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Invited review

A physiological and biochemical model for digestion in the ectoparasitic mite, *Psoroptes ovis* (Acari: Psoroptidae)

K.A. Hamilton[a,b], A.J. Nisbet[a,c], M.J. Lehane[b], M.A. Taylor[d], P.F. Billingsley[a,*]

[a]*Department of Zoology, University of Aberdeen, Tillydrone Ave., Aberdeen AB24 2TZ, Scotland, UK*
[b]*School of Biological Sciences, University of Wales-Bangor, Deiniol Road, Bangor, Gwynedd, LL57 2UW, Wales, UK*
[c]*Department of Veterinary Science, University of Melbourne, 250 Princes Highway Werribee, Victoria 3030, Australia*
[d]*Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB UK*

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Abstract

Mites are an important group of arthropod pests affecting crops, animals and humans. Despite this, detailed physiological studies on these organisms remain sparse due largely to their small size. Unifying models are required to draw together the diverse information from studies on different groups and species. This paper describes a model for digestion in the parasitic mite, *Psoroptes ovis*, the causative agent of psoroptic mange or sheep scab disease. The limited information about this species is supplemented with data from other acarines, especially house dust mites and ticks. We review the range of enzymes and allergens found in mites and consider their possible roles in digestion in mites, generally and in particular, *P. ovis*. Histological studies, enzyme biochemistry and molecular biology and experimental evidence suggest that *P. ovis* utilises a digestive system reliant upon acid peptidases functioning in a largely intracellular environment. The actions of the digestive enzymes are supplemented by the involvement of bacteria as potential direct and indirect sources of nutrition. It is possible that some extra-corporeal digestion also takes place. The interaction of bacteria and digestive enzymes on the skin surface of the sheep may be responsible for the excessive pathological reactions evident in clinical sheep scab.

Keywords: Digestion; *Psoroptes ovis*; Proteolytic; Enzymes; Allergens; House dust mites

1. Introduction

Psoroptes ovis (Acari: Psoroptidae) is an astigmatid, non-burrowing, obligate ectoparasite of sheep, goats and cattle. The mite completes its entire life cycle on the epidermis of its host. The mites' presence induces an immediate-type hypersensitivity response in the host, characterised by intense pruritus, extensive dermatitis, hyperkeratosis and secondary bacterial infection. The precise causes of the pathology are unknown but, in the light of other mite–host interactions, may depend upon material derived from the digestive system of the mite. In the ovine host, this condition is known 'sheep scab' or 'psoroptic mange' and is prevalent throughout Europe, parts of the USA, the South American continent and many other regions (Mathieson, 1995). Our aim is to develop a better understanding of the interactions between the host and the digestive physiology of this ectoparasite, with a view of evaluating the mite digestive system as a source of putative antigens and/or chemotherapeutic targets.

Within the subclass Acari (Fig. 1), most information on digestive systems and their structures has been gathered from haematophagous members of the order Ixodida (ticks) and house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, order Astigmata) (Evans, 1992). House dust mites are the most important source of allergens in the domestic environment and affect humans worldwide. House dust mite faecal pellets contain potent allergens, several of which are hydrolytic enzymes, probably of gut origin. They feed on dead skin, hair and other detritus, and on the microbes associated with this debris (Walter and Proctor, 1999). The ingestion of solid food is a common characteristic of many of the members of the order Astigmata, however, *Psoroptes* mites are adapted to feed on liquid diets consisting mostly of serous exudate

* Corresponding author. Tel.: +44-1224-272882; fax: +44-1224-272396.
E-mail address: p.billingsley@abdn.ac.uk (P.F. Billingsley).

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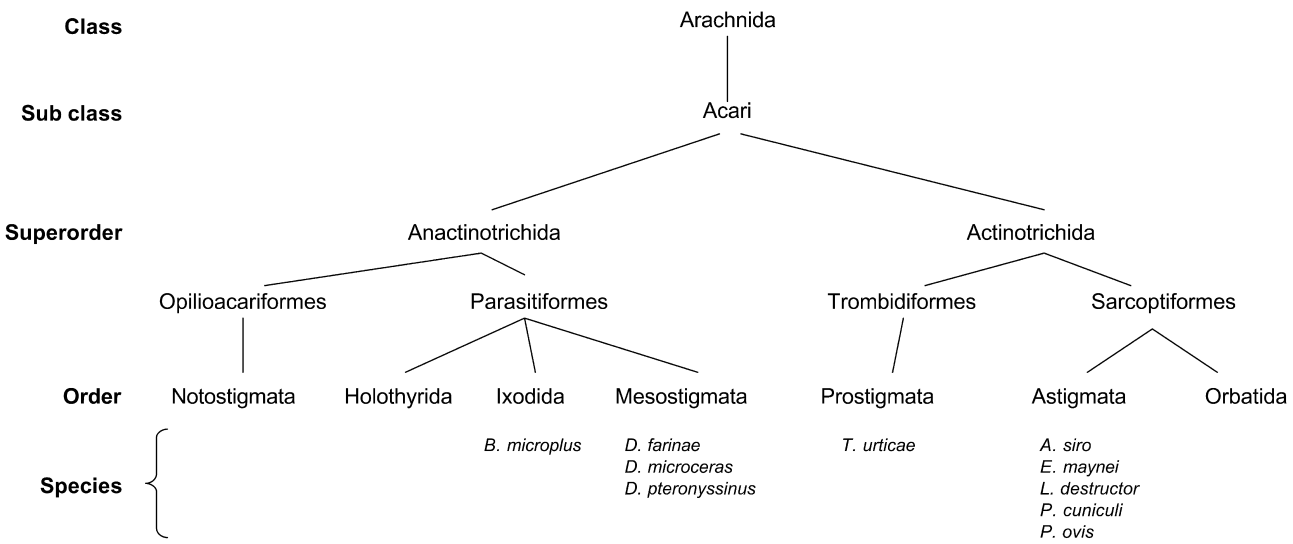

Fig. 1. Classification of the acarines. The class Arachnida divides into the subclass Acari and then into two major divisions, the Anactinotrichida and Actinotrichida, which eventually subdivide via intermediate groups into seven orders according to the classification of Evans (1992). The species of interest to this review are indicated below the orders to which they belong.

from the host. The only host-derived cellular material evident in the gut contents of *P. ovis* are eosinophils ingested along with the inflammatory exudate (Mathieson and Lehane, 2002). *Psoroptes cuniculi*, the causative agent of psoroptic otocariasis mostly in rabbits, is believed to be conspecific with *P. ovis* but phenotypically adapted to exist in the ears of its host (Ochs et al., 1999; Zahler et al., 2000). *P. cuniculi* ingests serous exudate in a similar fashion to *P. ovis* but also consumes host erythrocytes when feeding on rabbits (Rafferty and Gray, 1987).

