Molecular Analysis of *Sarcoptes scabiei*

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Abstract

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The mite *Sarcoptes scabiei* (Acari), causes sarcoptic mange or scabies that globally affects animals and humans. Although scabies and mange are recognised as important diseases in human and veterinary medicine the amount of molecular studies of *S. scabiei* have been limited, which has been attributed to a shortage of parasitic material.

This thesis is based on four studies that make use of molecular techniques with the aims to overcome the problems associated with a scarcity of mites, gain insights into the genetic background of the mite and to identify proteins important for host-parasite interactions. A better knowledge of parasite proteins that interact with the host is pivotal for understanding the mite's pathogenicity.

In order to accelerate gene discovery in *S. scabiei* an expressed sequence tag (EST) analysis of 1,020 ESTs was performed. Around half of the ESTs could be assigned with a putative gene identity. In the data set several proteases and allergens were identified, all of which possibly are involved in host-parasite interactions.

The EST analysis identified a transcript corresponding to the enzyme gluthathione-S-transerase (GST). GST has an active role in detoxification, and has been a target molecule for vaccine development and drug resistance in other parasitic diseases. A phylogentic study showed that it was a delta-class GST, which previously never had been described in the order Acari. Several recombinant versions of the delta-GST were expressed and all were enzymatically active.

Paramyosin, major *Sarcoptes* antigen 1 (MSA1) and atypical *Sarcoptes* antigen 1 (ASA1), were identified by immunoscreening, and later expressed as recombinant proteins for further characterisation. Paramyosin is an invertebrate muscle protein, associated with protective immunity in helminth infections. Sera from both dogs and pigs infected with *S. scabiei* reacted with recombinant paramyosin. ASA1 contained a MADF domain, which is not normally associated with antigens. In a Western blot analysis, 24% of the *S. scabiei* positive dogs were positive to ASA1. In contrast 82% of the dogs had antibodies towards MSA1. Immunohistochemistry (IHC) localisation of ASA1 and MSA1 supports the observation that these proteins are exposed to the host. In contrast, IHC showed that delta-GST is more confined to the mites.

Keywords: Acari, *Sarcoptes scabiei*, Mange, Scabies, EST, Gene expression, Gluthathione S-transferase, Immunolocalisation, Paramyosin

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Svensk sammanfattning

Molekylär analys av skabbkvalstret *Sarcoptes scabiei*

Det parasitära kvalstret *Sarcoptes scabiei* orsakar sjukdomen skabb som drabbar både djur och människor runt om i världen. Skabb orsakar betydande lidande hos drabbade värddjur och orsakar påtagliga produktionsbortfall i t.ex. svinbesättningar. En intensiv klåda är det kliniska symptom som dominerar sjukdomsbilden hos alla typer av infekterade värdar. I Sverige är skabb en välkänd dödsorsak hos räv, så kallad rävskabb, men den är även orsak till svår hudsjukdom hos hund, gris och andra djurslag. Infektion med *S. scabiei* ger upphov till en immunaktivering som resulterar både i ett cellmedierat svar och ett antikroppssvar. Trots att både kvalstret och sjukdomen varit kända sedan länge har den molekylära forskningen kring *S. scabiei* varit begränsad. En av anledningarna till detta är svårigheterna att få tillgång till *S. scabiei*-kvalster för vetenskapliga studier, och det finns inte heller något odlingssystem för dessa parasiter. De för närvarande tillgängliga alternativen är således att isolera kvalster från naturligt infekterade värdar eller att etablera en infektion på försöksdjur.

I de fyra delstudierna i denna avhandling har målen varit att med molekylärbiologiska metoder komma förbi problemen med den bristande tillgången på kvalster, skaffa större kunskap om den genetiska bakgrunden hos skabbkvalstret, samt att identifiera proteiner som är viktiga för interaktionen mellan värd och parasit. Den kunskap som fås genom att identifiera och karakterisera parasitrelaterade proteiner som interagerar med värddjuret är viktig för att öka förståelsen kring uppkomsten av skabb och de skadeverkningar som ses i samband med sjukdomen. Vidare kan de proteiner som rör värd-parasitinteraktioner komma att utgöra målmolekyler för utveckling av framtida vacciner, samt bidra till ökad precision i serologiskt diagnostiska system.

För att effektivt kunna beskriva nya gener hos *S. scabiei* genomfördes en *expressed sequence tag* (EST) analys vilken omfattade 1020 ESTs. En EST är en kort DNA-sekvens som i denna studie motsvarar uttryckta gener i kvalstret. Denna analys ledde till identifieringen av en mängd tidigare okända gener hos *S. scabiei*. Ungefär hälften av dessa ESTs kunde tilldelas en trolig genidentitet med utgångspunkt från jämförelser med tidigare kända sekvenser som finns tillgängliga i olika databaser. Många av dessa gener kunde antas vara inblandade i den basala cellmetabolismen och den cellulära organisationen hos parasiten. En tiondel av alla EST-sekvenser motsvarar emellertid tidigare obeskrivna gener vars funktion i kvalstret för tillfället är okända. Vidare identifierades även många intressanta gener som kodar för ett antal proteinnedbrytande enzymer (proteaser), och från andra organismer kända allergiframkallande proteiner (allergener). Alla dessa proteaser och allergener är potentiellt inblandade i värd-parasit interaktioner.

En av de gener som identifierades med hjälp av EST-analysen kodar för enzymet *glutathione-S-transferase* (GST). Detta enzym finns hos många organismer och bidrar med ett allmänt skydd genom dess roll i olika avgiftningsprocesser. GST

utnyttjas också som målmolekyl i samband med vaccin- och läkemedelsutveckling för ett antal parasitära sjukdomstillstånd. Detta föranledde en fortsatt karakterisering av GST från *S. scabiei,* då väldigt få GST-enzymer blivit rapporterade från ordningen Acari, dvs. fästingar och kvalster. En fylogenetisk analys visade att det rörde sig om ett delta-klass GST som aldrig tidigare beskrivits från Acari. Flera versioner av rekombinant uttryckta GST-polypeptider genererades och samtliga visade sig vara enzymatiskt aktiva. Vid en analys av antikroppssvaret hos naturligt *Sarcoptes*-infekterade hundar och grisar kändes inte enzymet igen, vilket tyder på att delta-GST inte är exponerat för immunsystemet. Detta bekräftades genom immunolokalisering av GST i vävnadssnitt med parasiter.

Parasiten *S. scabiei* inducerar hos värddjuren ett mycket starkt antikroppssvar som kan utnyttjas för att identifiera immunreaktiva *S. scabiei*-proteiner som uttrycks i bakterier. Flera proteiner identifierades genom så kallad *immunoscreening* med hjälp av antikroppar som har sitt ursprung från *S. scabiei*-exponerade värddjur. Tre av dessa har uttryckts i rekombinantversioner för vidare karakterisering. Dessa är paramyosin, *major Sarcoptes antigen 1* (MSA1) och *atypical Sarcoptes antigen 1* (ASA1). Paramyosin är ett muskelprotein hos invertebrater vilket har visat sig vara inblandat bl.a. i utvecklingen av den skyddande immuniteten vid olika maskinfektioner. Följaktligen studerades antikroppssvaret hos hund och gris mot rekombinanta varianter av paramyosin från *S. scabiei*. Dessa försök visade att vissa individer reagerade mot rekombinantproteinerna, och att rekombinant paramyosin därför eventuellt skulle kunna fungera som en serologisk markör för skabbinfektion. ASA1 innehåller en s.k. MADF-domän och de få andra tidigare funna proteiner som har en sådan domän är alla transkriptionsregulatorer. Observationen att ASA1 från skabbkvalstret ger upphov till ett kraftigt immunsvar hos värden är därför särskilt intressant. ASA1 uttrycktes i ett antal versioner och en av dessa användes för att studera immunsvaret hos drygt 100 hundar. Ett antikroppssvar mot ASA1 hittades hos 24 % av de skabbpositiva hundarna medan 82 % var MSA1-positiva. Med hjälp av immunohistokemisk analys kunde lokaliseringen av ASA1 och MSA1 i *S. scabiei* fastställas och det kunde konstateras att de båda antigenen förefaller vara exponerade för värddjuret.

Sammantaget har dessa studier av *S. scabiei* ökat kunskapen om vilka typer av gener som är aktivt uttryckta hos parasiten. Flera proteiner från *S. scabiei* som är inblandade i värd-parasit-interaktioner har också karakteriserats. I förlängningen kan dessa kunskaper bidra till förbättrad diagnostik och kontroll av parasiten.

Welcome and congratulations!

I am delighted that you could make it. Getting here was not easy, I know. In fact, I suspect it was a little tougher than you realise. To begin with, for you to be here now, trillions of drifting atoms had somehow to assemble in an intricate and intriguingly obliging manner to create you. It is an arrangement so specialised and particular that it has never been tried before, and will only exist this once. For the next many years, we hope, these tiny particles will uncomplainingly engage in all the billions of daft cooperative efforts necessary to keep you intact and let you experience the supremely agreeable, but generally under-appreciated state known as existence.

So thank goodness for atoms! But the mere fact that you have atoms and that they assemble in such a willing manner is only a part of what got you here. To be here now, alive in the 21st century, and smart enough to know it, you also had to be the beneficiary of an extraordinary string of biological good fortune. Survival on earth is a surprisingly tricky business, of the billions and billions of species of living things that have existed since the dawn of time, most, 99.99% are no longer around. Life on earth, you see, is not only brief but also dismayingly tenuous. Not only have you been lucky enough to be attached, since time and memorial, to a favoured evolutionary line but you also been extremely, make that miraculously, fortunate in your personal ancestry. Consider the fact that for 3.8 billion years, a period of time older than the earth's mountains and rivers and oceans. Every one of your forbearers, on both sides, has been attractive enough to find a mate, healthy enough to reproduce, and sufficiently blessed by fate and circumstances, to live long enough to do so. Not one of your ancestors was squashed, devoured, drowned, starved, stuck fast, untimely wounded, or otherwise deflected from its life's quest of delivering a tiny charge of genetic material to the right partner, at the right moment. In order to perpetuate the only possible sequence of hereditary combinations that could result, eventually, astoundingly, and all to briefly in you.

Bill Bryson – A short history of nearly everything (abbreviated foreword, Audible©)

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Mattsson, J.G., Ljunggren, E.L. & Bergström, K. 2001. Paramyosin from the parasitic mite *Sarcoptes scabiei*: cDNA cloning and heterologous expression. *Parasitology* 122, 555-562.

II. Ljunggren, E.L., Nilsson, D. & Mattsson, J.G. 2003 Expressed sequence tag analysis of *Sarcoptes scabiei*. *Parasitology* 127, 139-145.

III. Pettersson, E. U., Ljunggren, E.L. & Mattsson, J.G. 2005. Functional analysis and localisation of a delta-class glutathione-S-transferase from *Sarcoptes scabiei*. *International Journal for Parasitology* 35, 39–48

IV. Ljunggren, E.L., Bergström, K., Morrison, D.A. & Mattsson, J.G. 2005. Characterisation of an atypical antigen from *Sarcoptes scabiei* containing an MADF domain. *Submitted manuscript.*

Abbreviations

ASA1	atypical *Sarcotes* antigen 1
BIT	burrow ink test
BLAST	basic local alignment search tool
bp	base pair
cDNA	complementary DNA
CO	cytochrome C oxidase
dbEST	database of expressed sequence tags
DNA	deoxyribonucleic acid
EST	expressed sequence tag
GST	glutathione S-transferase
His_6	histidine-tag
HIV	human immunodeficiency virus
Ig	immunoglobulin
IMAC	immobilised metal affinity chromatography
ITS	internal transcribed spacer
kDa	kilo Dalton
MADF	domain in ASA1
MBP	maltose-binding protein
MHC	major histocompatibility complex
mRNA	messenger RNA
MSA1	major *Sarcoptes* antigen 1
mtDNA	mitochondrial DNA
ORF	open reading frame
PCR	polymerase chain reaction
pfu	*Pyrococccus furiosus*
RH	relative humidity
RNA	ribonucleic acid
rRNA	ribosomal RNA
SRCRP	scavenger receptor cysteine rich protein
Ssag	*Sarcoptes scabiei* antigen
STI	sexually transmitted infection
taq	*Thermus aquaticus*
Th	T-helper cell type
TIL	trypsine inhibitor like
UTR	untranslated region

Introduction

"As to diseases make a habit of two things - to help, or at least, to do no harm."
Hippocrates

The science of diagnosing, treating, or preventing disease and other damage to the body or mind is called medicine. This thesis deals with research within the branch of medicine called veterinary parasitology, i.e. the medical science that studies parasites in relation to disease in animals. Disease in all its forms has been a part of people's everyday life throughout history, and it is likely to follow us into oblivion. Regardless of whether diseases affect our domesticated animals or ourselves, there will be an ongoing struggle to find new treatments, improved diagnostics or vaccines, in order to minimize the damage inflicted by pathogenic viruses, bacteria, parasites and other ill-health causing conditions.

Arachnids (spiders, ticks and mites) form a large class of described arthropods, and most likely a large number of not yet described species. Spiders make up the majority of this group (over 35,000 described species), with mites and ticks being next (around 30,000 described species). Among these, the study object for this thesis is found, *Sarcoptes scabiei*. This ectoparasite is a pathogenic burrowing mite with the ability to infect a wide range of animals and humans. The disease it causes is known as mange when it affects animals or scabies in humans. It is regarded as a parasite of significant veterinary importance, since as it affects both farm and companion animals. For example, in pig herds, in Europe and North America, prevalences from 20% to 86% have been reported. In Europe, the estimated loss due to porcine mange is about 7 euro per finishing pig (Lowenstein *et al.*, 2004). Likewise, there are several reports from all over the world of outbreaks of mange as an epizootic disease in wild populations of e.g. bovids, canids, felids etc. (Pence & Ueckermann, 2002).

S. scabiei is also regarded as an important public health problem among humans. Estimations indicate that worldwide up to 300 million are affected by scabies at any given time (Taplin & Meinking, 1990). The mite is transmitted by close personal contact, including sexual contact. Primarily it is in developing countries that scabies is a major problem, as usually transmission is associated with overcrowding and poverty (Green, 1989). Other groups that are predisposed to contract scabies are infants, the elderly and patients with immunocompromised conditions (Chosidow, 2000). Thus, *S. scabiei* is an important pathogen, as a cause of suffering in humans and animals, as well as inflicting economical damage to farmers. Presently, there are effective treatments for both scabies and mange, but recently reports about increased resistance to the most common acaricides have been published (Currie *et al.*, 2004; Walton *et al.*, 2000). The problems linked to resistance are most likely to escalate with time if no preventive measures are taken.

Recombinant DNA technology has provided tools that have significantly increased our understanding of many organisms and various biological processes. Including molecular studies on *S. scabiei*, however, these did not appear until the

mid 90's. Particular important was the introduction of the polymerase chain reaction (PCR). This technology has by itself changed the face of biological and medical sciences. This thesis makes use of the recombinant DNA technology (1) to increase the knowledge about expressed genes in *S. scabiei* and (2) to identify *S. scabiei* antigens by sera from dogs with sarcoptic mange, (3) to generate recombinantly expressed proteins from *S. scabiei* and to further characterize their function in *S. scabiei*.

