate), alastin-orcein (elastase susbtrate) and SA$_3$pNA (N-Succinyl-L-alanyl-L-alanyl-L-alanine 4-nitroanilide) was confirmed in *D. farinae* and in six species of stored product mites; however, the majority of digestion occurred in basic pH. The low activity towards keratin and collagen suggests that these substrates are not important sources of energy for mites. However, it is hypothesized that the effective utilization of these substrates is mediated by symbiotic microorganisms growing on the substrate (Erban & Hubert 2010a). Although Ortego *et al.* (2000) did not report activity using SA$_3$pNA, activity on this substrate

was confirmed in all seven species of acaridid mites (Erban & Hubert 2010a). The fact that activity on SA$_3$pNA was similar to activity on ZRRpNA may indicate an interaction involving these two reactions (Erban & Hubert 2010a). The elastase-like activity was localized in gut of *L. destructor* using SA$_3$pNA and elastin-orcein. The *L. destructor* specimens feeding on elastin-orcein had a rose color in the whole midgut. It was similar to the yellow color generated by hydrolyzis of SA$_3$pNA (Erban & Hubert 2011). However, no significant color changes were observed in *in vivo* enzyme localizations in *L. destructor* specimens feeding on the keratin-azure (Erban & Hubert 2011) supporting poor or no keratinase activity in mite gut (Erban & Hubert 2010a).

The combination of measured proteolytic activities and biotests in this study showed no remarkable correlation. Some influences of protein addition into the diets were observed in *T. putrescentiae* and *A. siro*, which showed only intermediate protease activities, but the effect on population increase was very low. A similar small effect on population increase was observed on the addition of starch into diets (Erban *et al.* 2009a). In sum the nutritional benefit from bacterial cells (Erban & Hubert 2008) was higher than from pure proteins (Erban & Hubert 2010a) and saccharides (Erban *et al.* 2009a).

3.5. Is it Possible to Determine Feeding Guilds Based on Enzymatic Activities?

The feeding guilds described by Siepel and de Ruiter-Dijkman (1993) and Berg *et al*. (2004) are based on the enzymatic activities of chitinase, trehalase and cellulase (Siepel & de Ruiter-Dijkman 1993, Berg *et al.* 2004). Previous classifications into feeding guilds were also based on enzymatic activities that were probably digestive (Schuster 1956, Luxton 1972). Sustr & Stary (1998) claimed that the analyses of enzymatic activities in whole body homogenates were erroneous. These authors mentioned that the whole body homogenates may be influenced by non-digestive enzymes and/or enzymes of microbial origin. In addition, the discrepancies may be derived from *in vivo* versus *in vitro* enzyme action (Sustr & Stary 1998). Here it is suggested that defining feeding guilds based only on the presence or absence of enzymatic activity in the crude extract is too simplistic, may lead to discrepancies and may not correctly determine feeding guilds. A review of the literature in which feeding guilds are proposed based on enzymatic activity indicates three general limitations of prior research:

1) The authors did not mention that chitinase and trehalase are used in nature primarily for purposes other than digestion:

a) Chitinase – all animals having chitin must be equipped with chitinases, which are used for molting processes (growth); chitinolytic activity can be caused by the action of chitinase or other enzymes like lysozyme

b) Trehalase – (i) many invertebrates, especially insects, utilize this enzyme to degrade trehalose, which functions as a source of energy (i.e. flying) or a protectant against the effects of freezing or dehydration; (ii) presence of trehalase in invertebrates is joined mainly with chitin metabolism and chitin synthesis during molting processes

2) Many enzymes with an optimal pH similar to the physiological pH of the gut were considered to be digestive. This interpretation of an enzyme's function is questionable in cases where: (i) the enzymes were not purified from gut or feces; (ii) were not localized in gut or feces; or (iii) were not analyzed with respect to the physiological pH of gut.

3) Cellulase, chitinase, and trehalase activities were used to characterize feeding guilds. Cellulases are enzymes that are very rare in animals and are present in plants, algae, bacteria and fungi (Lynd *et al*. 2002). The evaluation of cellulases as digestive enzymes must be based on localization in the gut or determination of association with microorganisms rather than on only measuring cellulolytic activity. Nevertheless, the presence of cellulases in extracts indicates the presence of symbiotic bacteria in mites or the presence of digestive cellulases.