In this article, we review the current state of knowledge on the morphology, physiology, biochemistry and molecular biology of digestion in *Psoroptes* mites and discuss how knowledge in these areas relates to what is known about digestion in other species of astigmatid mites and parasitic Acari. Ultimately we consider the different components of the system to construct the first model for digestion in sheep scab mites, an essential first step in the design of agents to control the parasites.

2. Morphology of the digestive system

All mites possess two pairs of appendages, the chelicerae and palps (which together are referred to as the gnathosoma) located anteriorly on the head (Fig. 2a). These structures are used principally for capturing, tasting and ingesting food (Walter and Proctor, 1999). The palps, which are believed to be chemosensory, are used to locate and discriminate between dietary components. They are rich in sensory setae that are often concentrated distally (Evans, 1992).

The gnathosoma may assist the two-way flow of liquid (Mapstone et al., 2002). Saliva flows down a central salivary canal and is deposited on the host skin. The pump-like action of the pharynx (Mathieson and Lehane, 2002) may suck up the liquid food for transportation to the foregut (Mapstone et al., 2002).

The digestive system of Acarines is a tube divided into three sections: foregut, midgut and hindgut (Coons, 1978). The cuticle-lined foregut is divided into a heavily sclerotized muscular pharynx, which lies behind the gnathosoma and an oesophagus (Coons, 1978; Evans, 1992; Mathieson, 1995; Mathieson and Lehane, 2002). The midgut is of endodermal origin and is not cuticle lined. It is the site for synthesis and secretion of digestive enzymes and absorption of nutrients (Evans, 1992). The hindgut is typically cuticle-lined and is most likely involved in ion and water regulation (Evans, 1992).

2.1. Foregut

The foregut is divided into the pharynx and oesophagus (Coons, 1978; Evans, 1992; Mathieson, 1995; Mathieson and Lehane, 2002) (Fig. 2b). It is not known whether the foregut has any physiological function other than to deliver the food to the midgut. Dilator and constrictor muscles are located along the length of the pharynx, possibly to aid the movement of food material, by sucking and pumping actions (Mathieson, 1995; Mathieson and Lehane, 2002). In many mite species the pharyngeal lumen is capable of great extension and is characteristically folded into ridges (Brody et al., 1972; Evans, 1992). Food is pushed through the pharynx, by peristalsis, via the pharyngo-oesophageal valve to the oesophagus (Brody et al., 1972). Immediately anterior to the pharyngo-oesophageal valve in *P. ovis* is an area of thickened cuticle that is covered with pharyngeal teeth, which may represent a remnant structure indicative of a diet requiring trituration in an ancestral species (Mathieson and Lehane, 2002). In *P. ovis*, as with other mites, the oesophagus has a thin cuticle lining lying within encircling