Background

The following section in this thesis is included to give the reader some general information about the parasite and its biology. Central to the background are of course the topics animal mange and human scabies. There are many references to various aspects of human scabies, as a direct consequence of the amount of published literature within the field, i.e. there are many more reports on the topic human scabies than the topic animal mange. Furthermore, the studies included in this thesis primarily make use of biological material from members of the family Canidae (e.g. foxes and dogs), and thus the background focuses on sarcoptic mange in dogs, to provide the reader with relevant information in relation to the studies. These sections also contain some information about porcine mange and mange in wild animals. Porcine mange is important from a veterinary perspective, as the disease has extensive economical impact for farmers. At the end of the background, there is a section covering the subject of molecular approaches that have been used in the studies of *S. scabiei*. Understanding this part of the background requires some knowledge of recombinant DNA technologies. A reading suggestion is the book *Gene Cloning and DNA analysis*, which provides a basic introduction to recombinant DNA technologies (Brown, 2001).

History

The knowledge of scabies or mange has a long and interesting history: the disease has been recognised for at least 3,000 years. Presented here are some highlights on the subject c.f. (Roncalli, 1987)

The name *Sarcoptes scabiei* is derived from Greek and Latin words. The Greek words *sarx* (the flesh) and *koptein* (to cut) form the word *Sarcoptes*. The Latin word scabiēs comes from *scabere* that means “to scratch”, and in this respect the word reflects the dominant clinical symptom of an infection with *S. scabiei* which is an intense pruritus.

The oldest known references to the disease either in humans or animals date back to the Bible. For instance, in the third book of Moses (Chapter 13), a man suffering from skin disease was asked to show the lesions to a priest who, in turn, was supposed to be capable of differentiating scabies from leprosy. In Aristotle’s *De Historia Animalium* (347-343 B.C.) the next reference to scabies is found, and this was followed by several references from the Roman times. In *Georgics* (29 B.C.), a poetic agricultural manual by Virgil, a description of the problems associated with sarcoptic mange in sheep is described, and how the condition could be treated with tar, grease and washing. Based on Virgil’s description of mange in sheep, approximately 1550 years later the first illustration of the treatment of mangy sheep was made (Fig. 1). Note that it is impossible to conclude from the illustration, whether these animals were infected with *S. scabiei* or the close relative, the sheep scab mite *Psoroptes ovis*.

Fig. 1. The first illustration on the treatment of mangy sheep from a version of "Georgics" by Virgil published 1502 in Strasbourg. Here sheep with mange are treated with ointments, bloodletting and removal of crusts with a blade (Roncalli, 1987).

Although people had known of the existence of the sarcoptic mite and the disease scabies/mange, no one had linked the two together. It was not until 1687 that the Italian Bonomo, in his letter "Observations concerning the fleshworms of the human body", actually linked the origin of the disease scabies to the mite. Bonomo, together with his co-worker Cestoni, described several new findings about the mites. Using microscopy, they were the first to produce fairly accurate illustrations of the mites and to describe how they dug out their burrows. Interestingly, they discovered the existence of male and female mites, which proved that reproduction is through mating and not spontaneous generation. Bonomo's publication drew at the time a lot of attention; unfortunately the information was not kept for long within the medical world.

Early Swedish contributions were made in 1746 by Linnaeus, who described two species of the itch-mite, one specific to animals and the other specific to humans. The entomologist de Geer (1720-1778) was the first person to provide a very good rendition of a *Sarcoptes* mite (Thyresson, 1994).

During the late 18th and early 19th centuries prominent advances were made in veterinary parasitology. As an example in 1786, the German scientist J.E. Wichmann published a monograph, *Aetologie der Krätze*. Here he makes the important statement, that mites are the origin of mange in animals and scabies in humans. However, despite the knowledge in veterinary parasitology, many physicians in the beginning of the 19th century could still not accept the parasitic nature of scabies. The change came in 1834, when a Corsican medical student, S.F. Renucci, recovered *Sarcoptes* mites from patients with scabies. His rediscovery of

the mite lead to a chain of important works describing the morphology and biology of mites, and established the presence of different species of mites.

In the 20th century, new treatments and methods to control sarcoptic mange were developed. The uses of ectoparasiticides, along with new veterinary regulations, have substantially reduced the presence of mange in domestic animals in many countries. Although great achievements have occurred in the control and treatment of mange and scabies in the last century, there are still many people and groups of animals at risk of contracting the disease.

Biology

Classification

S. scabiei is an arthropod belonging to the class Arachnida, subclass Acari, order Astigmata, superfamily Sarcoptoidea and family Sarcoptidae (Fain, 1968). Typical features of members of the class Arachnida are that they have four pair of legs and their bodies consist of a cephalo-thorax and an abdomen. Members of the order Astigmata have no detectable spiracles or tracheal system, are weakly sclerotised and are slow moving mites. The sarcoptid mites, such as members of the families, Sarcoptidae of mammals, Teinocoptidae of bats and Knemidocoptidae of birds (Krantz, 1978), are all obligate parasites burrowing into the skin of their hosts. The family Sarcoptidae include besides *S. scabiei,* the mite of guinea pig (*Trixacarus caviae*), and the cat mite (*Notoedres cati*).

Morphology

The colour of *S. scabiei* is creamy white with brown sclerotized legs and mouthparts (Fig 2). The body is tortoise-like: the idiosoma (body) has a broad oval shape that is ventrally flattened and dorsally convex. The surface of the idiosoma is covered with fine striations and dorsally there are a number of variable fields of stout setae and spines. Additionally, in the female there are also fields of numerous cuticular spines, which are of taxonomic significance (Fain, 1968). The size ranges from 300 to 500 µm long by 230-420 µm wide in the female; the male is smaller, 213 µm-285 µm long by 162-210 µm wide (Fain, 1968). The coxa (joint of leg) is embedded in the ventral surface. The two anterior legs are short, and extend beyond the anterior-lateral margins of the prosoma (anterior part of body). In both sexes legs 1 and 2 are situated close to the gnathosoma (mouth region), and they terminate in a stalked pulvillus (pad), also known as a sucker (Mellanby, 1972). The two posterior legs do not extend beyond the lateral opisthosoma (posterior part of body) margins. Legs 3 and 4 of females and legs 3 of males terminate in a long seta (chitinous hair). The anus is terminal in both sexes.

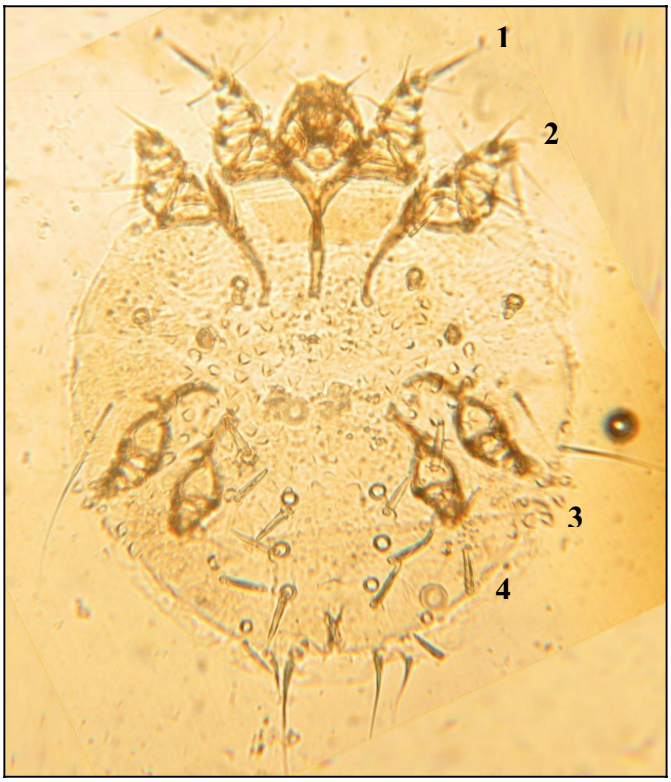

Fig. 2. Ventral view of *S. scabiei* taken from red fox (*Vulpes vulpes*). Numbers 1-4 indicate position of anterior and posterior legs. Magnification x 200. Photo: Erland Ljunggren.

Life cycle

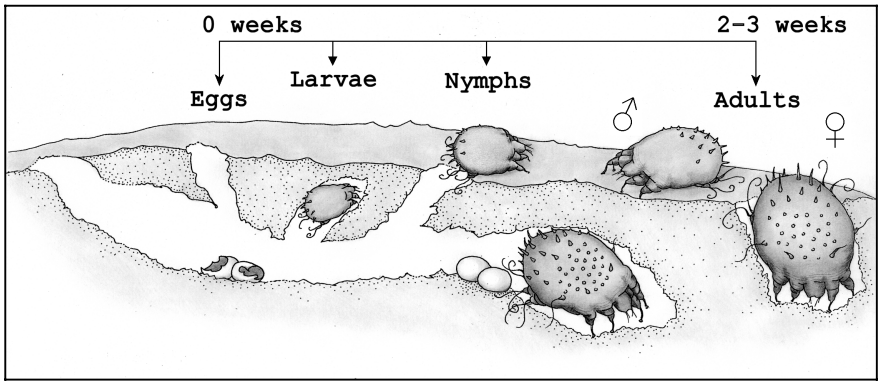

Fig. 3. The life cycle of *S. scabiei* comprises five lifestages: egg, larva, protonymph, tritonymph and adult. Illustration: Katarina Näslund.

The initial studies of the life cycle of *S. scabiei* (Fig. 3) were performed on humans with scabies. These studies shared a common problem, which is true of many studies past or present: the number of available parasites was relatively small. Another problem was the decision as to when to abort the investigations, as the patients ultimately needed treatment. At the time there was no experimental propagation system for *S. scabiei*, which would allow for repeatable observations. However, this was solved by the establishment of a culture population of *S. scabiei* derived from dogs on laboratory rabbits (Arlian *et al.*, 1984b), which made way for a more accurate study of the different life stages (Arlian & Vyszenski-Moher, 1988).

The life cycle of *S. scabiei* has five different developmental stages: eggs, larvae, protonymphs, tritonymphs, and adults. The duration of the life cycle appear to be dependent on the type of host species. Studies made on humans display variations in the developmental time from egg to adult: one reported 12-16 days (Mellanby, 1944) and another reported 17-21 days (Heilesen, 1946). In pigs, 10-15 days was needed for the completion of the life cycle. An even faster completion of the life cycle was observed in the experimental rabbit model, as here the development from egg to adult took 10-13 days (Arlian & Vyszenski-Moher, 1988). *S. scabiei* is described as a burrowing mite, since all life stages of the mite penetrate and inhabit the outer layers of the epidermis of the skin, where tunnels are excavated (Fig. 4) It is in these tunnels where the egg-carrying female lays 2-4 eggs daily, which will hatch in 3-5 days. From the hatched eggs the first instar, the six-legged larvae, emerge. Larvae either make short burrows from the breeding tunnel or, in most cases, tunnel up through the roof to the surface of the skin (Heilesen, 1946). Once on the surface of the skin, they move around to find a suitable skin crease or hair follicle, and either enter the follicle or dig a short burrow which nearly conceals the body. This type of short burrow is termed a moulting pocket, and it is here they develop into nymphs, the second instar protonymphs and the third instar tritonymps. The nymphal stages either remain in their moulting pockets or move on

the skin to make new moulting pockets. The moulting into adults requires an additional three to four days (Arlian & Vyszenski-Moher, 1988).

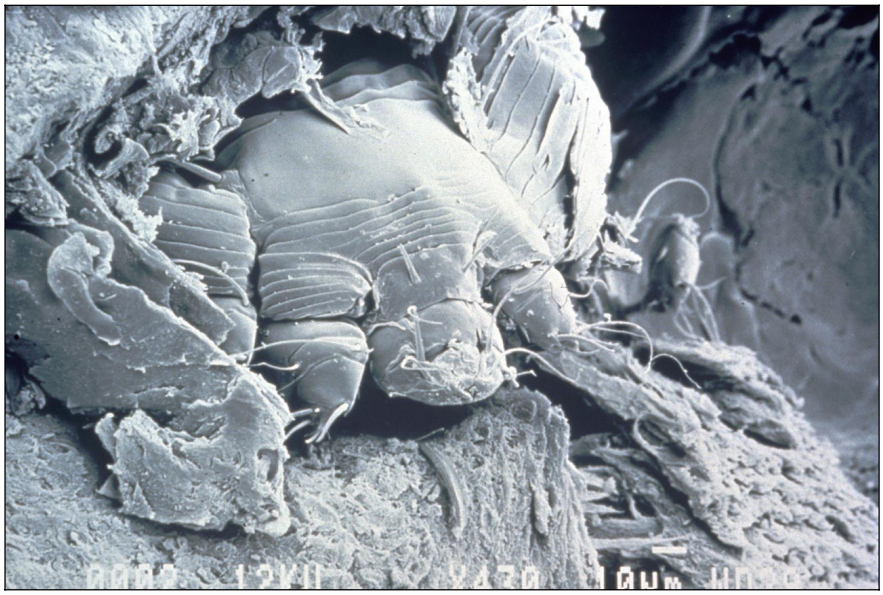

Fig. 4. S. scabiei in a burrow. Photo: Tapio Nikkilä

Infectivity, survival and transmission

Survival and infectivity for all life stages of *S. scabiei*, in an environment off the host, is dependent on the relative humidity (RH) and temperature. Sarcoptic mites from both humans and canines have been shown to survive for 24-36 hours in room conditions (21°C and 40-80% RH) without loosing any performance in infectivity (Arlian *et al.*, 1984a). The longest recorded survival time has been observed in females and nymphs isolated from dogs, at 10-15°C and a RH of 97% these mites survived between 1 to 3 weeks (Arlian *et al.*, 1989). Interestingly, mite activity is minimal at temperatures below 20°C whereas the mobility peaks at 35°C. Survival of females and nymphs is generally longer than for males and larvae. A high RH combined with a low temperature favours survival of mites isolated from dogs; consequently, higher temperature and lower RH shorten the lifespan. It was also noted that the time required for complete penetration of the epidermis increased as a function of the time off the host (Arlian *et al.*, 1984a). Additionally, there are indications from a series of host-seeking experiments that canine-derived mites dislodged from their host respond to thermal stimuli and the host odour by selectively seeking the source (Arlian *et al.*, 1984c). This host-seeking behaviour, coupled with long survival, suggests that environmental contamination is a potential source for contracting an *S. scabiei* infection. However, the actual risk of contracting an infection from the environment appeared to be low. In studies by Mellanby (1944) only four volunteers out of 272 contracted an infection after using warm beds just vacated by heavily infected patients. Several authors have stated that the main route of transmission of mites is through physical contact (Heilesen, 1946; Mellanby, 1972).

Host specificity

The majority of scabieologists agree with Fain (Fain, 1968; Fain, 1978) that *S. scabiei* is a single species, but that it is variable and adaptable. This conclusion follows from the observation that isolates from different hosts display few or no morphological differences. In contrast, there are acarolologists that have maintained that there are distinct species of sarcoptic mites that infect different hosts (Kutzer & Grunberg, 1969). This is supported by a cross-infection study, which described unsuccessful experimental attempts to transfer scabies mites from dogs to mice, guinea pigs, pigs, cattle, cats, goats and sheep (Arlian *et al.*, 1984b), which indicates that mites do not easily pass from one host species to another. Only New Zealand white rabbits were susceptible to the dog-derived mites and a long-term infestation was established. This model is the only current *in vivo* culture system for *S. scabiei* mites. These experiments imply that various host strains of *S. scabiei* are host specific by the demonstration of limited cross-infectivity between different host species. The underlying mechanisms for host specificity of different variants/populations of *S. scabiei* are not understood but are most likely to be explained by the interaction between both parasite and host factors (Arlian, 1989). Thus, it is common to specify from which host the mites have been isolated, this is done by adding the host name after the species name e.g. *S. scabiei* var. *vulpes* (fox), *S. scabiei* var. *hominis* (human) *S. scabiei* var. *canis* (dog) etc.