The above-mentioned limitations were primarily methodological in nature, but the ideas are correct. According to the arguments presented above, it is probably not possible to determine feeding guilds reliably based solely on enzymatic activities without solving the various methodological problems. Bowman & Childs (1982) suggested that cellulolytic activity seems to be a result of microorganisms and their exoenzymes in the mite gut. Here it is suggested that trehalase and, in particular, chitinase activity measured in mites is the result of enzymes that are utilized by mites during growth and molting. In addition, the digestive hydrolysis of chitin can be simulated by lysozyme (Skujins *et al.* 1973, Minic *et al.* 1998) or other bacteriolytic enzymes with similar enzyme action from mite gut or tissues. Therefore, it is necessary to localize and determine the enzymatic activity, especially in the case of chitinolytic activity.

3.6. Nutrient Sources of Stored Product and House Dust Mites

The results of this Ph.D. thesis showed that stored product and house dust mites are able to feed on higher plant material and on microflora (Erban & Hubert 2008, Erban *et al.* 2009a, Erban & Hubert 2010a, Erban & Hubert 2011). Therefore, these mites are panphytophagous

species based on Luxton's classification (Luxton 1972). These mites are well adapted to digest bacteria and fungi. They are able to utilize bacterial and fungal cell walls as well as bacterial and fungal cell content (Erban & Hubert 2008, Erban *et al.* 2009a, Erban & Hubert 2010a, Erban & Hubert 2011).

Based on the results in this Ph.D. thesis, I conclude that stored product mites often found in stored grain, such as *A. siro*, *A. ovatus*, *L. destructor* and *T. lini*, are enzymatically best adapted for the digestion of starch-rich substrates (Erban *et al.* 2009a). However, these mites also have higher activity of other enzymes, such as proteases (Erban & Hubert 2010a). In particular, *L. destructor* is a species with an extremely high enzymatic activity with respect to every enzyme studied (Erban & Hubert 2010a, Erban & Hubert 2011). On the other hand, *D. farinae* showed very low enzymatic activity with the exception of bacteriolytic activity (Erban & Hubert 2008). It was suggested that *D. farinae* is a species that is well adapted to the digestion of proteins and keratin; however, the results showed that this mite has poor proteolytic activity in comparison to the stored product species tested. This conflicts with the supposedly high proteolytic and keratinolytic activity of house dust mites (Erban & Hubert 2010a). *C. lactis* is a species that infests materials with high sugar content, especially dried fruit (Hughes 1976, OConnor 1979, Halliday 2003, Hubert *et al.* 2011). Therefore, this mite should have high saccharase activity. However, *C. lactis* exhibited only intermediate activity on sucrose (Erban *et al.* 2009a).

Why do different species of mites have different enzymatic activity? This may be due to differences in nutritional needs among different species. Therefore, very low protease and starch hydrolyzis activity in *D. farinae* may correspond to a lesser nutritional need; this may also be true for *C. lactis*, which has low saccharase activity. These enzymatic activities could correspond to the degree of mite mobility (personal observation). Therefore, the level of enzymatic activity may or may not correlate with the preferred food source but, rather, may be due to nutritional needs. This factor also corresponds with mite population growth. Such an explanation is more probable than the hypothesis that animals with larger body mass have lower enzymatic activity (Sustr & Stary 1998). Sustr & Stary (1998) mentioned that it is not possible to explain differences in amylolytic activity between species simply as a consequence of body size and the extent of chitinisation (Sustr & Stary 1998). On the contrary, in termites it is believed that the reduction in size of the midgut, as well as of the termite's whole body size, is one of key factors that has allowed termites to flourish after evolving from wood-feeding cockroaches. Termites have a prominently developed hindgut and a short midgut, suggesting a greater contribution of microbial cellulose digestion in the

hindgut (Watanabe & Tokuda 2010). Such adaptations are not possible in mites due to their morphology.