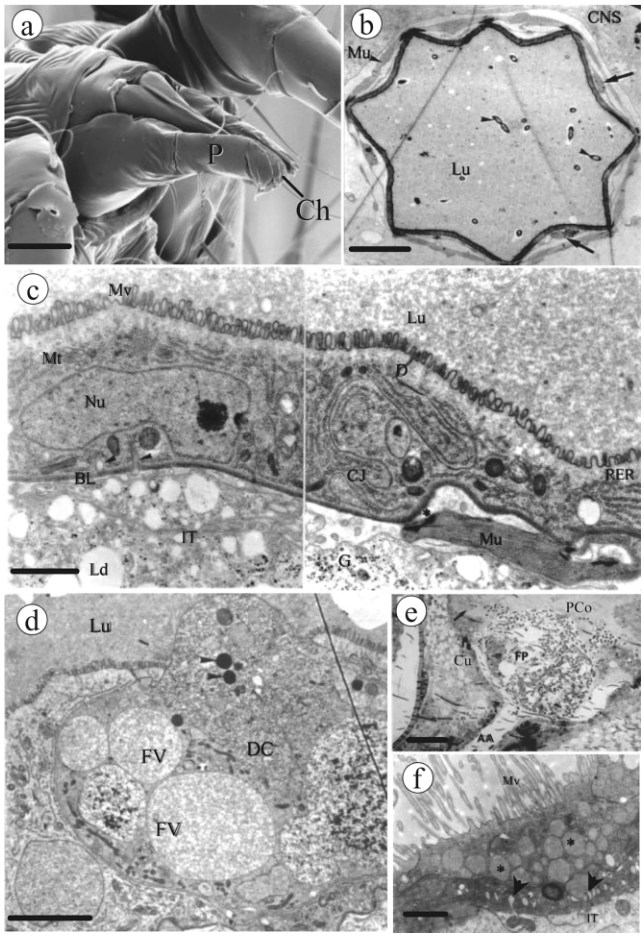

Fig. 2. Structure of the alimentary system of *P. ovis*. (a) The chelicerae (Ch) and palps (P) constitute the gnathosoma. The chelicerae run through the central canal shaped by the palps (bar = 36 μm). (b) The oesophagus forms an eight-pointed star arrangement in transverse section. Constrictor muscles (Mu) are attached at each point of the star (arrowhead, bacterial cells; CNS, central nervous system; bar = 3 μm). (c) Cross-sections through Type I epithelial cells. The cells typically possess short microvilli (Mv) adjacent to the gut lumen (Lu), a large basally located nucleus (Nu) encompassing a single nucleolus, small mitochondria (Mt) and extensive rough endoplasmic reticulum (RER). Convoluted lateral cell junctions (CJ) and apical desmosomes (D) are often observed throughout the midgut epithelium. The association of the basal lamina (BL) with the visceral muscle (Mu) is highlighted with an asterisk. Lipid (L) and glycogen (G) can be clearly observed in the underlying lobular intermediate tissue (IT) (bar = 1 μm). (d) A Type II digestive cell (DC) migrating from the epithelium to the lumen (Lu). Cells typically have few microvilli (arrowheads, lysosomes; FV, food vacuoles; bar = 2.5 μm). (e) Section through the post-colon (PCo) and anal atrium (AA) junction, demonstrating the long microvilli (arrow) of the post-colon giving way to the cuticle (Cu) lined anal atrium (FP, faecal pellet containing bacteria; bar = 6 μm). (f) Typical post-colon cell containing numerous vesicles (*) of unknown function. Long microvilli (Mv) and numerous intermediate tissue (IT) invaginations (arrowheads) suggests an absorptive function (bar = 1.2 μm).

epithelial cells and circular constrictor muscles. When seen in cross-section, the oesophagus forms an eight-pointed star arrangement and the constrictor muscles are attached to each point of the star (Woodring and Cook, 1962; Brody et al., 1972; Coons, 1978; Mathieson, 1995; Mathieson and Lehane, 2002). The oesophagus leads into the midgut where the cuticular lining is absent and the epithelial cells possess microvilli facing the lumen (Beetham, 1997; Mathieson and Lehane, 2002).

2.2. Midgut

The midgut consists of a stomach, colon and post-colon (Evans, 1992; Mathieson, 1995; Beetham, 1997; Mathieson and Lehane, 2002). The stomach can be divided into an anterior portion and two lateral ventriculi, which are believed to be the main sites for digestion and absorption (Hughes, 1954; Dinsdale, 1974; Evans, 1992; Mathieson, 1995; Mathieson and Lehane, 2002). Food from the oesophagus moves to the anterior stomach and then to the two lateral ventriculi and finally to the remainder of the midgut (Brody et al., 1972; Dinsdale, 1974; Coons, 1978; Mothes and Seitz, 1981; Beetham, 1997; Evans, 1992; Mathieson, 1995; Mathieson and Lehane, 2002).

Two distinct cell types (Types I and II) have been identified in the midgut epithelium of arachnids (Ludwig and Alberti, 1992a,b; Ludwig et al., 1994; Mathieson and Lehane, 2002) (Fig. 2c). In common with other members of the arachnid family, it is proposed that *P. ovis* uses both intracellular and extracellular digestion with each cell type playing a distinct role in either secretion (Type I) or intracellular digestion (Type II) (Ludwig and Alberti, 1992a,b; Ludwig et al., 1994; Mathieson and Lehane, 2002) (Fig. 2d).

Type I cells are the most abundant within the midgut epithelium and contain a large quantity of rough endoplasmic reticulum which is commonly concentrated in the apical region of these cells. They are located throughout the stomach of *P. ovis*. Type I cells are believed to be constitutively secretory in function and the lack of secretory vesicles in this cell type in *P. ovis* is consistent with this hypothesis (Evans, 1992; Mathieson, 1995; Mathieson and Lehane, 2002). Type II cells are commonly observed in the anterior part of the stomach but do not contain a dense network of rough endoplasmic reticulum suggesting that they play a limited role in synthesis and secretion. However, some vesicles are present in these cells which are potentially secretory. The digestive cells described are similar to those of other Acarines (Coons, 1978; Mothes and Seitz, 1981; Akov, 1982; Agyei et al., 1991).

P. ovis Type II cells possess an extensive network of apical tubules as well as numerous primary lysosomes and basally-located vacuoles, which contain material of a similar density to the lumen contents. These cells, which constitute approximately 8% of all the cells in the stomach of *P. ovis*, are believed to absorb fluid from the gut lumen by pinocytosis (Mathieson and Lehane, 2002). The Type II cells may ultimately detach from the gut epithelium and become free floating in the gut lumen of the mite where they begin to degenerate (Brody et al., 1972; Evans, 1992; Mathieson and Lehane, 2002). At this stage the cells frequently contain large secondary lysosomes and vesicles

(Mathieson and Lehane, 2002). Digestive cells in tick gut lumens behave in a similar fashion with gut epithelial cells detaching and moving into the lumen where they function as holocrine secretory cells (Akov, 1982).

The colon is lined with epithelial cells, which have a similar structure to Type I cells, but with numerous large vesicles and secondary lysosomes (Fig. 2f). In the post-colon the epithelia possess longer microvilli suggesting that re-absorption occurs in these areas (Hughes, 1950; Herman and Preus, 1972; Evans, 1992). Cells in this area also often contain large vesicles. Large quantities of faecal pellets are present in the lumen of the post-colon (Fig. 2g) and the microvilli of the lining epithelium are often tightly adjacent to the faecal pellets supporting the idea of absorption in these areas (Mathieson and Lehane, 2002). Faecal pellets, which are enveloped in a peritrophic matrix of unknown origin, often contain a much higher concentration of bacteria than observed elsewhere in the digestive system. The pellets, measuring ca. 15 μm in diameter, leave the digestive system via the cuticle-lined hindgut and are voided from the anal atrium onto the surface of the host (Mathieson, 1995; Mathieson and Lehane, 1996, 2002).