If more recent data from population genetics studies of host specificity are taken into account, the result is still ambiguous. An analysis of a 450 bp nuclear ribosomal marker, the internal transcribed spacer 2 (ITS-2) in 21 mites derived from mite populations from dog, cattle, fox, lynx, wombat, chamois and dromedary detected no differences in the ITS-2 region of the mites between the different host species (Zahler *et al.*, 1999). In another study of genetic variation in the ITS-2 marker and a part of the mitochondrial 16S rRNA gene in 28 mites isolated from chamois and fox populations, no specific host association of the mites could be detected (Berrilli *et al.*, 2002). Also, no variation was detected by sequencing mitochondrial 12S rRNA from mites isolated from wombats (Skerratt *et al.*, 2002). In contrast, a structural division of the mite populations was detected which was attributed to geographic isolation. Both of these studies suggest that *S. scabiei* is a single spieces.

However, Walton and colleagues analysed population diversity in more than 700 mites taken from humans and dogs in Australia and the Americas using a genotyping system based on three hyper-variable microsatellite loci (Walton *et al.*, 1999; Walton *et al.*, 1997). The subsequent multi-locus analysis revealed that mites were segregating into one dog-associated population and one human-associated population. Overall these studies suggest that mites isolated from humans and dogs are genetically distinct although the species from both hosts were isolated from the same geographical locations. Walton's results imply that the species constituted of several different distinct genotypes. This was somewhat contradicted in an extended genotype analysis (see section Zoonotic aspects), which demonstrated the presence of gene flow between populations of *S. scabiei* var. *hominis* and populations of *S. scabiei* var. *canis* (Walton *et al.*, 2004a). In conclusion, although

S. scabiei appear to be a single species, there is a remaining uncertainty concerning host specificity and classification.

Epidemiology

Scabies

Prior to the development and introduction of effective treatments scabies was a disease that was highly prevalent in most populations (Arlian, 1989). Nowadays scabies is still found in almost every part of the world and, estimates indicate that worldwide up to 300 million people are affected by scabies at any given time (Taplin & Meinking, 1990).

There have been many studies on different aspects of the epidemiology of scabies, trying to identify some distinguishing characteristics of the disease. Scabies has been shown to be more common in very young children, which has been explained by an increased risk of exposure and the absence of immunity (Alexander, 1984). More authors have found the highest prevalence in children up to 2 years (Montgomery, 1985) and childen under the age of 6 years (Wakhlu *et al.*, 1988). Other groups with an increased risk of contracting the disease are the elderly in nursing homes (Parish *et al.*, 1991) and people that are immunodeficent, such as patients with HIV (Meinking *et al.*, 1995; Taplin & Meinking, 1997; Thappa & Karthikeyan, 2002).

As scabies is a sexually transmitted infection (STI), just being sexually active is another factor that elevates the risk of contracting scabies. Sexual contact among adults is probably the most important route of transmission. In a recent study, covering the years 1988-2002, from a sexually transmitted infections unit in Spain, the prevalence of scabies among the 9751 attendees was 1.5% (Otero *et al.*, 2004). Scabies affects men and women equally, but there are observations of some variability between sexes (Otero *et al.*, 2004; Taplin & Meinking, 1990).

Ethnicity is another proposed factor that could increase susceptibility to scabies infection (Alexander, 1978; Funaki & Elpern, 1987), but no significant evidence for this exists. Observed differences in prevalence between people of different ethnic backgrounds have been suggested to be the result of different social customs and lifestyles (Walton *et al.*, 2004b). Contrary to common knowledge, good hygiene practices and access to water do not appear to decrease or eliminate scabies (Burgess, 1994). Evidence for this is found in reports about outbreaks in institutions, where washing facilities are common and the hygienic standards are high (Holness *et al.*, 1992). However, high incidence of scabies is associated with poverty and overcrowding (Currie & Carapetis, 2000; Taplin *et al.*, 1983). Studies also show that there is an increased incidence of scabies during the winter months (Kimchi *et al.*, 1989; Mimouni *et al.*, 2003).

Epidemiological studies have also suggested a cyclic nature in the occurrence of scabies outbreaks. The estimated length of these periods varies between 10 to 30 years (Christophersen, 1978; Shrank & Alexander, 1967). Perhaps the most important transmission events of *S. scabiei* occur within families. In molecular

studies of intra-family transmission of *S. scabiei* mites, it was confirmed that mites isolated from people in the same family displayed little deviation in genotypic homogeneity, whereas large differences could be observed between different families (Walton *et al.*, 1999).

Mange

An infection of *S. scabies* in animals is known as mange or sarcoptic mange. Globally, the mite is of significant veterinary importance as it causes disease in companion animals, commercially important animals and epizootic disease in wild animal populations (Pence & Ueckermann, 2002).

S. scabiei infections are recurrent in commercially important animals. As an example, in pig herds in Europe and North America are reported to have prevalences range from 20% to 86%. In Europe, the estimated loss due to mange is 7 euro per finishing pig (Lowenstein *et al.*, 2004). Many countries have documented the occurrence of mange in pig herds, including Belgium (Smets *et al.*, 1999), Denmark (Jensen *et al.*, 2002), Finland (Heinonen *et al.*, 2000), Germany (Damriyasa *et al.*, 2004), the Netherlands (Hollanders *et al.*, 1995), Australia (Davies *et al.*, 1991a; Davies *et al.*, 1991b), the United States of America (Davies *et al.*, 1996) and Sweden (Jacobson *et al.*, 1999; Wallgren & Bornstein, 1997). Globally, sarcoptic mange is the most important ectoparasitic disease among pigs. Animals with chronic skin lesions, which usually have a heavy burden of parasites, constitute the main route of transmission within pig herds (Davis & Moon, 1990). There are several veterinary concerns regarding sarcoptic mange in pigs, including problems with diagnosis, effective control of the parasite and the negative economic effect of mange due to disturbances in weight gain.

In wildlife, sarcoptic mange has been reported from numerous different animals, in recent years, including reports of mange in populations of red foxes (*V. vulpes*) in Europe (Bates, 2003; Sreter *et al.*, 2003), raccoon dogs (*Procyon lotor*) in North America (Fitzgerald *et al.*, 2004), lynxes (*Lynx lynx*) in Europe (Ryser-Degiorgis *et al.*, 2002), ibexes (*Capra ibex*) in Europe (Leon-Vizcaino *et al.*, 1999), camels (*Camelus dromedaries*) in Africa (Bornstein *et al.*, 1997), and wombats in Australia (*Vombatus ursinus*)(Martin *et al.*, 1998).

Sweden was for a long time spared from sarcoptic mange in wildlife, however that changed with the first documented case in 1972 in wild red fox followed by a second case in 1975. The result of the epizootic spread was devastating, and within 8 years sarcoptic mange could be found all over mainland Sweden, and 50% of the red fox population was lost (Bornstein, 1995). Although red foxes were most heavily affected by the epizootic outbreak, other wildlife species like lynx and martens (*Martes martes*) also died from the infection (Morner, 1992). The primary routes of transmission of sarcoptic mange in red foxes are by direct contact between animals or by environmental contamination in the bedding of dens.

Furthermore, recently there have been reports of cases of scabies and sarcoptic mange on, the previously *S. scabiei* free island, Gotland. This means that *S. scabiei*

is now prevalent throughout mainland Sweden and on the two largest islands, Öland and Gotland.

Dogs
Sarcoptic mange in a group of dogs is considered to be highly contagious; more than 50% of exposed dogs will eventually develop signs of the disease. There are claims that no sex, age or breed predisposition occurs in dogs (Bornstein, 1995). This is in contrast to other observations that mange is more common in puppies/young and old or debilitated dogs (Walton *et al.*, 2004b). Mite transmission in dogs is reported to be through direct contact or via contaminated bedding (Walton *et al.*, 2004b). The prevalence of mange in domestic animals in some industrialised countries has declined in recent years, probably as a result of routine application of insecticides for tick and flea control (Walton *et al.*, 2004b).

Zoonotic aspects

There are several reports of humans contracting *S. scabiei* from animal sources (Chakrabarti, 1990; Chakrabarti *et al.*, 1981a; Chakrabarti *et al.*, 1981b; Estes *et al.*, 1983; Menzano *et al.*, 2004). Two categories of people are more prone to contract zoonotic scabies: those who work and handle domestic animals and dog-owners. Transmission of animal scabies is observed in e.g. pig farmers and slaughterers (Burgess, 1994; Chakrabarti, 1990). The most frequently observed form of animal transmitted scabies in humans is from infected dogs (Moriello, 2003). Commonly, canine scabies is introduced to a family when it obtains a new dog (Meijer & van Voorst Vader, 1990; Orkin & Maibach, 1985). Untreated dogs are prone to develop a crusted form of the disease, which results in the shedding of mite-containing crusts and scales; these may serve as a potential source of infection of the family. An experimental transfer of mites isolated from dogs to humans resulted in a severe itch within 24 hours (Estes *et al.*, 1983). In all described cases of scabies contracted from animals is that the disease is self-limiting (Burgess, 1994; Chakrabarti, 1990). In humans this form of scabies is a transient disease, meaning that the *S. scabiei* mites from animals will not establish a long lasting infection. Due to the self-limiting character of the disease, treatment of canine scabies is usually not required, since affected people are free of symptoms within days. However, atypical cases have been observed here lesions continued to appear from 5 weeks (Smith & Claypoole, 1967) up to 13 weeks (Arlian *et al.*, 1984b), in such cases specific therapy would be beneficial.

A recent study of sequences from the mitochondrial (mt) genes coding for 16sRNA and cytochrome C oxidase subunit I (COI) in northern Australia (Walton *et al.*, 2004a), has shown the prescence of relationships between *S. scabiei* mtDNA haplotypes, host species and geographical location. Their study demonstrated, by utilising 15 microsatellite markers for a multi-locus genotyping analysis, that the gene flow between *S. scabiei* populations in humans and mite populations in dogs is extremely rare, but that these crosses do occur. This genetic division of dog and human derived scabies mites also imply that control programs for human scabies in areas where populations of human mites and populations of dog mites coexists

should primarily focus on the human-to-human transmission (Walton *et al.*, 2004a).

Clinical manifestation

The major problem with diagnosis of dermal diseases is that the clinical manifestations, whether in humans or animals, often resemble each other. Scabies or mange is no exception, and it has been refereed to as the "great imitator" regarding clinical signs associated with dermal disease (Burgess, 1994). The clinical signs of scabies vary depending on patient age and what form of the disease was contracted. The scenario is similar regarding mange, but the clinical signs vary depending on which species is affected (Burgess, 1994). Regardless of species, the most pronounced clinical presentation of scabies or mange is generalised pruritus.

Human scabies

Typically, classical scabies starts with an intense generalised pruritus that may affect most parts of the body, and which is frequently more intense at night. Two forms of skin eruption occur concurrently: (1) those associated with the burrows and their vicinity, and which consist of papular and vesicular lesions some which become postular or bullous; and (2) a more generalised itchy papular eruption, not associated with burrows or mite activity. These papular eruptions are most commonly observed around the axillae, thighs, buttocks, lower legs including ankles, and the wrists extending along the forearms. Sometimes nodular reactions occur which can persist for months after treatment of the disease. These nodules are usually dark pink to brown, firm and pruritic. The classical localisations of mites in humans are in the webs of fingers, the volar aspects of the wrists and arms, and the extensor aspects of the elbow, buttocks, ankles, and genital areas (Arlian, 1989; Burgess, 1994; Walton *et al.*, 2004b).

In rare cases, classical scabies may progress into an extreme form of scabies known as crusted scabies or Norwegian scabies. This condition is characterised by development of hyperkeratotic skin crusts, which may be loose, scaly and flaky or thick and adherent. The condition may be accompanied by an irregular and diffuse erythroderma. This severe form of scabies is associated with predisposing conditions in the affected person. Scabies patients co-infected with the human immunodefieciency virus (HIV) and immunosuppression have been linked with severe crusted scabies (Brites *et al.*, 2002; Orkin, 1993; Valks *et al.*, 1996). Crusted scabies is also more contagious than classical, scabies due to the high number of mites in the infected host; there are reports of a mite burden of 4,700 mites/g human skin (Currie *et al.*, 1995).

Sarcoptic mange in dogs

The first clinical signs of an *S. scabiei* infection in dogs are lesions in the form of red papules visible on sparsely haired regions of the body. The most prominent symptom is an intense pruritus, accompanied by scratching and inflammation of the skin. The pruritus has been observed to be worse at night, which is also observed in human cases. The incubation period has been observed to vary between three to

four weeks or four to eight weeks. Untreated canine scabies may lead to loss of hair, crusting and scaling of the skin, and hyperkeratosis (Bornstein, 1995). Distributions of lesions vary, but common sites are muzzle, ear, face, legs and tail (Walton *et al.*, 2004b). Anorexia, depression, weight loss, and secondary bacterial pyoderma are observed in chronically infected dogs (Scott & Horn, 1987).

Immunology

In order to understand the pathogenicity of *S. scabiei* it is necessary to have an understanding of the mechanisms involved in the immune response during primary (naïve host) and secondary (sensitised host) infections with the mite. Protective immunity to these parasites has been described in several hosts, including humans, dogs and rabbits (Arlian *et al.*, 1996b; Arlian *et al.*, 1994a; Arlian *et al.*, 1994b; Mellanby, 1972). The development of immunity is triggered by the host immune response in the presence of *S. scabiei* antigens, and is mediated by both humoral (Th2-response) and cellular mechanisms (Th1-response).

Humans

In human scabies the clinical symptoms of a primary infection becomes apparent after about 4-6 weeks (Mellanby, 1944), and it has been suggested that the asymptomatic latency period may reflect the amount of antigenic stimuli (Estes & Estes, 1993). In naïve hosts this is accompanied by cellular infiltrates in the epidermis, containing mast cells lymphocytes, eosinophils and other inflammatory cells (Falk & Eide, 1981; Reunala *et al.*, 1984; Van Neste, 1981; Van Neste & Staquet, 1986). There is also an increase of circulating IgE antibody levels (Dahl *et al.*, 1985; Falk, 1980; Morsy *et al.*, 1993), especially in patients with crusted scabies (Arlian *et al.*, 2004). In a secondary infection the onset of the host immune response is much more rapid and clinical symptoms are evident within 24 hours (Mellanby, 1944) The observed elevated IgE levels in scabies patients and the rapid response to a secondary infection is evidence of a type I immediate hypersensitivity reaction (Dahl, 1983; Falk, 1980). The antibodies IgG and IgM are also elevated in serum from scabies patients (Morsy *et al.*, 1993). Moreover, in sensitised hosts the number of parasites were significantly reduced, and 60% of the cases were protected from a secondary infection.