The bacteriolytic activity observed in *D. farinae* showed that this mite has adapted to digest bacteria. On other hand, similar species, such as *D. pteronyssinus*, have a lesser degree of adaptation to feed on bacteria (Erban & Hubert 2008). This observation contradicts the hypothesis stated in the previous paragraph. However, these two *Dermatophagoides* species are not identical, even though they live in the same habitat and in mixed populations Valerio *et al*. (2005) found that 16S ribosomal DNA from *D. farinae* contained a significantly greater number of copies of DNA encoding 16S ribosomal RNA than did *D. pteronyssinus*, indicating a greater diversity of bacterial strains in *D. farinae*. This suggests a higher degree of bacteriophagy in *D. farinae* than in *D. pteronyssinus* (Valerio *et al.* 2005, Erban & Hubert 2008).

It appears that, although *D. farinae* has low proteolytic activity, it is able to digest bacteria with higher efficiency. It is probable that house dust mites tend to rely on microphagy more than the other species. A similar situation is suggested for *P. ovis*, which is most likely an ancestor of *Dermatophagoides* spp. (Hamilton *et al*. 2003). In addition, it could be stated that *D. farinae* is the most unique mite of the species studied, not only in relation to its gut acidobasic properties and proteolytic activity, but also for its high degree of bacteriophagy.

The hypothesis of OConnor (1979) that *Dermatophagoides* spp. is derived from species inhabiting the nests of birds is consistent with the fact that these mites inhabit house dust, where keratin and collagen are widely distributed. Therefore these mites may have evolved relationships with keratinophilic bacteria and fungi before synanthropization in the nests of birds. A similar situation is possible for other species of mites that penetrated human environments through the nests of mammals. Because the largest group of stored product mites is derived from the nests of mammals, especially rodents (OConnor 1979, OConnor 1982), these mites may have adapted the ability to digest food stored by the rodents as well as the ability to interact with keratinolytic and collagenolytic microorganisms growing in hair and skin found in the nest. This hypothesis is supported by the fact that many stored product species are also found in house dust (Colloff & Spieksma 1992, Colloff 2009).

The organization of animals into feeding guilds should be based on habitat. Siepel & de Ruiter-Dijkman (1993) claimed that in the laboratory environment where food is plentiful, there is no need for selective efficiency and species will likely feed on the components easiest to digest (cell contents). Therefore, it is not surprising that species reared in the laboratory even on algae are found to digest fungi in the field (Siepel & de Ruiter-Dijkman 1993). The

biotests performed in this thesis were based on the enrichment of dietary substrates that are a target for appropriate digestive enzymes. From these results, biotests, and enzyme analyses it appears that a favorable digestive method or, in some environments, the essential digestive strategy for the synanthropic acaridid mites is microphagy (i.e. bacteriophagy and mycophagy). Mites are able to feed on many types of food stored for human consumption as a direct food source and also through symbiotic interactions. Mite populations increased rapidly with the addition of bacteria to the diet. Thus, bacteria offer an even higher nutrient benefit than does complex food, which is used in laboratory cultures (Erban & Hubert 2008), or food enriched in starch, sucrose (Erban *et al.* 2009a) or protein (Erban & Hubert 2010a).

4.   CONCLUSIONS

A wide range of the enzymatic activities were characterized for a spectrum of synantrophic acaridid mite species. These *in vitro* enzymatic results are supported by biotests and by the *in vivo* observation of enzymatic activity in the mite gut.

Mites are small animals and it is not possible to dissect the gut from the body. In order to study digestive enzymes, we determined the acidobasic properties of the gut of 12 different species. The gut contents of acaridid mites were determined to be within a pH range of 4 to 7. Enzymatic activity outside of this pH range is not considered to be related to digestion.

The presence of lysozyme-like (bacteriolytic) activity was confirmed in the WME and SGME of 14 species of synanthropic acaridid mites. The highest activity of digestive bacteriolytic activity was found in *L. destructor*, *C. arcuatus* and *D. farinae*. *D. farinae* showed higher degree of bacteriophagy than *D. pteronyssinus*. The results demonstrate that mites are able to feed on bacteria and are equipped with enzymes that can digest and/or protect the mites from bacteria. The results of this Ph.D. thesis showed that the interaction of house dust and stored product mites with bacteria is more important than previously suggested. This is particularly important for mites that live in an environment where the food source is low in complex nutrients and feeding on microbes is necessary. The mites are able to feed on the decomposing tissues and utilize bacteria growing there.