3. Biochemical aspects of digestion

The rigid cuticle and small size of mites precludes the dissection of their digestive systems for methodical analyses of the biochemical components and their localisation. Much of the information available regarding the digestive systems of astigmatid mites has been derived from house dust mites. The ability to produce laboratory cultures of these mites and to collect their faeces in their 'spent growth medium' (SGM) has allowed several groups to identify enzymatic activities associated with the faeces and SGM (e.g. Stewart et al., 1991). The presence of these enzymes in faeces suggests a role in the digestion of food and a localisation in the digestive tract (Stewart et al., 1998). This approach has led to the description of a number of serine, aspartyl and cysteine proteinases, aminopeptidases and carboxypeptidases present in whole body extracts of the astigmatid mould mite *Tyrophagus putrescentiae* (Ortego et al., 2000). The activities of several of these enzymes (notably the serine proteinases and carboxypeptidases) were 40-fold to 100-fold higher in SGM than in the whole body extracts, an observation that was attributed by the authors to the concentration of these enzymes within the peritrophic matrix-enclosed faecal pellets. Enzymes activities, which were present in the whole body extracts but absent from the SGM (e.g. aspartyl proteinases) were regarded as enzymes which were not involved in digestion in this species. However, the high concentration of proteolytic enzymes in faecal pellets and prolonged exposure of SGM to high relative humidity and ambient temperatures is a very harsh environment for the survival of proteins, including those involved in digestion, and their absence from SGM cannot be taken as an indication that they were not present and functional in the mite gut lumen. For example, a bacteriolytic enzyme in *D. pteronyssinus* and *D. farinae* was present in whole body extracts and SGM, suggesting gut-derivation (Mathaba et al., 2002). The lytic activity of *D. pteronyssinus* whole mite extract against *Micrococcus lysodeikticus* was 10-fold higher than that of SGM. The reduction in activity in SGM was the result of proteolytic action in the faeces.

Faecally derived enzymes from house dust mites, which are often potent allergens, including cysteine proteinases (Chua et al., 1988), serine proteinases (Stewart et al., 1991; Yasueda et al., 1993; King et al., 1996) amylase (Lake et al., 1991) and a bacteriolytic enzyme (Mathaba et al., 2002) are summarised in Table 1.

Below we discuss the current state of knowledge of digestive enzymes in *Psoroptes* species and their relationship with the enzymes described in other mite species. Table 2 summarises the enzymatic activities identified so far for *Psoroptes* mites.

3.1. Proteolytic enzymes

Proteinases are classified into four groups: serine, cysteine, aspartyl and metallopeptidases according to the amino acid residues and co-factors within their active sites and inhibitors directed to these sites (Barrett, 1980). Activities representative of all four groups have been described in mites and are discussed in detail below.

3.1.1. Cysteine proteinases

The group 1 allergens described from house dust mites (Der p 1, Der f 1; Table 1) are cysteine proteinases with sequence homology to cathepsin B and are important medically because of their ability to bind human IgE. The group number is assigned to each allergen in the order of identification and the same number is generally used to designate homologous allergens of related species (Hoffman et al., 1994). Der p 1 is excreted within the faecal pellet and 80% of individuals who are allergic to house dust mites produce Der p 1 specific IgE (Chapman et al., 1983; Krillis et al., 1984; Furmonaviciene et al., 2000; John et al., 2000). Der p 1 has been localised to the oral cavity, midgut epithelium, gut contents, hindgut tissues and faecal pellets of *D. pteronyssinus* and appears to be synthesised by the cells lining the gastrointestinal tract (Tovey et al., 1981; Thomas et al., 1991; Rees et al., 1992). The enzyme is thought to be important in digestion of skin cells (Stewart et al., 1991) through the hydrolysis of skin collagens and keratin (Colloff, 1993). Der p 1 mRNA encodes a cysteine proteinase containing one potential *N*-glycosylation site (Chua et al., 1988). There is considerable sequence homology between the group 1 antigens but even small sequence changes can result in changes in enzyme characteristics. Thus, the proteolytic activities of Der p 1 and Der f 1 (from *D. farinae*) were similarly inhibited by egg white cystatin and E-64, but only Der f 1 activity was

Table 1
Functional classification of known mite allergens

Allergen group	Functional classification	M_r (kDa)	Nomenclature		References[a]
1	Cysteine proteinase	25	Der f 1 Der m 1	Der p 1	1
			Eur m 1	Lep d 1	
2	Unknown	14	Aca 2		2
			Der f 2	Der p 2	
			Eur m 2	Gly d 2	
			Tyr p 2	Lep d 2	
3	Trypsin-like (serine proteinase)	29	Der f 3	Der p 3	3
			Eur m 3		
4	Amylase-like	56	Der p 4	Eur m 4	4
5	Unknown	14	Blo t 5	Der p 5	5
			Lep d 5		
6	Chymotrypsin-like (serine proteinase)	25	Der p 6	Der f 6	6
7	Unknown	22–30	Der p 7	Der f 7	7
			Lep d 7		
8	Glutathione-S-transferase	26	Der p 8		8
9	Collagenase-like (serine proteinase)	24	Der p 9		9
10	Tropomyosin	36	Der f 10	Der p 10	10
			Lep d 10	Blo t 10	
11	Paramyosin	98	Der f 11	Blo t 11	11
12	Unknown	14	Blo t 12		12
13	Fatty acid-binding protein	15	Aca s 13	Blo t 13	13
			Lep d 13		
14	Apolipophorin-like protein		Der f 14	Der p 14	14
			Eur m 14		
15	Chitinase	98	Der f 15		15
16	Gelsolin-like	55	Der f 16		16
17	Ca^{++} binding EF protein		Der f 17		17
18	Chitinase	60	Der f 18		18
19	Antimicrobial peptide		Blo t 19		19

Nomenclature: Aca, *A. siro*; Der p, *D. pteronyssinus*; Blo t, Blomia tropicalis; Eur m, *E. maynei*; Der f, *D. farinae*; Gly d, *Glycyphagus domesticus*; Der m, *Dermatophagoides microceras*; Tyr p, *T. putrescentiae*; Lep d, *L. destructor*.