Dogs

In dogs with a primary infection of sarcoptic mange the cellular infiltrates in scabetic lesions show high levels of mast cells among other mononuclear cells, which indicates a Th2 response in naïve animals (Arlian *et al.*, 1996b; Arlian *et al.*, 1997; Stemmer *et al.*, 1996). Symptoms of a primary infection develop within 2-5 weeks. In secondary infections there is a marked increase of cellular infiltrates containing mainly lymphocytes monocytes and neutrophils, most likely induced by Th1 cytokine signalling. Histological analyses of scabietic lesions also indicate infiltration of T cells, as well as both CD1+ Langerhans cells and other major histocompatibility complex (MHC) class II-positive cells within 12-48 hours (Lalli *et al.*, 2004). Symptoms of a secondary infection appear within 1-2 weeks (Arlian *et al.*, 1996b). Additionally, resistant dogs exhibited a stronger cell-mediated and weaker circulating antibody response than non-resistant dogs (Arlian *et al.*, 1994a).

Both in primary and secondary infections in dogs a humoral immune response develops. In serum from naïve and sensitised dogs the overall strongest antibody responses are from IgE and IgG (Arlian & Morgan, 2000; Bornstein & Zakrisson, 1994).

Diagnosis and treatment

Primary diagnosis of scabies poses a real challenge for physicians and veterinarians primarily because the clinical signs and symptoms of scabies and mange infections are hard to distinguish from other dermatological conditions. In humans, the infection resembles other diseases that are caused by insects such as lice, fleas, bedbugs and a wide range of other skin diseases like eczema, impetigo, dermatitis herpetiformis, allergic reactions and syphilis (Alexander, 1984). Hitherto, there is only one definitive diagnosis of scabies that is commonly practised: a microscopic examination of skin scrapings to physically verify the presence of *S. scabiei* mites.

Non-serological diagnosis

Several techniques have been developed in order to assist veterinarians and physicians in the diagnosis of scabies. The unifying factor of these techniques is to identify *S. scabiei* mites, their eggs or faecal pellets. Furthermore, all of them have a low sensitivity. Here follows a brief outline of some of these methods. (1) A traditional way of scabies diagnosis is to examine a possible burrow and with the point of a needle try to extract and identify individual mites. (2) The most commonly used technique to diagnose scabies is microscopic examination of skin scrapings taken from lesions using a scalpel blade dipped in oil to collect the skin particles. In normal scabies the number of parasites is usually low (11-13), which hampers detection. It has been reported that >50% of scrapes can be negative in dogs with sarcoptic mange (Morris & Dunstan, 1996). (3) Dermoscopy, which is direct microscopic examination of the skin to identify structures made by mite activity or live mites (Prins *et al.*, 2004). (4) Skin biopsy, a technique that rarely identifies mites or traces of them like eggs and faeces. In a study of 19 dogs, not a single histomorphological feature was identified that could serve as reliable marker of infection in the absence of a sectioned mite (Morris & Dunstan, 1996). Hence, histological examination of skin biopsies is frequently inconclusive. (5) Burrow ink test (BIT) (Woodley & Saurat, 1981), in which ink is rubbed unto the examination area and then removed with alcohol. If burrows are present then these will appear as dark irregular tracks. BIT has a high incidence of false negatives and is obviously not used on animals with fur. (6) PCR has been tested to amplify *S. scabiei* DNA from cutaneous scales from patients with clinically atypical eczema (Bezold *et al.*, 2001). (7) In dogs, the potential of the pinnal-pedal scratch reflex has been assessed as an aid in diagnosis of sarcoptic mange (Mueller *et al.*, 2001). (8) Treatment with an acaracide is sometimes used when trial therapy is necessary to differentially diagnose the disease (Folz *et al.*, 1984). (9) In pigs, the scratching behaviour has been studied and the use of a scratch index implemented. The use of a scratch index is similar to the pinnal-pedal scratch reflex in dogs, it is not a diagnostic method but may function as an aid in diagnosis and monitoring of the disease (Hollanders *et al.*, 1995).

Serological diagnosis

The fact that clinical symptoms of scabies resemble those of other skin diseases, and problems in demonstrating mites on infected hosts have led to the development of serological tests. This is possible because the *S. scabiei* mites has been shown to induce a humoral antibody response in affected hosts (Arlian & Morgan, 2000; Arlian *et al.*, 1996a; Arlian *et al.*, 2004; Arlian *et al.*, 1996b; Arlian *et al.*, 1994a; Bornstein & Zakrisson, 1994; Falk, 1980). So far, enzyme linked immunosorbent assays (ELISA) for the demonstration of antibodies to *S. scabiei* have been developed for pigs and dogs (Bornstein *et al.*, 1996; Jacobson *et al.*, 1999; Lower *et al.*, 2001). The canine ELISA (Bornstein *et al.*, 1996) has been extensively tested and is routinely used for the diagnosis of sarcoptic mange (Curtis, 2001; Lower *et al.*, 2001). The basis for these ELISAs are dependent on a steady supply of antigen preparations; which are made from mites isolated from infected hosts. Due to a documented cross reactivity between isolates of *S. scabiei* from different hosts (Arlian *et al.*, 1996b), the serological diagnosis of sarcoptic mange in dogs is possible to make with mites isolated from the red fox.

Although efforts are being made, no commercial ELISA has been developed for diagnosis of human scabies. The main problem with a human ELISA is to obtain human-derived mites. However, recently the cross reactivity between IgG antibodies in human scabies cases and red fox mite whole antigen extracts was investigated in an ELISA. It was found that IgG antibodies in human scabies patients cross react with *S. scabiei* isolated from foxes, but the sensitivity was found to be too low (48%) for the establishment of an ELISA system (Haas *et al.*, 2005).

Cross reactivity between *S. scabiei* and house-dust mite antigens

The favourable cross reactivity observed in antigen extracts made from one strain of *S. scabiei* mites can be used to detect serum antibodies elicited by another strain. As mentioned above, fox *S. scabiei* antigen extracts are used in a serological test to detect serum antibodies in pigs infected with a host-specific strain (Hollanders *et al.*, 1997; Wallgren & Bornstein, 1997).

Studies show that there also is cross reactivity between antigens of *S. scabiei* and of the house-dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Arlian *et al.*, 1988; Falk & Bolle, 1980). These house-dust mites and *S. scabiei* are closely related arthropods and it is therefore not surprising that the different species share common antigens. As a consequence, exposure to house-dust mite allergens may induce immunity in *S. scabiei* infected hosts. This has been shown in an experimental trial, where 71 % of the rabbits immunized with extracts of *D. farinae* and *D. pteronyssinus* showed resistance to a subsequent infection of *S. scabiei* var. *canis* mites. The marked reduction in parasite loads was interpreted as resulting from development of cross-protective immunity (Arlian *et al.*, 1995). It is well known that house-dust mites give raise to atopy in humans and dogs, and that there is a correlation between scabies and atopic disease. As a consequence, most studies regarding cross reactivity between *S. scabiei* and various species of house-dust mite allergens are focused on the IgE response.

Treatment and acaricide resistance

There are a number of active compounds for the effective treatment of scabies and sarcoptic mange, used either as topical solution or taken orally. In short, some of the used compounds are: (1) Ivermectin is widely used, and is the most important drug in veterinary medicine for treatment of sarcoptic mange; in some countries it is also approved for therapeutic use in humans. (2) Benzyl benzoate 25%. (3) Crotamiton 10%. (4) Gammabenzene hexachloride 1%. (5) Sulphur compounds. (6) Permethrin, which has low toxicity and is therefore considered the treatment of choice in many parts of the world (Walton *et al.*, 2004b). Control programs exist to manage porcine mange, which also include treatment with macrocyclic lactones.

Although, there still are effective scabicidal treatments, there are reports of increased resistance towards the most commonly used compounds. Possible emergence of *S. scabiei* permethrin resistance has been reported from northern Australia (Walton *et al.*, 2000). Clinical and *in vitro* ivermectin resistance has been reported in *S. scabiei* mites isolated from two patients with crusted scabies in northern Australia (Currie *et al.*, 2004).

Molecular approaches in *S. scabiei* research

Despite the fact that the cause of scabies has been known for a long time, and the importance of *S. scabiei* as a pathogen in humans and animals, there are very few molecular studies focusing on different aspects of the mite's biology (Kemp *et al.*, 2002). It was not until the late 90's that the first articles employing molecular techniques like PCR in scabies research were published (Walton *et al.*, 1997; Zahler *et al.*, 1999). The primary reason for this was that molecular work on *S. scabiei* has been hampered by the problems associated with obtaining sufficient quantities of mites to perform such studies. This was in part solved by the *in vivo* cultivating system on laboratory rabbits (Arlian *et al.*, 1984b); however, the ethics of using such a system may be debated.

Currently, there is a shift in the research of *S. scabiei*, with the introduction of recombinant DNA technology applications. These applications have rendered previously impossible molecular experiments possible to perform. The main contribution of the recombinant DNA technology is that researchers are not dependent on large numbers of mites for molecular studies on *S. scabiei*. The use of PCR together with modern extraction protocols allows amplification of DNA from individual mites. Furthermore, advances in studies of biological processes of *S. scabiei* have been made with the construction of cloned complementary DNA (cDNA) expression libraries. These have in turn made generation of expressed sequence tags (EST) possible as well as immunoscreening experiments.

DNA libraries and immunoscreening

A cDNA library represents the information encoded in the messenger RNA (mRNA) of a specific tissue or organism. These mRNA molecules represent expressed genes for the given tissue or organism. Since RNA molecules in their

natural form are exceptionally unstable and difficult to amplify, the information encoded by the RNA is converted into stable double-stranded DNA (cDNA) that is inserted into a self-replicating lambda vector. The cloned cDNAs in the cDNA library are available for expression in bacterial and eukaryotic cells. This feature has made access to cDNA libraries invaluable, as tools for novel gene discovery, for immunology studies and a range of other applications.

In 1997 Walton and colleagues described the construction of a genomic library based on DNA from *S. scabiei* mites obtained from 23 patients diagnosed with crusted scabies. Briefly, the *S. scabiei* genomic DNA was digested to completion with *Eco* RI and ligated into an λgtl0 vector. This library was then used for the identification of multiple hypervariable microsatellies in the *S. scabiei* genome (Walton *et al.*, 1997). We described the first successful construction of an *S. scabiei* cDNA library (Paper I), which was based mRNA from mites isolated from red foxes (*V. vulpes*). In 2003, several *S. scabiei* cDNA libraries constructed from mites isolated from infected human patients were reported (Fischer *et al.*, 2003a; Fischer *et al.*, 2003b; Harumal *et al.*, 2003).

Immunoscreening is a technique where antibodies are used to screen a cDNA library to identify polypeptides with antigenic properties. The method is useful for identification of molecules involved in the host-parasite relationship, and may identify molecules corresponding to candidate antigens, useful as vaccine components and/or as molecules in serological assays. The combination of a parasite cDNA library, which is screened with an appropriate serum, has been extensively used to identify and characterize antigenic proteins from a variety of parasites (Hirata *et al.*, 2005; Lizotte-Waniewski *et al.*, 2000).

Recently in *S. scabiei* research, immunoscreening identified two immunodominant clones from the randomly primed pGEX4T-2 library with sera from rabbits infected with *S. scabiei* mites (Harumal *et al.*, 2003). The two clones were designated *Sarcoptes scabiei* antigen 1 (Ssag1) and *Sarcoptes scabiei* antigen 2 (Ssag2). Ssag1 was found to be the *S. scabiei* homologue of the major house-dust mite allergen M-177 originally sequenced from *Euroglyphus maynei*. Ssag2 was almost identical to one EST clone generated in our EST-project. The clones were expressed to obtain the polypeptide products; these were thereafter used in a vaccination trial with rabbits. Even though the challenge trials with Ssag1 and Ssag2 in rabbits failed to produce protective immunity, the work showed that challenge trials with cloned *S. scabiei* antigens are possible (Harumal *et al.*, 2003).

S. scabiei *expressed sequence tags*

Generation of ESTs was first reported in 1991 as an alternative to the whole-genome sequencing of human DNA (Adams *et al.*, 1991). ESTs are short (300-500 bp) single-pass sequence reads from randomly selected cDNAs. ESTs are usually generated in large batches; a recent estimate is that a single scientist can generate approximately 2000 ESTs in a month (Whitton *et al.*, 2004). As ESTs are generated from a cDNA library they represent a snapshot of all the expressed genes for a given organism (or developmental stage or cell-type etc.), and thus ESTs are

used to define and survey expressed genes. The generation of ESTs has been described as a cost-effective approach compared to e.g. whole-genome sequencing for the discovery of new genes (Adams *et al.*, 1991). The EST approach for the identification of potential targets for e.g. the control of a parasite is a much more "crude" method than immunoscreening, but it will generate much more spin-off data about the genetic background of an organism. Usually, EST data are submitted to dbEST, a public database for EST information (Boguski *et al.*, 1993). dbEST is a divison of GenBank© (Benson *et al.*, 2004), a comprehensive database containing public available DNA sequences from more than 140000 organisms; the sequence information in the database is also continuously updated.

Since 1991 several EST analyses have been performed on cDNA libraries from parasites, including worms and protozoans like *Schistosoma mansoni* (Franco *et al.*, 1995; Santos *et al.*, 1999), *S. japonicum* (Fung *et al.*, 2002)*, Toxoplasama gondii* (Ajioka *et al.*, 1998; Li *et al.*, 2003; Li *et al.*, 2004), *Eimeria tenella* (Wan *et al.*, 1999), *Brugia malayi* (Blaxter *et al.*, 2002), and *Neospora caninum* (Li *et al.*, 2003).

In an EST analysis of a more closely related parasite to *S. scabiei*, the sheep scab mite *P. ovis* 507 cDNA clones have been sequenced (Kenyon *et al.*, 2003). The sequence analysis revealed 49 clusters and 231 singletons, with 109 of the singletons sharing similarity with submitted sequences in GenBank. Interestingly, 9% of the ESTs showed apparent homology to known house-dust mite allergens, including cysteine protease, glutathione S-transferase (GST), tropomyosin, paramyosin and fatty acid binding protein. The most abundant transcripts were identified house dust mite allergens and had similarity to GSTs. Other interesting genes from *P. ovis* were free radical scavenging enzymes, a heat shock protein and more cysteine proteases cathepsin L and cathepsin B. A total of 47.5% of the ESTs did not share any similarity with known sequences in GenBank and were considered novel. However six clusters were formed, with at least eight sequences for each cluster indicating that some of these are highly expressed genes (Kenyon *et al.*, 2003).

To date, a total of 1039 (25 March 2005) ESTs generated from *S. scabiei* cDNA libraries have been submitted to dbEST. The majority of those (904) are from our EST-analysis of *S. scabiei* from the red fox (Paper II). Besides this EST analysis, a pilot-scale study, which generated 145 ESTs from a λZAP library made from mites isolated from humans, has been reported (Fischer *et al.*, 2003b). Of these 145 sequences, 39 lacked apparent homologues in GenBank, but 103 showed significant matches to sequences in GenBank. Additionally, three sequences were established to be human cDNA contamination and hence were excluded from the data set. Particularly interesting sequences were *S. scabiei* homologues to: (1) the *D. pteronyssinus* allergen, GST, a potential candidate for a scabies allergen; (2) Paramyosin; (3) three clones corresponding a segment of Ssag1; (4) the cysteine protease cathepsin-L from *Boophilus microplus*; and (5) COI and COII (Fischer *et al.*, 2003b). Furthermore, this small-scale EST analysis is said to being followed by a large EST project, where 50000 cDNA clones are being sequenced (Fischer *et al.*, 2003b). No EST data from this project have been submitted to dbEST, but an

analysis of sequences from 19488 clones, from the large scale EST project, revealed abundant transcripts corresponding to allergens found in house-dust mites. The subsequent analysis led to the proposal of multigene families of inactivated serine and cysteine proteases in *S. scabiei* that may be involved in an immune evasion strategy (Holt *et al.*, 2003; Holt *et al.*, 2004).