Examination of starch and sucrose digestion in the nutritive biology of synanthropic acaridid mites showed that the mite gut is equipped with enzymes that digest these saccharides. Our results showed that the typical stored product mites (*Acarus siro*, *A. ovatus* and *T. lini*) and *L. destructor* are better adapted to digest starch. In contrast, other mites, such as *D. farinae*, *C. arcuatus* and *S. rodionovi* (syn. *C. redickorzevi*), are associated with sucrose digestion. *T. putrescentiae* and *C. lactis* exhibited low or intermediate activity on both substrates. The biotest results using a starch dietary additive suggest that starch enrichment increases the population growth of the mites. However, our biotest results did not confirm this observation; we conclude that population growth was negatively affected by some microbes and that population growth was accelerated by the addition of sucrose to the diet. Nevertheless, all the mites utilize starch and sucrose as a source of nutrients.

Our results suggest that the primary mite digestive proteases are chymotrypsin-like proteases and aminopeptidases. It was surprising that a low enzymatic activity corresponded to trypsins and cysteine proteases. The low level of collagen and keratin digestion of substrates at the mite midgut pH suggests that keratin and collagen are not so important direct

sources of nutrients. Thus, we hypothesize that the effective utilization of nutrients from skin, hair, nails and feathers by mites is possible through the symbiotic interactions of mites with keratinolytic and collagenolytic bacteria and fungi.

The analysis of digestive enzymes in mites is applicable to their nutritional biology, with some limitations. Although mites are able to digest basic carbohydrates and proteins, in some environments they require the help of microbes. Our results, in addition to recent studies, highlight the importance of symbiotic interactions between mites and microorganisms, which serve as a nutrient source. Microphagy is a favorable digestive strategy for mites living in environments where they feed on the skin, hair, nails and feathers from humans and animals. *Dermatophagoides* spp. and *L. destructor* were the most unique mites tested. The different enzymatic properties of *Dermatophagoides* spp. are probably due to environmental constraints. *L. destructor* has a uniquely high activity of digestive enzymes, which was several-fold higher than in the other species tested.

Based on the degree of enzymatic activity, the species are divided into different groups. The level of enzymatic activity may be dependent on the preferred food source. Our results showed that typical stored product mites, which are often found in cereals, exhibit higher enzymatic activity than house dust mites. This may be dependent not only on the food preference, but also on the metabolic demands of the species. We have observed that the stored product mites are more active than the house dust mites or *C. lactis* (personal observation). In particular, *L. destructor* is a very active mite; therefore, its extremely high enzymatic activity may be result of high nutritional demand.

Finally, it is imperative to point out that it is not possible to reliably characterize feeding guilds based only on enzymatic activity. Nevertheless, in accordance with the feeding classifications of Luxton (1972), the synanthropic acaridid mites are best classified as panphytophagous species. Because stored product and house dust mites are believed to have originated within the Oribatida, the ancestral stored product and house dust mite may be similar to panphytophagous oribatid mites.

5.   REFERENCES

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7.   SUPPLEMENTARY MATERIAL – FIVE PEER-REVIEWED ARTICLES

The articles that comprise results part of the Ph.D. thesis are listed in the following order.

Article 1

Erban, T., and J. Hubert. 2008. Digestive function of lysozyme in synanthropic acaridid mites enables utilization of bacteria as a food source. *Experimental and Applied Acarology* 44: 199-212.

Article 2

Erban, T., M. Erbanova, M. Nesvorna, and J. Hubert. 2009. The importance of starch and sucrose digestion in nutritive biology of synanthropic acaridid mites: alpha-amylases and alpha-glucosidases are suitable targets for inhibitor-based strategies of mite control. *Archives of Insect Biochemistry and Physiology* 71: 139-158.

Article 3

Erban, T., and J. Hubert. 2010. Determination of pH in regions of the midguts of acaridid mites. *Journal of Insect Science* 10.42.

Article 4

Erban, T., and J. Hubert. 2010. Comparative analyses of proteolytic activities in seven species of synanthropic acaridid mites. *Archives of Insect Biochemistry and Physiology* 75: 187-206.

Article 5

Erban, T., and J. Hubert. 2011. Visualization of protein digestion in the midgut of the acarid mite *Lepidoglyphus destructor. Archives of Insect Biochemistry and Physiology* 78: 74-86.