[a] References: Group 1, (Chua et al., 1988; Smith et al., 1999); 2, (Heymann et al., 1989; Chua et al., 1990; van Hage-Hamsten et al., 1995; Eriksson et al., 1998; Park et al., 2000; Gafvelin et al., 2001); 3, (Stewart et al., 1992a,b; King et al., 1996; Smith and Thomas, 1996a; John et al., 2000); 4, (Stewart et al., 1992a; Olsson and van Hage-Hamersten, 2000); 5, (Yasueda et al., 1986; Tovey et al., 1989; Lin et al., 1994; Hsu et al., 1996; Arruda et al., 1997a); 6, (Yasueda et al., 1993; Hales et al., 2000); 7, (Shen et al., 1993); 8, (O'Neill et al., 1994); 9, (King et al., 1996); 10, (Aki et al., 1995; King et al., 1996); 11, (Tsai et al., 1999); 12, (Puerta et al., 1996); 13, (Yasueda et al., 1986; Caraballo et al., 1997); 14, (Fujikawa et al., 1996); 15, (McCall et al., 2001); 16 and 17, (Tategaki et al., 2000); 18 and19, (Tategaki et al., 2000).

inhibited by chestnut cystatin (CsC), ginko and pine extracts (Pernas et al., 2000).

Cysteine proteinase activity displaying the characteristics of a cathepsin B-like molecule was demonstrated in soluble extracts of *P. ovis* and, using a biotinylated cysteine proteinase inhibitor, a major band of 29 kDa was visualised after SDS-PAGE of the enzyme:inhibitor complex (Nisbet and Billingsley, 2000). Indeed, a range of cysteine proteinases in *P. ovis* extracts show hydrolytic activities against gelatin, fibronectin and IgG (Kenyon and Knox, 2002). A potential role for these proteinases in the degradation of host skin and in anticoagulant activity is therefore clear. Der p 1 possesses several biochemical properties related to its enzymatic activity which make it a potent allergen (Chapman et al., 1985; Chua et al., 1988; Ando et al., 1991; Hewitt et al., 1995, 1998; Cambra and Berrens, 1996) and it seems quite feasible that the cysteine proteinases described in *P. ovis* could serve the same function in the pathogenesis of sheep scab disease. A notable difference between *P. ovis* cysteine proteinases and those from house dust mites is the inability of the former to degrade keratin (Kenyon and Knox, 2002), which may reflect the different diets of the two species.

Expressed sequence tags (EST) of *P. ovis* have revealed several cathepsin B and L-like cysteine proteinase sequences, the most abundant of which had 69% amino acid identity to Der p 1 (Kenyon unpublished cited in Kenyon and Knox (2002)). A cDNA clone (*Pso o 1*) encoding a Der p 1-like cysteine proteinase from *P. ovis* has been isolated. The recombinant protein (Pso o 1) was recognised by antibodies induced during experimental infection suggesting its importance in the immune response to the mite. IgG titres were higher for Pso o 1 than for whole mite extract again suggesting Pso o 1 as a major and/or immunodominant antigen, similar to its closely related house dust mite homologue (Lee et al., 2002b).

Table 2
Enzyme activities described from *Psoroptes* mites

Enzyme	P. ovis	P. cuniculi
Phosphatase		
Acid	+	+
Alkaline	+	+
Phosphoamidase	+	+
Esterase		
Esterase (C4)	+	+
Esterase lipase (C8)	+	+
Lipase (C14)	+/−	−
Proteinases		
Aspartyl proteinase	+	+
Cysteine proteinase	+	+
Metalloproteinase[a]	+	n/a
Leucine aminopeptidase	+	+
Valine aminopeptidase	+	+
Glycosidase		
β-Galactosidase	+	+
β-Glucuronidase	+/−	+
β-Glucosidase	+	+
N-Acetyl-β-glucosaminidase	+	+

Adapted from (Nisbet and Billingsley, 1999a,b, 2000, 2002).
'+', present; ' − ', absent; 'n/a' not assessed.
[a] See Kenyon and Knox, 2002.

3.1.2. Serine proteinases

A number of serine proteinases have been identified in extracts of house dust mites and their SGM (King et al., 1996). The group 3 allergens (Der p 3) possess similar activity and sequence homology to trypsin (Smith and Thomas, 1996b), while two other house dust mite-derived serine proteinases, Der p 6 and Der p 9, possess chymotrypsin- and collagenase-like activities, respectively (Yasueda et al., 1993; King et al., 1996). Der p 9 has activity characteristics similar to cathepsin G, preferentially cleaving substrates containing either C-terminal phenylalanine or leucine residues (King et al., 1996). The substrate specificity of Der p 9 may have evolved to digest collagenous substrates in human skin cells and is similar in characteristics to an enzyme from the cattle warble fly *Hypoderma lineatum*, whose larvae burrow into skin (King et al., 1996).

Serine proteinase-like enzymes are present in the storage mites *Lepidoglyphus destructor* and *T. putrescentiae*, implying that biochemically similar allergens occur in different mite species. However, while elastase-like activity has been demonstrated in *D. pteronyssinus* and *D. farinae*, it is absent from *T. putrescentiae* extracts (Stewart et al., 1992b, 1998; Ortego et al., 2000) again indicating that molecular adaptation to diet occurs in mites as it does in insects (Terra et al., 1996).

Mite extracts have been assayed using a variety of natural and synthetic substrates to determine the possible role(s) of serine proteinases in the digestive system of *P. ovis* and *P. cuniculi* (Nisbet and Billingsley, 1999a,b, 2000; Kenyon and Knox, 2002). To date, no serine proteinase activity has been demonstrated in extracts of either species and there might be several reasons for this: (a) serine proteinases may not play any role in digestion in *Psoroptes* spp.; (b) there may be host- or parasite-derived serine proteinase inhibitors in the extracts that mask activity in the assays or (c) serine proteinase activity may be concentrated and voided in the faecal pellets (Ortego et al., 2000) but only whole body extracts have been assayed. In this regard, the development of an in vitro feeding system for *P. ovis* will be an important step forward, allowing the feeding of specific proteinase inhibitors to the mites to determine the role of different enzymes in digestion and the collection of faecal pellets for enzyme analysis.