Outline and aims of the study

S. scabiei is the causative agent of sarcoptic mange and scabies, which world-wide are recognised as important diseases in humans and animals alike. Although the negative effects of sarcoptic mange and scabies are well documented, there are few molecular studies that aim to elucidate the interactions between parasite and host. A better understanding of this molecular interplay would be beneficial for the understanding of the pathogenesis observed in *S. scabiei* infections. Furthermore, identification of key molecules in the infection process may ultimately provide tools for the control of the mite, such as improved diagnostic systems and vaccines. The main reason for the scarcity of molecular studies of *S. scabiei* is a lack of parasite-derived material. So far, the rather limited amount of mites that can be obtained must be isolated from an infected host, as there is no *in vitro* cultivating system available for *S. scabiei*. However, recent advances in the field of molecular biology have made it possible to perform such studies on a limited number of mites.

Some of these advances include recombinant DNA technology, which enables the possibility of transfering genomic material from one organism to another. This can be done in order to give the receiving organism new genetic traits or abilities. The receiving organism can be used as tool to produce a protein that it normally lacks the ability to produce. In many cases the bacterium *Escherichia coli* is used for the production of proteins that are rare or in some cases nearly impossible to obtain from the original source. Relatively large amounts of bacteria can easily be grown at low cost and can thus be used for the efficient and manageable production of the desired proteins. For a detailed description of recombinant gene technologies, see for instance (Brown, 2001).

My over all objective was to apply some of these new technologies to the age-old problem of infection by *Sarcoptes* mites, particular with reference to the problems of veterinary parasitology. The aims of this study were to:

- overcome the problems associated with a lack of parasite material;
- extend the knowledge of the molecular repertoire in *S. scabiei*;
- identify and characterise *S. scabiei* proteins involved in parasite-host interactions.

To achieve these objectives four different studies were performed. The establishment of an *S. scabiei* cDNA library was the basis for overcoming the scarcity of parasite-derived material (I-IV). In order to accelerate gene discovery and to expand the molecular knowledge of *S. scabiei*, an EST-analysis was performed (II). Based on the EST data, one particular cDNA and its protein were studied in detail (III). Two studies utilised immunoscreening techniques to identify *S. scabiei* proteins involved in parasite-host interactions (I, IV). Recombinant proteins were produced and characterised in three of the studies (I, III, IV).

Comments on material and methods

Detailed descriptions of the materials and methods used are given separately in each paper (I-IV).

S. scabiei antigen preparations

S. scabiei antigen preparations have been used in this study as antigens in Western blots (I, III, IV) and for the generation of rabbit hyperimmune sera (I, III, IV). For the isolation of mites and preparation of antigens, the methods originally described by Bornstein and Zakrisson were used (Bornstein & Zakrisson, 1994). Pieces of skin from wild red foxes with naturally contracted sarcoptic mange were put in Petri dishes and left at room temperature under an electric light source. Living mites of both sexes and of different developmental stages migrated to the inside of the lids of the Petri dishes. After 2-4 hours the lids were collected and the mites killed by freezing (-20 ˚C) and new lids were put in place to continue the harvest. Frozen mites were thawed and homogenized in a glass grinder together with phosphate buffered saline pH 7.4 at 4 ˚C; the mite slurry was subsequently ultrasonicated. Following a centrifugation at 3800 x *g* for 30 minutes, the supernatant was collected and filtered through a 0.2 µm filter. The protein concentration of the preparation was determined by the Bradford method and the preparation was stored at -20 ˚C.

The *S. scabiei* cDNA library and host contamination

The *S. scabiei* cDNA library described in study I, was generated from mRNA from mites that were isolated from wild red foxes as described above. However, all mites were killed and stored at -70°C prior to RNA preparation. Preferably, these mites should have been from an *in vitro* cultivating system without any previous contact with any source of biological contamination, e.g. a host. In the absence of such a cultivating system, mites must be isolated from a parasitised host. The parasitic nature of *S. scabiei,* its feeding behaviour and the fact that the organism spends its life in the upper skin layer of a host, make the parasite predisposed to carry biological material from its host.

A way to minimize host contamination has been tested with the closely related sheep scab mite *P. ovis* (Evans *et al.*, 2003). Prior to the construction of a DNA library, intended for the identification of microsatellites, sheep scab mites were starved for 48-72 hours. This step would allow time for most of the ingested host tissue to clear the mites' guts. Furthermore, the mites were washed and micro-dissected into three parts and these were washed again to minimize host contamination. The outcome was that out of the 26 microsatellite primer sets tested nine could be confirmed to be of mite origin and 17 were of host origin (Evans *et al.*, 2003). Although, the *P. ovis* study described the isolation of genomic DNA and not mRNA, it illustrates the difficulties in obtaining mites free of host contamination, despite extensive precautions. A similar study has so far not been

performed with *S. scabiei* mites but it is likely that the outcome would be similar. Thus, so far there is no way to guarantee mites to be free of host contamination.

However, in study I where the whole parasite was used, biological material located on the parasite may have been reduced by adding washing steps prior to the mRNA purification protocol. As expected, this step would not remove whatever the parasite had consumed from its host, and this biological material thus constituted a source of contamination. In the EST analysis, 1,020 clones were sequenced and five of these could clearly be shown to be of host origin (II). This means that host mRNA contamination was present during the construction of the cDNA library. Furthermore, the presence of contaminating host mRNA has also been observed in the recent EST-analysis performed with a *S. scabiei* var. *hominis* cDNA library, where 3 transcripts out of 145 could be verified to be of host origin (Fischer *et al.*, 2003b). The contamination appeared to be low, with less than <0.5% of the transcripts corresponding to foreign genes in the *S. scabiei* var. *vulpes* cDNA library and ~2% in the *S. scabiei* var. *hominis* cDNA library. Nevertheless, contaminations of host origin in *S. scabiei* cDNA libraries are a real problem that should be considered in order to avoid errors in any downstream application or analysis. Note: we have also demonstrated traces of contaminating host IgG in the *S. scabiei* antigen preparations.

Assigning a putative identity to an EST

As described in study II, EST transcripts equal to or longer than 100 bp were compared with public databases (GenBank non-redundant nucleotide and protein databases) using the BLAST family of algorithms with default parameters (BLOSUM62, gap existence and extension penalties 11 and 1, E= 10 and word size 3 without complexity filtering) (Altschul *et al.*, 1990; Gish & States, 1993). Where applicable, ESTs were then assigned a putative functionality. The threshold for significant/informative matches was set to a BLASTx similarity score value ≥50 and probability (P) $P \leq 10^{-4}$. Sequences without significant database matches were considered novel (Porcel *et al.*, 2000).

BLAST searches produce an E-value, which is the number of "false positives" expected to result from the database search. These can be easily converted to probabilities, and it is these probabilities that are used to define a "significant" match between an EST and a sequence in the database. However, the choice of the region defining significant matches is somewhat arbitrary, in the sense that published choices vary considerably, and there is no consensus for a suitable value. In an attempt to use an objective criterion, the main consideration is the number of hypotheses being tested (i.e. 1,020 in this case, one for each EST), which means that the conventional probability of p=0.05 for each test is unsuitable. The multiple hypothesis-testing problems is often dealt with using the Bonferroni correction, which simply divides the chosen probability by the total number of hypotheses being tested and uses this new probability for each test (Fig. 5, where all points below the line will be considered significant and all those above the line are non-significant). A more powerful alternative is the sequential Bonferroni correction, which adjusts the probability for each EST individually (Glantz & Reinhardt,

1996). For the ESTs here, this procedure indicates that P=0.0001 is a suitable cut-off (see graph); and this was therefore used to define ESTs with significant matches.

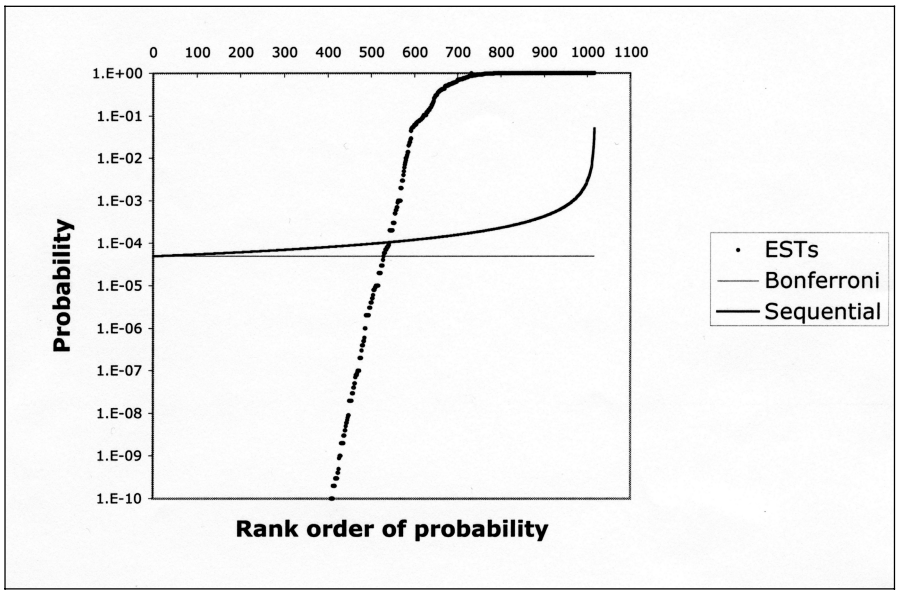

Fig. 5. Graph showing a comparison of the Bonferroni and sequential Bonferroni corrections used to define a "significant" match between an EST and a sequence in the database. Sequential Bonferroni adjusts the probability for each EST individually, and this procedure indicated that P=0.0001 is a suitable cut-off. This was therefore used to define ESTs with significant matches.

The highest numbers of matches for the EST dataset were with genes from the fruit fly *Drosophila melanogaster*. However, at that time the annotations of *D. melanogaster* entries in GenBank from the genome project were lagging behind. Many of the coding sequences identified in the *D. melanogaster* genome project had not been assigned any useful name or description of the protein encoded, even when the gene, the protein and its function has been fully described in previous work with *D. melanogaster*. For the majority of the top-scoring *D. melanogaster* matches, we used KEGG (Ogata *et al.*, 1999) in combination with FlyBase (http://flybase.bio.indiana.edu; 9-Apr-2005) and Gadfly (http://flybase.net/annot/; 9-Apr-2005) to be able to assign putative functions to our ESTs.

DNA polymerases used for the construction of expression plasmids

Throughout this study we have used two enzymes for the amplification of DNA by PCR: the *Pfu* DNA polymerase and Ampli*Taq* DNA polymerase. Ampli*Taq* DNA polymerase has mainly been used for verification purposes, for instance to verify positive clones after cloning experiments. Only one time has Ampli*Taq* DNA polymerase been used for subcloning purposes and that was for the construction of the expression plasmid pPU57 (I) that express miniaturised paramyosin. The

reason for this was that the *Pfu* DNA polymerase failed to amplify PCR fragments corresponding to the N-terminal and central region from the paramyosin template. *Pfu* DNA polymerase is otherwise the preferable choice for subcloning purposes, as it has been shown to exhibit superior thermostability and proofreading properties compared to other thermostable polymerases, including Ampli*Taq* DNA polymerase (Vieille & Zeikus, 2001). *Pfu* DNA polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. PCR fragments amplified with *Pfu* DNA polymerase will therefore minimise the occurrence of errors compared to fragments amplified with Ampli*Taq* DNA polymerase (Vieille & Zeikus, 2001). The sequences of our expression constructs were normally verified by sequencing.

Production of recombinant proteins from *S. scabiei* in *E. coli*

As already indicated, the supply of antigen is problematic since there is no *in vitro* propagation system for *S. scabiei*. Furthermore, there are several potential pitfalls associated with the expression of foreign proteins in various host systems, including folding difficulties as well as toxicity problems (Hannig, 1998; Makrides, 1996). To overcome these obstacles we cloned several genes coding for *S. scabiei* proteins, and developed a general expression system in *E. coli* for the production of recombinant proteins.

Our system is based on a modified version of the expression vector pMAL-c2. A sequence coding for six consecutive histidines followed by a stop codon was introduced between the *Pst*I and *Hind*III sites of the pMAL-c2 vector from New England Biolabs (Beverly, MA) using standard techniques. The resulting vector was designated pPU16. In this system, the *S. scabiei* cDNA is spliced to a sequence that codes for the maltose binding protein (MBP) and a hexahistidine tag (His_6). This results in the expression of a recombinant fusion protein with dual affinity tags. The MBP is fused to the N-terminal part of the recombinant protein and the His_6-tag is fused to the C-terminal part. The MBP stabilises the recombinant protein and increases the expression yield. This has in part been attributed to the function of MBP as a molecular chaperone that promotes the solubility and stability of proteins that are fused to it (Kapust & Waugh, 1999). To further enhance the expression of soluble proteins, we used a minimal growth medium supplemented with a range of metal ions (Pryor & Leiting, 1997) and an incubation temperature of 18 ºC during the induction of protein expression. This also resulted in high growth densities. After harvest and cell lysis, the recombinant fusion protein was either affinity purified on an amylose resin column or, preferably, by immobilised metal affinity chromatography (IMAC). The latter technique is based on the interaction between the His_6-tag and metal ions that have been immobilised by chelation. The vector pPU16 was used as the backbone for the expression of several recombinant *S. scabiei* proteins, including paramyosin (I), GST (III), major *Sarcoptes* antigen 1 (MSA1) (IV), and atypical *Sarcoptes* antigen (ASA1) (IV).

Results and discussion

The *S. scabiei* cDNA library and isolation of cDNA clones (I-IV)

S. scabiei *cDNA library (I-IV)*

The prerequisite for all studies included in this thesis has been access to an *S. scabiei* cDNA library (I). The genetic sequence data generated in the studies all originate from this library. The cDNA library was constructed from mites of different developmental stages and of both sexes isolated from wild red foxes. In order to assess the quality of the primary cDNA library, two different tests were used (I). The first was a background test using a blue/white plaque-screening protocol. Less than 1.5% of the plaques were blue, suggesting that more than 98.5% of them carried an insert. In the second test the sizes of inserts were evaluated. A total of 20 randomly selected plaques from the primary library were used in a PCR, and their inserts were amplified with vector specific primers. All of the analysed plaques carried inserts, half of them had inserts between 0.5 kb and 1.0 kb long, 6 had inserts between 1.1 and 1.8 kb and 4 had inserts above 1.8 kb. The largest insert was close to 3 kb. Approximately 10^6 clones were obtained in this primary library.

The benefits of using cDNA libraries for molecular studies of *S. scabiei* have recently resulted in the generation of several *S. scabiei* var. *hominis* libraries (Fischer *et al.*, 2003a; Fischer *et al.*, 2003b; Harumal *et al.*, 2003). Access to the *S. scabiei* var. *vulpes* (I) and var. *hominis* libraries have, as shown in this thesis, proved to be, and will continue to be, useful for studies of the biology of *S. scabiei,* including host-parasite interactions. Although there are several advantages to use a cDNA library for the molecular study of *S. scabiei*, some aspects of the mite's biology have to be considered, including the host-origin of the mites (see section Host specificity). As discussed in Paper I, the source of *S. scabiei* mites for construction of cDNA libraries, or for the purification of specific molecules, will rarely be any problem. Still, the unique interface between a particular host species and *S. scabiei* mites derived from that host has to be taken into consideration in certain applications.