3.1.3. Aspartyl peptidases

Cathepsin D-like aspartyl peptidase activity has been identified and characterised in extracts of *P. ovis* and *P. cuniculi* (Nisbet and Billingsley, 1999a,b). The activity was optimal at acidic pH and inhibited by pepstatin A. Using haemoglobin or a synthetic aspartyl proteinase substrate, extracts of *P. cuniculi*, which ingest erythrocytes from rabbit hosts, contained higher activities of aspartyl peptidase than *P. ovis* which had been fed on sheep, suggesting that diet plays a critical role in determining the relative activities of digestive enzymes in the mites (Nisbet and Billingsley, 1999a). Aspartyl proteinase activity has also been detected in extracts of *Acarus siro*, *Dermanyssus gallinae* and *Tetranychus urticae* (Nisbet and Billingsley, 2000). Cathepsin D-like enzymes are utilised as digestive enzymes by haematophagous and phytophagous hemipterans (Houseman and Downe, 1983) and dipterans (Lemos and Terra, 1991) and coleopterans (Blanco-Labra et al., 1996). Consistent with the absence of apparent serine protease activity in *Psoroptes* mites, hemipteran insects appear to have lost the ability to express serine proteases in the midgut as an evolutionary consequence of adaptation to a diet rich in serine proteinase inhibitors (Houseman and Downe, 1983; Terra et al., 1996). Cathepsin D-like enzymes and Cathepsin B and L-like cysteine proteinases are also principal proteolytic enzymes of lysosomes and aspartyl proteinases have been implicated in blood-meal digestion by specialised digestive cells in the guts of ticks (Akov, 1982). Analysis of the digestion patterns of ingested host immunoglobulin by *P. ovis* also indicated that these types of enzymes are involved in digestion in sheep scab mites (Pettit et al., 2000).

3.1.4. Metalloproteinases

Extracts of *P. ovis* are rich in metalloproteinase activity. The metalloproteinase inhibitor 1,10 phenanthroline inhibits proteolytic activity of *P. ovis* extracts against fibrinogen, IgG, fibronectin and azocoll (Kenyon and Knox, 2002). The actions of metalloproteinases on fibrinogen and IgG are of particular interest as they may facilitate mite survival through anticoagulant action and inhibition of host immune responses, respectively (Kenyon and Knox, 2002).

In addition, several metallo-exopeptidase activities,

including leucine (LAP) and valine (VAP) aminopeptidases, are present in *P. ovis* and *P. cuniculi*, (Nisbet and Billingsley, 1999a,b). In *P. cuniculi*, LAP activity in soluble extracts was characterised, on the basis of substrate and inhibitor specificity and cation sensitivity, as a cytosolic leucyl aminopeptidase corresponding to the M17 family of metalloproteinases (Nisbet and Billingsley, 2002). A cDNA clone encoding an M17 LAP was isolated from a *P. ovis* cDNA library and the derived amino acid sequence possessed the cation- and bestatin-binding residues in the correct conformation for a typical cytosolic LAP (EC 3.4.11.1). No signal sequence was evident at the N terminal of the protein, confirming the intracellular nature of the molecule (Nisbet et al., unpublished). A second, membrane-associated, aminopeptidase activity remains uncharacterised. Blood-meal induced, gut-associated LAP activity has previously been described from acarine species (Kerlin and Hughes, 1992), and so *Psoroptes* LAP may be present in the gut as part of the proteolytic machinery of digest cells or as a luminal enzyme (Nisbet and Billingsley, 1999a). LAP activity is present in *Dermatophagoides* spp. (Stewart et al., 1992a) and the faecal pellets of a stored product mite, *T. putrescentiae*, suggesting a digestive role for this enzyme (Ortego et al., 2000).

Carboxypeptidases A and B were 50-fold more active in faecal extracts than in whole body extracts of *T. putrescentiae* and this is considered indicative of their digestive function as endoperitrophic enzymes (Ortego et al., 2000). Carboxypeptidase A and B have been detected in *D. farinae* and *D. pteronyssinus* whole mite extract, although only carboxypeptidase B has been detected in the SGM. No carboxypeptidase activity has yet been described in the house dust mite *Euroglyphus maynei* (Stewart et al., 1991). Carboxypeptidase activity in *P. ovis* extracts has not yet been described but, as carboxypeptidases are often associated with lysosomal proteolysis, it is possible that they are employed by *Psoroptes* mites as exopeptidases in conjunction with aminopeptidases.

3.2. Glycosidases

In comparison with plant-parasitic and stored product mites, *P. ovis* and *D. gallinae* exhibited a limited range of glycosidases that are present at low activities. Low activities of β-galactosidase, β-glucuronidase and β-glucosidase in *P. ovis* extracts are probably indicative of the highly proteinaceous nature of its diet. Group 4 allergens from house dust mites possess properties similar to amylase (Lake et al., 1991), but an equivalent enzyme has not been identified from *P. ovis*.

3.3. Esterases

C14 lipase activity in *D. gallinae*, *P. ovis*, *A. siro* and *T. urticae* extracts was very low in comparison to C4 esterase and C8 esterase lipase (Nisbet and Billingsley, 2000). C4 and C8 esterases are present in the salivary glands and gut of the tick *Boophilus microplus* where they are assumed to play a digestive role (Kerlin and Hughes, 1992). Lipase was also present in very small quantities in extracts of *B. microplus* guts and in the faeces of house dust mites (Stewart et al., 1991; Kerlin and Hughes, 1992).

Overall, the biochemical nature of the enzymes detected so far from *P. ovis* extracts reflects the morphological and histological observations that much of the digestion of the liquid diet takes place intracellularly through endoproteinases derived from lysosomes with further processing by cytosolic and/or membrane bound exopeptidases. Fragments of ovine IgG present in *P. ovis* homogenate support the findings that aspartyl proteinase and metalloproteinase activity could be responsible for digestion within the *P. ovis* mite (Pettit et al., 2000).