Isolation of cDNA clones (I-IV)

The studies included in this thesis can be divided into two groups depending on the strategy used for isolation of cDNA clones: Firstly, immunoscreening that is a more direct method to find clones corresponding to immunoreactive proteins; immunoscreening was used to isolate paramyosin (I) and ASA1 (IV). Secondly, the randomised discovery of *S. scabiei* genes through the generation of ESTs (II) that identified the delta-GST (III). Both of these methods are well established and have been used in several molecular studies of various parasites (see section Background).

In paper I, the initial screening of the amplified cDNA library with hyperimmune rabbit sera identified a number of antibody reactive plaques. The deduced amino acid sequences for seven of these clones corresponded to myosin. Also, for three of the clones the sequences were similar to paramyosin. The last two clones were both unique, and one corresponds to major *Sarcoptes* antigen 1 (MSA1; (Mattsson *et al.*, 1999)). The library was also screened with sera from dogs naturally infected with *S. scabiei*. From this screening, three clones corresponding to paramyosin were isolated. Immunoscreening of the cDNA library was also used to identify ASA1 in paper IV. The initial screening was performed with sera from five *S. scabiei* dogs. One of these sera identified two antibody-reactive plaques. In the re-screening of single plaque clones one of the two clones was excluded as it turned out to be a false positive. The protein product of the remaining clone was denoted ASA1.

In paper III, the information gained from the EST dataset (II) was utilised. In the dataset a clone denoted SAS0751 corresponded to a glutathione-S-transferase, which was further characterised (III), together with paramyosin (I) and ASA1 (IV).

EST data vs. immunoscreening

Paramyosin, myosin and ASA1 were all identified in immunoscreening experiments (I, IV). Interestingly, only paramyosin lacked an apparent homologue in the EST dataset. This demonstrates that immunoscreening and the generation of ESTs have functioned as complementary methods in this study. The EST dataset contains several clones corresponding to myosin and one clone in part corresponding to the ASA1 ORF. In contrast, Fischer *et al.*'s (2003) EST analysis of a *S. scabiei* var. *hominis* cDNA library revealed a homologue to paramyosin but did not identify any clones corresponding to myosin or ASA1 (Fischer *et al.*, 2003b). Furthermore, 13 of the clones identified by Fischer and co-workers showed the best BLASTn scores with *S. scabiei* var. *vulpes* ESTs. This demonstrates that EST data from variants of *S. scabiei* also are most likely to be complementary to each other.

Novel gene discovery in *S. scabiei* by EST analysis

Features of the EST dataset

Paper II mainly discusses the generation of ESTs for the purpose of accelerating gene discovery, and to gain insight into the biology of *S. scabiei*. A total of 1,020 ESTs were generated from the *S. scabiei* var. *vulpes* cDNA library in order to study expressed genes. The ESTs were generated by single-pass sequencing, which was performed from the 5'-end of randomly selected cDNA clones. A total of 116 sequences of poor quality were removed from the dataset, including five clones that were excluded since they clearly were of host origin. The BLASTx analysis did not reveal any other top scoring matches with sequences from red fox or other members of the Canidae family. This suggests a low level of host cDNA contamination.

A cluster analysis of the 904 sequences submitted to dbEST formed a total of 76 clusters and 576 singletons. The largest cluster contained 27 sequences and included transcripts coding for COI. The second largest cluster comprised 19 sequences and displayed similarity to a class of cysteine-rich proteins. The redundancy of the cDNA library was established to be 37% using those sequences that assembled into clusters. The number of clones corresponding to ribosomal RNAs (rRNA) was 3.3%. For comparison, sequences corresponding to rRNAs from the *S. scabiei* var. *hominis* cDNA library were 3.4% (Fischer *et al.*, 2003b).

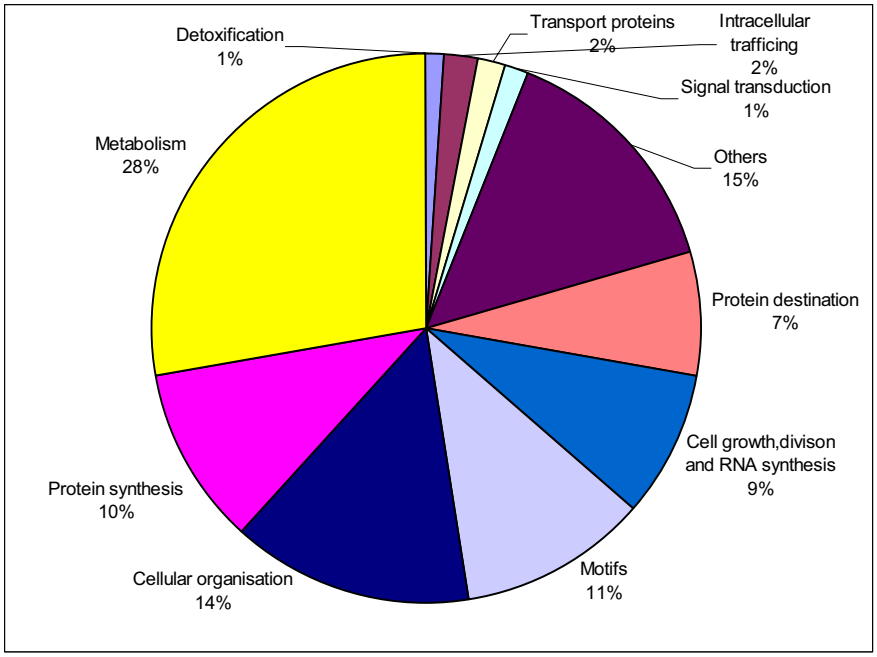

Fig. 6. Pie chart showing the functional classification of S. scabiei ESTs. Transcripts with putative identity in *S. scabiei* were divided into functional categories. Transcripts involved in metabolism constituted the largest group. The group denoted "others" contained ESTs that matched proteins with a known function but were not easily classified in any of the major groups and were too few to form unique groups.

Sequence similarity searches in public databases revealed that 48% of the *S. scabiei* ESTs could be assigned with a putative identity. Depending on the identity, these transcripts have been classified into different groups according to function (Fig. 6). The majority of matches were with sequences with a known or partly described function. However, a considerable fraction corresponded to evolutionarily conserved sequences of unknown function. This means that such sequences can be found in many different genera but have not yet been attributed any function. The largest fraction of transcripts with a putative identity coded for proteins involved in metabolism (28%). Transcripts corresponding to proteins involved in cellular and structural organization were the second largest group. Another large group of transcripts corresponded to proteins involved in translation. Predictably, this group was dominated by ribosomal proteins but also included

initiation and elongation factors. Signal transduction, detoxification, intracellular trafficking and transport proteins were the smallest groups, only adding up to a total of 6% (Fig. 6). An example from the detoxification group is the clone SAS0751 that shared similarity with a glutathione-S-transferase, which was further studied in Paper III. Additionally, another GST from the *S. scabiei* var. *hominis* library (Fischer *et al.*, 2003b) was identified by EST analysis; this GST was included in a phylogenetic analysis of GSTs from *S. scabiei* (III).

Additionally, the nucleotides A + T were observed to be more frequently occurring in the var. *vulpes* ESTs and var. *hominis* ESTs than the nucleotides G + C. The A + T nucleotides were also more common in the *S. scabiei* ESTs in comparison with the average composition of all sequences in the dbEST and GenBank nucleotide databases (unpublished data). The A + T content in our EST dataset was 65.4%, a figure far from the 80% A + T content observed in the A + T biased *Plasmodium falciparum* genome (Aravind *et al.*, 2003). A high A + T content might have practical implications for various down-stream applications, for instance A + T rich regions may pose a challenge to design efficient new sequencing or PCR primers.

Allergens and proteases

Several of the ESTs are similar to previously described allergens/antigens from various mites (II). Particularly interesting is a number of ESTs sharing apparent homology with various proteolytic enzymes. Clone SAS0725 encoded for a cysteine protease that shares its highest similarity with Der f 1, a group 1 allergen from the house-dust mite *D. farinae*. The group 1 allergens belong to the papain-like cysteine protease family and are primarily found in mite faeces (Yasuhara *et al.*, 2001). In addition, five clones corresponded to Eur m 1 (Kent *et al.*, 1992), which represent another group 1 cysteine protease, albeit from the house dust mite *E. maynei* (Thomas & Smith, 1998). The group 1 enzymes represents a major type of house-dust mite allergen in humans and the cysteine protease activity is thought to induce the pathogenic processes of allergy (Shakib & Gough, 2000; Shakib *et al.*, 1998). In all types of organisms cysteine proteases play many important roles. They are essential for general catabolic functions as well as protein processing. However, in parasites they may also be pivotal for immunoevasion, cell and tissue invasion or destruction, excystment/encystment and exsheathing (Sajid & McKerrow, 2002). In *S. scabiei* there are no reports of active proteases involved in immunoevasion. However, families of inactivated cysteine and inactivated serine proteases have recently been described and implicated to be important for mite immunoevasion (Holt *et al.*, 2003; Holt *et al.*, 2004).

We could also identify cDNAs coding for other classes of proteolytic enzymes. One of these corresponded to an aspartic proteinase similar to a cathepsin D. In *P. ovis*, cathepsin D-like enzymes have been localized to the digestive cells and are involved in the lysosomal degradation of nutrients (Nisbet & Billingsley, 2000). Another cathepsin found in the *S. scabiei* EST sequence set was a lysosomal cysteine protease corresponding to cathepsin L. A recombinant form of cathepsin L from the tick *B. microplus* hydrolyses a range of substrates, including synthetic as

well as natural proteins like haemoglobin, vitellin, and gelatin (Renard *et al.*, 2000; Renard *et al.*, 2002). Furthermore, an *S. scabiei* var. *hominis* EST has also been identified to be homologous to cathepsin L from *B. microplus* (Fischer *et al.*, 2003b). Cathepsin L-like proteases are also candidate vaccine antigens for protection against helminth infections (Harmsen *et al.*, 2004).

We also fully sequenced the five Eur m 1-like *S. scabiei* EST clones. The longest ORF was then expressed as a recombinant MBP-Eur m 1-like protein in *E. coli*. Only after affinity purification could we detect trace amounts of the recombinant protein. The identity of the protein was verified by mass-spectrometry analysis at the Proteomics unit at the Biomedical Centre, Uppsala University. It appeared as if the bacterial expression system had problems in handling this recombinant protein. One could speculate that a fully active cysteine protease could wreak havoc with the bacteria. Still, even expression of a part of the Eur m 1-like protein was toxic to *E. coli* (unpublished data).

Abundant transcripts

In the *S. scabiei* dataset a total of 32 ESTs shared similarity with a scavenger receptor cysteine rich protein (SRCRP) from purple sea urchin (*Strongylocentrotus purpuratos*). Many of these carry a trypsin-inhibitor like domain (TIL), and they constitute more than 3% of the investigated clones, suggesting that the mRNA levels from this family of genes are highly abundant in *S. scabiei*. A cluster analysis was performed with these sequences, which resulted in three related clusters. No exact function can be assigned to these clusters (II).

The value of the EST data

One of the most valuable types of information that can be gained from any EST dataset is help choosing candidate genes for future studies. Some of the transcripts identified in our study are obvious choices, such as proteases and various allergen-related proteins. Not only are these types of proteins fundamental to a basic understanding of the parasite–host interaction, but they can also be turned into valuable tools for the immunological control of *S. scabiei*. Transcripts that correspond to proteins of unknown function, in particular those that appear very frequently in the EST collection, represent an intriguing group. Over-representation in the dataset suggests that the parasite requires large amounts of their protein products, which merits them for further investigations. The rapid growth of the different molecular databases will certainly be helpful in identifying the roles for the hitherto unknown transcripts/proteins, as demonstrated by data-mining strategies (Conklin *et al.*, 2000; Dalton *et al.*, 2003). Nevertheless, biochemical studies are inevitable if we want to begin to understand the molecular interplay between *S. scabiei* and its hosts. In this thesis, paper III is the best example of how to progress with EST data if one wants to do a more detailed study.

Sequence analysis and characterisation of *S. scabiei* proteins

The three proteins paramyosin, GST and ASA1 have in this study been further characterised (I, III, IV). The main reason for an extended analysis was that they have been implicated in the experiments (I, IV) and/or in the literature (I, III) to have immunogenic and/or immunomodulatory properties.

Paramyosin (I)

Paramyosin is a major structural component of many filaments isolated from invertebrate muscles. Since paramyosin has been recognized also as an important antigen for various parasitic helminths, it was decided to investigate the *S. scabiei* paramyosin in some detail. The predicted initiation codon is surrounded by A at position –3, and T at position +4, a context that is identical to paramyosin cDNAs from *D. melanogaster* (Becker *et al.*, 1992) and *Onchocerca volvulus* (Dahmen *et al.*, 1993). The ORF encodes a protein of 876 amino acids with a molecular weight of 102.5 kDa. All paramyosin proteins have predicted non-helical amino- and carboxy-termini (the first 27 residues and the last 31 residues in the *S. scabiei* paramyosin) that flanks a alpha-helical major part of the protein. As for other paramyosins our *S. scabiei* paramyosin was found to have heptad repeats characteristic of a coiled-coil alpha helix. The first and fourth of the amino acid residues in the repeats are usually apolar and while charged residues can be found in the other positions. In addition there was a 28-residue repeat, which is also characteristic for paramyosin (Becker *et al.*, 1992; Laclette *et al.*, 1991; Landa *et al.*, 1993; Limberger & McReynolds, 1990). Three different versions of recombinant *S. scabiei* paramyosin were expressed and included in a Western Blot analysis, to evaluate the role of paramyosin as a marker for *S. scabiei* infection, which will be further discussed below.

Glutathione-S-transferase (III)

The full cDNA sequence included a complete ORF of 684 bp that encoded a putative protein of 227 amino acids in length and with a predicted molecular weight of 26 kDa. GST is a dimeric cytolytic enzyme found in all eukaryotic organisms (Sharp *et al.*, 1991). The enzyme is multifunctional, catalysing the conjugation between different forms of the tripeptide glutathione, e.g. reduced gluthathione (GSH) (Krause *et al.*, 2001; Ouaissi *et al.*, 2002), and many structurally different substrates (Ouaissi *et al.*, 2002; Yang *et al.*, 2003). GST form a superfamily whose proteins are encoded by several multigene families. GST participates in a number of different reactions that are important in the defence of cells against oxidative attacks by oxygen and radicals (Sharp *et al.*, 1991). The enzyme often has immunomodulatory properties, and GST secreted from parasites could play a part in evasion of the immune response of the host (Ouaissi *et al.*, 2002).

A phylogenetic analysis of the identified GST showed that the new *S. scabiei* sequence described in this thesis is a member of the delta-class of GSTs (Fig. 1, III). This is the first example that delta-class GSTs occur in organisms other than insects. Furthermore, our analysis places a GST isolated from an *S. scabiei* var.

hominis cDNA library (Fischer *et al.*, 2003b), in the mu-GST class (Fig. 2., III). We could thus demonstrate for the first time that two classes of GSTs occur within the Acari, and also within a single species of the Acari. The mu classification was also confirmed in a recent report (McGoldrick *et al.*, 2005). In our laboratory, two additional types of *S. scabiei* GSTs have recently been cloned (Pettersson and Mattsson, unpublished data). One of them is identical to the mu-class GST isolated by Fischer et al (2003) and the second remains to be classified.

Furthermore, all bacterially produced GST proteins showed specific activity in the various enzyme assays conducted (Table 1, III). Recombinant enzymes purified using the GST and glutathione interaction showed higher specific activity compared to those purified using the His-tag. This is probably due to the selective enrichment of correctly folded enzymes in the former protocol. The HiTrap purification in the other protocol does not discriminate between the active and inactive forms of the enzyme. We could also see a possible influence on the activity depending on whether the His-tag was positioned at the N-terminus or C-terminus.

Atypical Sarcoptes *Antigen 1 (IV)*

Sequencing established the insert length to be 2 847 bp. The insert contained a 5'-UTR, a 3'-UTR and a 2,157 bp long ORF corresponding to a protein product of 719 amino acids (Fig. 1, IV). The predicted molecular weight was 79 kDa. The ASA1 sequence was compared with sequences in the GenBank non-redundant databases. A BLASTx comparison revealed a 26% identity with a protein named GA10629-PA of unknown function ($E = 1 \times e^{-6}$; scorebit 57.8; accession No. EAL25440) sequenced from *Drosophila pseudoobscura*. A BLASTp comparison combined with conserved domain (CD) search (Marchler-Bauer *et al.*, 2003) with the translated ASA1 ORF identified the presence of a MADF domain ($E = 4 \times e^{-14}$). Interestingly, the first protein described with a MADF domain, the Adh distal factor-1 (Adf-1) from *D. melanogaster* is a transcription factor that binds the promoters of a diverse group of genes (England *et al.*, 1992; England *et al.*, 1990). The domain is a distantly related member of the Myb helix-turn-helix family of DNA binding proteins (Cutler *et al.*, 1998). Although the list of MADF-containing proteins has since then expanded, very few of them have been experimentally characterised, as the vast majority originate from the ORF predictions of major genome sequencing projects. However, the few examples characterised are all transcriptional regulators from *D. melanogaster* (Barbash *et al.*, 2003; Bhaskar *et al.*, 2000; Clark & McKearin, 1996). So far, the MADF domain has not been found in mammals.

Outside of the MADF domain ASA1 has a low compositional complexity with several blocks of triplets and even quadruplets of polar amino acids (Asn, Gln and Ser). As a result no significant similarities were found besides the MADF domain.

Four different versions of recombinant *S. scabiei* ASA1 were expressed and included in a Western Blot analysis, to evaluate the role of ASA1 as an *S. scabiei* antigen, which will be further discussed below.

Western blot analysis

To study the antigenic properties of paramyosin, GST and ASA1, several versions of each protein were recombinantly expressed. These recombinant proteins were analysed by Western blot using rabbit hyperimmune sera and sera from naturally *S. scabiei* infected dogs and pigs. A recombinant protein that is recognised by sera from naturally infected hosts suggests that the protein is somehow involved in host-parasite interactions, since a positive reaction indicates that the protein is exposed to the host immune system.

Paramyosin (I)

The antigenicity of the MBP-paramyosin 145 kDa protein was confirmed by Western blotting using rabbit hyperimmune sera raised against native *S. scabiei* antigens (Fig. 2B, I). The Western blot data indicated that only a fraction of the induced protein was of full-length. This could be due to either *E. coli*-induced degradation of the recombinant protein or the result of translation-associated problems. Switching to other strains of *E. coli* did not produce any significant effect on the ratio of the full-length protein versus shorter fragments. Sera from experimentally infected dogs as well as pigs reacted with the recombinant paramyosin (Fig. 3A and B, I). However, some of the tested sera did not react with the recombinant paramyosin (Fig. 3B, I).

In contrast, MBP-paramyosin Δ*Xba*I with the N-terminal part of the *S. scabiei* paramyosin in frame with the MBP was expressed to very high levels (Fig. 2A, I). This fusion protein contained about 40% or the first 344 amino acid residues of the *S. scabiei* paramyosin, and the resulting product was readily recognized by rabbit antisera (Fig. 2B, I).

The miniaturised paramyosin of 17 kDa, corresponding to the non-helical ends of *S. scabiei* paramyosin and a central alpha-helical domain, was readily expressed as a fusion with MBP, and there were almost no smaller sized fusion proteins present in the induced samples. MBP-miniaturised paramyosin was analysed by Western blot with sera from *S. scabiei* infected dogs and swine. The results were, however, rather weak and inconclusive. In contrast, the miniaturised protein was recognised by rabbit antisera.

The conclusion is that paramyosin is an important immunogen in scabies, although the precise interaction between the host's immune system and *S. scabiei* paramyosin remains to be clarified. A recombinant paramyosin could potentially be used as an infection marker in immunoassays, e.g. ELISA. However, due to the large number of evolutionarily conserved epitopes in paramyosin, the chances are that cross-reacting antibodies from other types of parasitic infections might interfere with the assay. It should be noted that no sera from animals with crusted scabies were included in the study.

GST (III)

In Western blot experiments, antibodies in the rabbit hyperimmune sera reacted both with recombinant GST and with the native GST from *S. scabiei* protein extracts (Fig. 4, IV). The molecular mass of the recombinant GST corresponded to the expected value of 29 kDa. When analysing the *S. scabiei* protein extracts, the polyclonal sera reacted with a band of the predicted molecular mass for native *S. scabiei* GST (26 kDa). However, none of the sera from mange-infected dogs and pigs contained specific IgG or IgE antibodies towards GST. Thus, we could not discriminate between sarcoptic animals and healthy animals. As in the previous examples, no animals with crusted scabies were analysed.

ASA1 (IV)

With Western blotting we could detect bands corresponding to the expected protein sizes from all four of our ASA1 constructs. In this assay we used a rabbit hyperimmune serum raised against native *S. scabiei* antigens. However, the reaction with MBP-ASA1 was weak and only a small fraction of the purified protein was of full-length. In the same way, the protein profiles for ASA1c also displayed what looked like truncated peptides. However, the reaction towards ASA1c was stronger. As a contrast, both ASA1a and ASA1b were efficiently expressed, and only minor amounts of non-full-length proteins were enriched in the affinity purification.

The *S. scabiei* positive dog serum originally used in the immunoscreening to identify the ASA1 clone was used to test the ASA1a and ASA1b proteins. The best response with this serum was seen towards ASA1a. Interestingly, this is also the part that contains the MADF domain. In contrast, the ASA1c peptide, corresponding to amino acids 480-719 and included the C-terminus of ASA1, was the part that had the lowest compositional complexity, and which probably contributed to expression difficulties. Based on immunoscreening and Western blot data, we selected ASA1a for an evaluation with a panel of 107 sera from dogs. The Western blot analysis also included native *S. scabiei* protein extracts and the MSA1(Mattsson *et al.*, 1999). The latter was included as a reference antigen. A total of 15 sera had IgG that reacted with the recombinant ASA1a, and 53 of the samples were positive for MSA1. Of the 107 samples, 62 samples reacted with the *S. scabiei* protein extract whereas 22 samples gave an unspecific background staining. We could also show that one of the ASA1a-responsive sera had IgE reactive antibodies, but none of these 15 sera had IgE antibodies that reacted with MSA1 or the *S. scabiei* protein extract.

ASA1 is clearly an immunogenic protein that is recognised by *S. scabiei* infected dogs. At the same time, the presence of the MADF domain presents something of a dilemma since that domain is normally found only in proteins associated with transcription control. We do not know if ASA1 is involved in transcriptional control, or if ASA1 has an alternative function and is the result of a gene that evolved from recombination of pre-existing domains (Copley *et al.*, 2002).

Immunolocalisation experiments (III, IV)

To further characterise the various proteins, we tried to localise them in skin biopsies containing *S. scabiei* mites. For this examination we used samples from naturally infected wild red foxes, and polyclonal antisera specific for the recombinant versions of the delta-class GST, ASA1 and MSA1 proteins. Immunolocalisation of the proteins provides information on their abundance, and distribution in the mite, and dissemination in the host tissue. Taken together this information will also provide insights into the possible exposure of the proteins to the host's immunesystem.

The anti-GST sera stained discrete portions of the mites (Fig. 5, III), while pre-immune serum showed no staining. Specifically, the integument of the epidermis and cavities surrounding vital organs were stained. Minor staining was observed in the intestine. We could not detect any staining on the mite surface or in the host tissue. This is in agreement with the Western blot data, where no GST reactive sera from *S. scabiei* infected animals could be found. In addition, no signal peptide typical for a secretion signal was found in our sequence analysis.

The staining of sections through red fox skin infected with *S. scabiei* showed that anti-ASA1a and anti-MSA1 antibodies generated in rabbits bound strongly to the mites. The polyclonal antisera stained numerous vital organs and tissues in the mite's body cavity. The only part of *S. scabiei* that omitted staining was the thin cuticle, including legs and mouthparts (Fig. 5 and 6, IV). This was also true for visible structures such as stout setae. The anti-ASA1a serum also stained what appeared to be the inside of the burrow wall, suggesting that the mite somehow deposits the protein there. However, ASA1 resembles GST in the respect that we could not identify a secretion signal. The staining of the burrows inside could be an experimental artefact due to the method used, but no staining of any host tissue beyond the burrow could be detected (Fig. 5, IV) This was also true for the sections analysed with anti-MSA1 serum (6, IV). It appears that deposited eggs were stained by anti-MSA1 serum in contrast to eggs *in utero*. The presence of any signal peptide in MSA1 is not known, since the full-length cDNA (gene) has not been cloned. Control sera from naïve rabbits did not stain the mites or red fox tissue.

Harumal *et al*. (2003) have identified an immunodominant antigen, which they called Ssag1, which is homologous with MSA1 (Mattsson *et al.*, 1999). They have also used immunostaining in order to localise Ssag1 in biopsies from human patients with crusted scabies. Their localisation experiments of MSA1/Ssag1 are in concordance with ours.

Whether the density of infection also influences the localisation of various proteins is not known. However, it is tempting to speculate that a higher mite density also increases the risk of exposure to a wider range of mite-derived proteins compared to more restrained disease conditions.

Concluding remarks

Many research areas in human and veterinary medicine are hampered by shortages in biological material. Some examples include infectious organisms that are virtually impossible to cultivate, certain proteins that are extremely rare or only present during a short time frame in the life span of an organism, or the absence of phenotypic characters useful for identification or subtyping. These and other areas have been and continue to be affected by the molecular methods at hand. In many respects these have truly revolutionised our possibilities to explore and study the biological world. In my project I have used a range of molecular tools to study a parasite that, due to its parasitic life-style and relatively low abundance, poses several experimental challenges.

The difficulties of obtaining scabies mites regularly and in sufficient quantities have, in part, been overcome by the generation of an *S. scabiei* cDNA library. The library enabled me to study part of the genetic background and other aspects of *S. scabiei*. The access to the library and the use of recombinant DNA technologies made production of large quantities of recombinant *S. scabiei* proteins possible.

An EST analysis of the *S. scabiei* cDNA library generated novel sequence data corresponding to 904 expressed genes in the parasite. Nearly half of these could be assigned a putative identity. Thus, the EST analysis has provided basic knowledge of genes expressed in the parasite. Several identified genes code for proteins that possibly are important in the molecular interplay between *S. scabiei* and its host. Examples of such molecules from the dataset are proteases and previously identified allergens. Importantly, EST sequence data constitute a platform for *S. scabiei* gene information that provides guidance in choosing candidate genes for future studies. The characterisation of a GST from *S. scabiei* is an example of the valuable use of information gained from the EST analysis.

By utilising an immunoscreening technique several immunodominant clones were isolated. Immunoscreening identified the *S. scabiei* proteins paramyosin and ASA1. These findings demonstrate that the cDNA library can assist in isolation of important *S. scabiei* antigens that are exposed to the hosts.

The use of bioinformatics has been pivotal for the analysis of sequence data generated in this thesis. By combining data from several types of databases I was able to link my data to a much larger network of biological systems.

Recombinant *S. scabiei* paramyosin was recognised by sera from dogs and pigs infected with *S. scabiei*. This indicates that paramyosin is an important immunogen in *S. scabiei* infections. Recombinant ASA1 was also shown to have immunogenic properties, as it was recognised by sera from dogs infected with *S. scabiei*. Thus, both paramyosin and ASA1 appear to be involved in host-parasite interactions. Paramyosin has previously been shown to interact with the host's immune system in several parasite infections. In contrast, ASA1 represents a class of proteins not normally associated with antigenic properties.

The EST corresponding to *S. scabiei* GST was fully characterised, and through a phylogenetic analysis we could show that it belonged to the delta class. This class has previously been believed to be unique to insects. Active forms of the *S. scabiei* GST were expressed in *E. coli,* and after purification we could do a thorough enzyme kinetic analysis. Western blot data suggested that the delta GST was not exposed to the hosts during *S. scabiei* infections and this was further supported by our immunolocalisation experiments. This finding was in contrast to the situation with ASA1 and MSA1 for which our data suggest are readily exposed to the immune system of the host.

It is important to emphasise that the results presented here are only a mere scratch on the surface of understanding the biology of *S. scabiei* and the parasite's interactions with its host. Today the information in databases of genes and proteins is not only growing at an impressive rate, but more and more of data are also interconnected across various databases. It is perhaps my entries to these global databases that have been the most important scientific contribution described in this thesis. Specialists from various other disciplines, regardless of their specific interests, can now access the data and compare their gene or protein information with molecular data from *S. scabiei.*

Future research

Based on information generated in this thesis, further studies of *S. scabiei'*s biology, and potentially important proteins in its infection processes, already have commenced or will be initiated. More studies using molecular tools for the study of *S. scabiei* is obviously needed as this field still is in its infancy. Here follows some suggestions for future research in relation to the findings presented in this thesis.

As previously described, *S. scabiei* derived proteases and allergens most likely interact with the host immune system. The EST analysis identified several transcripts of these genes in *S. scabiei*. The cloning and expression of the Eur m 1 fragment has been the first effort to commence the characterisation of an *S. scabiei* protease. However, to understand their role in the pathogenesis of scabies/mange these proteins need to be further characterised in relation to the host immune response.

The available information of gene and protein data in databases is continuously expanding. It would therefore be useful to make new sequence comparisons with those transcripts from the EST project that presently lacks homologues in the databases. Thus, new comparisons may lead to more ESTs being assigned with a putative identity. Transcripts that appear very frequently in the EST collection today but correspond to proteins of unknown function are of continued interest. Their abundance indicates that the parasite requires large amounts of their protein products, which qualifies them for further investigations.

To overcome the observed problems in bacterial expression systems, such as for example degradation of recombinant proteins and folding problems, it would be beneficial to evaluate other expression systems. For instance, this is important for the further study of the cysteine rich/TIL-domain containing proteins identified in the EST study.

The delta-class GST described here was not recognised by sera from naturally infected animals. Still, other members of the GST family may be of importance for immunomodulation of the host. GSTs are, on the other hand pivotal detoxification enzymes that possess a wide range of substrate specificities. A *B. microplus* GST was recently shown to be inhibited by various acaricides (da Silva Vaz *et al.*, 2004) and the malaria vector *Anopheles gambiae* has elevated levels of GST activity in insecticide resistant lines (Chen *et al.*, 2003) and references therein. Clearly, more information is needed about various *S. scabiei* GSTs and their role in drug metabolism. This is also highlighted by the recent description of clinical and *in vitro* ivermectin resistance in *S. scabiei* mites isolated from human patients in northern Australia (Currie *et al.*, 2004).