Based upon their presence in SGM and whole body extracts and on allergenicity, a range of other compounds have been identified from house dust mites and can be considered putative but not proven digestive components (Table 1). These include the group 8 allergens which are glutathione-S-transferases (GSTs), ubiquitous multifunctional proteins involved in the detoxification of endogenous and xenobiotic compounds and which are also involved in the protection of cells from oxidative stress (Nauen and Stumpf, 2002). GSTs also function as carrier proteins (ligandins) participating in the transport of hydrophobic compounds (Pickett and Lu, 1989; Armstrong, 1991; O'Neill et al., 1994; Salinas and Wong, 1999; Lee et al., 2002a). GSTs from the cockroach *Blatella germanica* (Bla g 5) and the house dust mite *D. pteronyssinus* (Der p 8) are potent allergens (O'Neill et al., 1994; Arruda et al., 1997b; Pomes et al., 1998; Fernandez-Caldas, 1999). A *P. ovis* GST cDNA sequence was homologous to the class of GSTs of the cattle tick *B. microplus* (Rosa de Lima et al., 2002) and house dust mite *D. pteronyssinus* (Lee et al., 2002a), but a digestive function could not be ascribed to the molecule.

4. Role of bacteria in *P. ovis* digestive processes

Bacteria are important to arthropods as food sources, symbionts or pathogens (Marquardt, 1996). Symbiotic bacteria are found in most higher animals where they commonly provide supplementary nutritional components essential for maintenance of the host species, especially those surviving upon a nutritionally limited diet (Douglas, 1989). Guts of animals are often favoured sites for colonisation by microbes (Graf and Ruby, 1998) and usually remain colonised for the lifetime of the host (Douglas, 1989). Symbiotic microorganisms may aid digestion via the production of enzymes (Douglas et al., 2001) or the production of essential nutrients as has been demonstrated with the aphid *Aphis fabae*. The bacteria *Buchnera* spp. present in the gut of the aphid provide the essential amino acids that are not present in their diet of plant sap (Munson

et al., 1991; Douglas et al., 2001). Insects that feed on a nutritionally restricted diet of blood, for example triatomines, rely on bacteria present in their gut to provide essential vitamins including certain B vitamins (Beard et al., 2000). Similar to *P. ovis*, there is not one single species of bacteria that is always present within any single triatomine species, and it is possible that several bacteria can function in a symbiotic capacity (Beard et al., 2000). Certain groups of bacterial species or strains may have characteristics that allow them to fulfil the symbiotic needs of their host (Beard et al., 2000). Symbiotic bacteria have yet to be described in *P. ovis* but the mite clearly has very close associations with bacteria in its natural environment (Table 2). The bacterial flora of the *P. ovis* gut is abundant but not consistent in its species composition (Mathieson and Lehane, 1996, 2002; Hogg and Lehane, 1999, 2001). The relationship between the bacterial flora of the sheep skin and *P. ovis* gut is largely unexplored but *P. ovis* appears always to harbour a bacterial species capable of producing extracellular lipases (Mathieson and Lehane, 1996, 2002; Hogg and Lehane, 1999, 2001). Lipid is a major constituent of the sheep epidermis (Lloyd et al., 1979; Britt et al., 1985; Sinclair and Filan, 1989), and mites may use the lipolytic bacteria as a food source or exploit the bacterial lipases to digest the available host lipids either extra-corporeally or within their guts. *Bovicola* (*Damalinia*) *ovis*, a chewing louse of sheep, ingests large quantities of skin-dwelling bacteria and free lipid, and this may also be the case with *P. ovis* (Murray and Edwards, 1987).

Bacteriolytic enzymes are present in extracts of house dust mite and SGM and are distinct from known lysozymes. Recently, a number of bacteriolytic proteins, with preference for gram-positive bacterial substrates, were demonstrated in extracts from *D. pteronyssinus* and *D. farinae* (Mathaba et al., 2002). A cDNA for one of these bacteriolytic proteins indicated that the enzyme, which is likely to be involved in digestion and defence, was derived from bacteria within mites rather than the mites themselves.

Changes in the virulence measured by the clinical signs of sheep scab disease caused by different populations of mite may also result from differential pathology of gut flora of the mites (Hogg and Lehane 1999, 2001). However, no clear pattern has yet emerged of the elements in the species relationship that result in the pathological or sub-patent infestations. While host genetic background accounts in part, for mite pathology there is the intriguing possibility that knowledge of bacterial involvement in the scab lesion will lead to novel targets for therapeutic approaches.

5. Allergy, pathogenesis and food supply

Mite allergies are an important and growing medical and veterinary problem. Allergy to house dust mites is extremely common among humans with bronchial asthma and allergic rhinitis, (Stewart et al., 1991). The majority of allergens comprise low molecular weight glycoproteins (5–50 kDa) excreted in the faecal pellet, which are then able to enter the respiratory system of the affected individual (Tovey et al., 1981; Arlian et al., 1984; van Hage-Hamstern et al., 1992; Thomas, 1993). Der p 1 increases epithelial permeability (Herbert et al., 1995; John et al., 2000) by targeting proteins in the tight cell junctions (Herbert et al., 1991; Roche et al., 2000), a process that is blocked by the cysteine proteinase inhibitor E-64 (Wan et al., 2000). Der p 1 also inactivates alpha$_1$-antitrypsin, thereby allowing the release of neutrophil elastase and initiating the self-destruction of the alveoli in the lung (Crystal, 1990; Cambra and Berrens, 1996; Machado et al., 1996; Roche et al., 1997; Comoy et al., 1998; John et al., 2000; Wan et al., 2000). The IgE response initiated by the presence of Der p 1 is further exacerbated by cytokines (IL4 and IL5) and leukocytes associated with a Type II response (Chambers et al., 1998; Hales et al., 2000). In addition, Der p 3 has the ability to initiate complement C3 and C5, contributing to the pathology of the allergenic response (Olsson and van Hage-Hamersten, 2000).

Following a challenge infestation with *P. ovis*, there is significant reduction in lesion growth suggesting that mite products can induce protective immunity to *P. ovis* in sheep (van den Broek et al., 2000). Secretory/excretory products from the mite induced *P. ovis*-specific IgE indicating that mites can provoke an immediate Type I hypersensitivity reaction in sheep. Lesion-associated and cutaneous basophils are also present during the infestation, indicating that *P. ovis* induces a cutaneous basophil hypersensitivity reaction. The actual mechanism involved in protective immunity through hypersensitivity still remains unclear.

As in other mite species, the faecal pellets of *P. ovis* are probably involved in inducing the allergenic response typical of sheep scab and provide the mite with its main source of food, the serous exudate associated with the pathology (Sinclair, 1990). The clinical response of the sheep to *P. ovis* is more likely to be due to the production of antigenic faecal material containing active proteases than to the presence of the mite itself (Hogg and Lehane, 2001). Faecal pellets of *Psoroptes* mites contain guanine, (2-aminohypoxanthine) a compound described (by its toxicological data) as an ‘irritant’ to eyes and skin and probably a range of allergens such as cysteine and aspartyl proteinases that are common in other species. Salivary components, if deposited onto the host skin, may also initiate or exacerbate inflammatory response and leakage of serous exudates.

The relationship between allergy, immunity, pathology and the mite digestive system is slowly emerging. Sera from *P. ovis*-infested steers, some of which had acquired resistance, bound antigens present throughout the mite digestive system rather than those associated with the exoskeleton or other organs (Beetham, 1997). It is also notable that after successful acaricide treatment the immunological response of the host to the *P. ovis* antigens

stopped, indicating that dead mites are not immunogenic (Losson, 1999).

The scab lesion is probably further exacerbated by the presence of bacteria exploiting the local pathological environment and the nature of the scab may be determined in part by the bacterial species present. For example, *Pseudomonas aeruginosa* excretes an elastase that digests proteins of intracellular junctions and extracellular matrix (Roche et al., 2000). *Serratia marcescens*, *Panteoa agglomerans* and some pseudomonads produce extracellular lipase, *Burkholderia* spp. are able to colonise midguts of arthropods. All these species have been identified in association with *P. ovis* in vivo (Hogg and Lehane1999, 2001) (Table 3). The origins of the bacteria still remain uncertain, although *S. marcescens* generally has a pathogenic role instead of a symbiotic/mutualistic role. The diversity of bacteria associated with the *P. ovis* mite is a lot greater than that which appears on the surface of the sheep's skin, suggesting the mite is harbouring its own bacteria flora. Details of the bacterial flora present on the surface of the sheep's skin during the diseased condition and the *P. ovis* mite midgut are outlined in Table 3.

Table 3
Bacterial flora present on the surface of a sheep during different conditions

Fleece condition	Bacteria species	Location
Healthy	*Acinetobacter calcoaceticus*	Skin surface
	Bacillus cereus	
	Bacillus thuringiensis	
	Mirococcus spp.	
	Pseudomonas spp.	
	Staphylococcus cohnii	
	Staphylococcus xylosus	
P. ovis infestation	*Alloiococcus otitidis*	Mite midgut
	Acinetobacter spp.	Skin surface
	Burkholderia spp.	Skin surface and mite midgut
	Bacillus spp.	Mite midgut
	Bradyrhizobium japonicum	Mite midgut
	Corynebacterium spp.	Mite midgut
	C. baltica-related bacterium	Mite midgut
	P. agglomerans	Mite midgut
	P. rubiacearum	Mite midgut
	Propionibacterium acnes	Mite midgut
	Pseudomonas spp.	Skin surface and mite midgut
	Pseudomonas tolaasii related-proteobacterium	Mite midgut
	Uncultured eubacterium	Mite midgut
	Salinicoccus roseus	Mite midgut
	S. marcescens	Mite midgut
	Staphylococcus aureus	Skin surface and mite midgut
	Staphylococcus intermedius	Mite midgut
Wet-inducing fleece rot	*P. aeruginosa*	Skin surface

Includes bacteria associated with the *P. ovis* mite.

Based upon data from (Merritt and Watts, 1978; Chin and Watts, 1992; Lyness et al., 1994; Mathieson and Lehane, 1996; Hogg and Lehane, 1999; Hamilton et al. unpublished).

6. Model for digestion in *P. ovis*

When first becoming established on a host, both *P. cuniculi* and *P. ovis* probably feed on the loose stratum corneum and on any lipid secretions present (Rafferty and Gray, 1987). The gnathosomes are embedded in the outer epidermis, but not in deeper epidermis or dermal tissue (Kirkwood, 1985), suggesting that *P. ovis* ingests epidermal lipid.

As the mites actively wander across the surface of the host, antigenic, allergenic and enzymatically active material is deposited causing an inflammatory response on the skin (Rafferty and Gray, 1987; Pruett et al., 1998). As a result, skin breakages occur which result in the release of serum exudates and erythrocytes on which mites feed (Uhlir, 1991; Pruett et al., 1998).

Ingestion of the fluid and particulate diet is followed by digestion within the midgut. This involves internalisation of the food, probably by pinocytosis, into Type II digestive cells where proteolysis is initiated by lysosomally derived endopeptidases (aspartyl and cysteine proteinases, possibly metalloproteinases) and is followed by lysosomal and cytosolic exopeptidases (including cysteine proteinases and aminopeptidases). Proteolysis may also involve luminal enzymes derived from secretory Type I cells and membrane bound enzymes. Within the entire digestive system of *P. ovis*, a significant population of luminal bacteria have been identified, many of which are gram negative, including *S. marcescens*, *Propionibacterium*, *Phyllobacterium rubiacearum*, *P. agglomerans* and *Curacaobacter baltica* (Mathieson, 1995; Hogg and Lehane, 1999); however, their exact mutualistic or dietary roles have still to be determined.

An understanding of the physiology of mite digestion is an essential component of the research into potential and alternative means of controlling parasitic mites. For targeted therapies, such as drugs and vaccines or diagnostics for *P. ovis* control in sheep, the isolation and characterisation of putative mite antigens and active molecules is a necessary precursor to successful control. Understanding the physiological context in which these molecules function is crucial to the sustainability of such approaches.

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