Finally, the potential for protective immunity induced by paramyosin and some of the other proteins discussed in this thesis should be evaluated, since they could have potential roles in a future vaccine against *S. scabiei* infections.

References

Adams, M.D., Kelley, J.M., Gocayne, J.D., Dubnick, M., Polymeropoulos, M.H., Xiao, H., Merril, C.R., Wu, A., Olde, B. & Moreno, R.F. (1991). Complementary DNA sequencing: expressed sequence tags and human genome project. *Science 252*, 1651-1656.

Ajioka, J.W., Boothroyd, J.C., Brunk, B.P., Hehl, A., Hillier, L., Manger, I.D., Marra, M., Overton, G.C., Roos, D.S., Wan, K.L., Waterston, R. & Sibley, L.D. (1998). Gene discovery by EST sequencing in *Toxoplasma gondii* reveals sequences restricted to the Apicomplexa. *Genome Research 8*, 18-28.

Alexander, A.M. (1978). Role of race in scabies infestation. *Archives of Dermatology 114*, 627.

Alexander, J.O. (1984). Arthopods and human skin. Springer, Berlin, pp. 227-292.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology 215*, 403-410.

Aravind, L., Iyer, L.M., Wellems, T.E. & Miller, L.H. (2003). Plasmodium biology: genomic gleanings. *Cell 115*, 771-785.

Arlian, L.G. (1989). Biology, host relations, and epidemiology of *Sarcoptes scabiei*. *Annual Review of Entomology 34*, 139-161.

Arlian, L.G. & Morgan, M.S. (2000). Serum antibody to *Sarcoptes scabiei* and house dust mite prior to and during infestation with *S. scabiei*. *Veterinary Parasitology 90*, 315-326.

Arlian, L.G., Morgan, M.S. & Arends, J.J. (1996a). Immunologic cross-reactivity among various strains of *Sarcoptes scabiei*. *The Journal of Parasitology 82*, 66-72.

Arlian, L.G., Morgan, M.S., Estes, S.A., Walton, S.F., Kemp, D.J. & Currie, B.J. (2004). Circulating IgE in patients with ordinary and crusted scabies. *Journal of Medical Entomology 41*, 74-77.

Arlian, L.G., Morgan, M.S., Rapp, C.M. & Vyszenski-Moher, D.L. (1996b). The development of protective immunity in canine scabies. *Veterinary Parasitology 62*, 133-142.

Arlian, L.G., Morgan, M.S., Vyszenski-Moher, D.L. & Stemmer, B.L. (1994a). *Sarcoptes scabiei*: the circulating antibody response and induced immunity to scabies. *Experimental Parasitology 78*, 37-50.

Arlian, L.G., Rapp, C.M. & Morgan, M.S. (1995). Resistance and immune response in scabies-infested hosts immunized with Dermatophagoides mites. *The American Journal of Tropical Medicine and Hygiene 52*, 539-545.

Arlian, L.G., Rapp, C.M., Stemmer, B.L., Morgan, M.S. & Moore, P.F. (1997). Characterization of lymphocyte subtypes in scabietic skin lesions of naive and sensitized dogs. *Veterinary Parasitology 68*, 347-358.

Arlian, L.G., Rapp, C.M., Vyszenski-Moher, D.L. & Morgan, M.S. (1994b). *Sarcoptes scabiei*: histopathological changes associated with acquisition and expression of host immunity to scabies. *Experimental Parasitology 78*, 51-63.

Arlian, L.G., Runyan, R.A., Achar, S. & Estes, S.A. (1984a). Survival and infectivity of *Sarcoptes scabiei* var. *canis* and var. *hominis*. *Journal of the American Academy of Dermatology 11*, 210-215.

Arlian, L.G., Runyan, R.A. & Estes, S.A. (1984b). Cross infestivity of *Sarcoptes scabiei*. *Journal of the American Academy of Dermatology 10*, 979-986.

Arlian, L.G., Runyan, R.A., Sorlie, L.B. & Estes, S.A. (1984c). Host-seeking behavior of *Sarcoptes scabiei*. *Journal of the American Academy of Dermatology 11*, 594-598.

Arlian, L.G. & Vyszenski-Moher, D.L. (1988). Life cycle of *Sarcoptes scabiei* var. *canis*. *The Journal of Parasitology 74*, 427-430.

Arlian, L.G., Vyszenski-Moher, D.L. & Gilmore, A.M. (1988). Cross-antigenicity between *Sarcoptes scabiei* and the house dust mite, *Dermatophagoides farinae* (Acari: Sarcoptidae and Pyroglyphidae). *Journal of Medical Entomology 25*, 240-247.

Arlian, L.G., Vyszenski-Moher, D.L. & Pole, M.J. (1989). Survival of adults and development stages of *Sarcoptes scabiei* var. *canis* when off the host. *Experimental and Applied Acarology 6*, 181-187.

Barbash, D.A., Siino, D.F., Tarone, A.M. & Roote, J. (2003). A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America 100*, 5302-5307.

Bates, P. (2003). Sarcoptic mange (*Sarcoptes scabiei* var *vulpes)* in a red fox (*Vulpes vulpes*) population in north-west Surrey. *The Veterinary Record 152*, 112-114.

Becker, K.D., O'Donnell, P.T., Heitz, J.M., Vito, M. & Bernstein, S.I. (1992). Analysis of *Drosophila* paramyosin: identification of a novel isoform which is restricted to a subset of adult muscles. *The Journal of Cell Biology 116*, 669-681.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Wheeler, D.L. (2004). GenBank: update. *Nucleic acids research 32*, D23-26.

Berrilli, F., D'Amelio, S. & Rossi, L. (2002). Ribosomal and mitochondrial DNA sequence variation in Sarcoptes mites from different hosts and geographical regions. *Parasitology Research 88*, 772-777.

Bezold, G., Lange, M., Schiener, R., Palmedo, G., Sander, C.A., Kerscher, M. & Peter, R.U. (2001). Hidden scabies: diagnosis by polymerase chain reaction. *The British Journal of Dermatology 144*, 614-618.

Bhaskar, V., Valentine, S.A. & Courey, A.J. (2000). A functional interaction between dorsal and components of the Smt3 conjugation machinery. *The Journal of Biological Chemistry 275*, 4033-4040.

Blaxter, M., Daub, J., Guiliano, D., Parkinson, J. & Whitton, C. (2002). The *Brugia malayi* genome project: expressed sequence tags and gene discovery. *Transactions of the Royal Society of Tropical Medicine and Hygiene 96*, 7-17.

Boguski, M.S., Lowe, T.M. & Tolstoshev, C.M. (1993). dbEST--database for "expressed sequence tags". *Nature Genetics 4*, 332-333.

Bornstein, S. (1995). *Sarcoptes scabiei* infections of domestic dog, red fox and pig - clinical and serodiagnostic studies. Department of Parasitology. Swedish

University of Agricultural Sciences and National Veterinary Institute ISBN 91-576-4951-0, Uppsala, pp. 63.
Bornstein, S., Thebo, P. & Zakrisson, G. (1996). Evaluation of an enzyme-linked immunosorbent (ELISA) for the serological diagnosis of canine sarcoptic mange. *Veterinary Record 141*, 8-12.
Bornstein, S., Thebo, P., Zakrisson, G., Abu-Samra, M.T. & Mohamed, G.E. (1997). The demonstration of serum antibodies to *Sarcoptes scabiei* in naturally infected camels: a pilot study. *Journal of Camel Practice and Research 4*, 183-185.
Bornstein, S. & Zakrisson, G. (1994). Humoral Antibody response to Experimental *Sarcoptes scabiei* var. *vulpes* Infection in the Dog. *Veterinary Dermatology 4*, 107-110.
Brites, C., Weyll, M., Pedroso, C. & Badaro, R. (2002). Severe and Norwegian scabies are strongly associated with retroviral (HIV-1/HTLV-1) infection in Bahia, Brazil. *AIDS 16*, 1292-1293.
Brown, T.A. (2001). *Gene cloning and DNA analysis: an introduction*. Blackwell Science, 376.
Burgess, I. (1994). *Sarcoptes scabiei* and scabies. *Advances in Parasitology 33*, 235-292.
Chakrabarti, A. (1990). Pig handler's itch. *International Journal of Dermatology 29*, 205-206.
Chakrabarti, A., Chatterjee, A., Chakrabarti, K. & Sengupta, D.N. (1981a). Human scabies from contact with water buffaloes infested with *Sarcoptes scabiei* var. *bubalis*. *Annals of Tropical Medicine and Parasitology 75*, 353-357.
Chakrabarti, A., Dutta, A.K., Mandal, S.B., Chakraborty, A.K. & Sengupta, D.N. (1981b). Transmission of scabies from goats to human subjects. *Indian Journal of Dermatology 25*, 7-13.
Chen, L., Hall, P.R., Zhou, X.E., Ranson, H., Hemingway, J. & Meehan, E.J. (2003). Structure of an insect delta-class glutathione S-transferase from a DDT-resistant strain of the malaria vector *Anopheles gambiae*. *Acta Crystallographica. Section D, Biological Crystallography. 59*, 2211-2217.
Chosidow, O. (2000). Scabies and pediculosis. *Lancet 355*, 819-826.
Christophersen, J. (1978). The epidemiology of scabies in Denmark, 1900 to 1975. *Archives of Dermatology 114*, 747-750.
Clark, K.A. & McKearin, D.M. (1996). The *Drosophila* stonewall gene encodes a putative transcription factor essential for germ cell development. *Development 122*, 937-950.
Conklin, D., Yee, D.P., Millar, R., Engelbrecht, J. & Vissing, H. (2000). Mining of assembled expressed sequence tag (EST) data for protein families: application to the G protein-coupled receptor superfamily. *Briefings in Bioinformatics 1*, 93-99.
Copley, R.R., Letunic, I. & Bork, P. (2002). Genome and protein evolution in eukaryotes. *Current Opinion in Chemical Biology 6*, 39-45.
Currie, B.J. & Carapetis, J.R. (2000). Skin infections and infestations in Aboriginal communities in northern Australia. *The Australasian Journal of Dermatology 41*, 139-143; quiz 144-135.

Currie, B.J., Harumal, P., McKinnon, M. & Walton, S.F. (2004). First documentation of in vivo and in vitro ivermectin resistance in *Sarcoptes scabiei*. *Clinical Infectious Diseases 39*, e8-12.

Currie, B.J., Maguire, G.P. & Wood, Y.K. (1995). Ivermectin and crusted (Norwegian) scabies. *The Medical Journal of Australia 163*, 559-560.

Curtis, C.F. (2001). Evaluation of a commericially available enzyme-linked immunosorbent assay for diagnosis of canine sarcoptic mange. *Veterinary Record 148*, 238-239.

Cutler, G., Perry, K.M. & Tjian, R. (1998). Adf-1 is a nonmodular transcription factor that contains a TAF-binding Myb-like motif. *Molecular and Cellular Biology 18*, 2252-2261.

da Silva Vaz, I., Jr., Torino Lermen, T., Michelon, A., Sanchez Ferreira, C.A., Joaquim de Freitas, D.R., Termignoni, C. & Masuda, A. (2004). Effect of acaricides on the activity of a *Boophilus microplus* glutathione S-transferase. *Veterinary Parasitology 119*, 237-245.

Dahl, J.C., Scwartz, B., Graudal, C., Christophersen, J. & Henrisken, S.A. (1985). Serum IgE antibodies to the scabies mite. *International Journal of Dermatology 24*, 313-315.

Dahl, M.V. (1983). The immunology of scabies. *Annals of Allergy 51*, 560-566.

Dahmen, A., Gallin, M., Schumacher, M. & Erttmann, K.D. (1993). Molecular cloning and pre-mRNA maturation of *Onchocerca volvulus* paramyosin. *Molecular and Biochemical Parasitology 57*, 335-338.

Dalton, J.P., Brindley, P.J., Knox, D.P., Brady, C.P., Hotez, P.J., Donnelly, S., O'Neill, S.M., Mulcahy, G. & Loukas, A. (2003). Helminth vaccines: from mining genomic information for vaccine targets to systems used for protein expression. *International Journal for Parasitology 33*, 621-640.

Damriyasa, I.M., Failing, K., Volmer, R., Zahner, H. & Bauer, C. (2004). Prevalence, risk factors and economic importance of infestations with *Sarcoptes scabiei* and *Haematopinus suis* in sows of pig breeding farms in Hesse, Germany. *Medical and Veterinary Entomology 18*, 361-367.

Davies, P.R., Bahnson, P.B., Grass, J.J., Marsh, W.E., Garcia, R., Melancon, J. & Dial, G.D. (1996). Evaluation of the monitoring of papular dermatitis lesions in slaughtered swine to assess sarcoptic mite infestation. *Veterinary Parasitology 62*, 143-153.

Davies, P.R., Moore, M.J. & Pointon, A.M. (1991a). Sarcoptic mite hypersensitivity and skin lesions in slaughtered pigs. *The Veterinary Record 128*, 516-518.

Davies, P.R., Moore, M.J. & Pointon, A.M. (1991b). Seasonality of sarcoptic mange in pigs in South Australia. *Australian Veterinary Journal 68*, 390-392.

Davis, D.P. & Moon, R.D. (1990). Dynamics of swine mange: a critical review of the literature. *Journal of Medical Entomology 27*, 727-737.

England, B.P., Admon, A. & Tjian, R. (1992). Cloning of *Drosophila* transcription factor Adf-1 reveals homology to Myb oncoproteins. *Proceedings of the National Academy of Sciences of the United States of America 89*, 683-687.

England, B.P., Heberlein, U. & Tjian, R. (1990). Purified *Drosophila* transcription factor, Adh distal factor-1 (Adf-1), binds to sites in several *Drosophila*

promoters and activates transcription. *The Journal of Biological Chemistry 265*, 5086-5094.
Estes, S.A. & Estes, J. (1993). Scabies research: another dimension. *Seminars in Dermatology 12*, 34-38.
Estes, S.A., Kummel, B. & Arlian, L. (1983). Experimental canine scabies in humans. *Journal of the American Academy of Dermatology 9*, 397-401.
Evans, M.L., Dawson, A.D., Wall, R., Burke, T. & Stevens, J. (2003). Isolation of *Psoroptes* scab mite microsatellite markers (Acari:Psoroptidae). *Molecular Ecology Notes 3*, 420-424.
Fain, A. (1978). Epidemiological problems of scabies. *International Journal of Dermatology 17*, 20-30.
Fain, A. (1968). Etude de la variabilité de *Sarcoptes scabiei* avec une révision des Sarcoptidae. *Acta Zoologica et Pathologica Antverpiensia 47*, 1-196.
Falk, E.S. (1980). Serum immunoglobulin values in patients with scabies. *The British Journal of Dermatology 102*, 57-61.
Falk, E.S. & Bolle, R. (1980). IgE antibodies to house dust mite in patients with scabies. *The British Journal of Dermatology 103*, 283-288.
Falk, E.S. & Eide, T.J. (1981). Histologic and clinical findings in human scabies. *International Journal of Dermatology 20*, 600-605.
Fischer, D.C., Holt, D., Wilson, P., Davis, J., Hewitt, V., Johnson, M., McGrath, A., Currie, B.J., Walton, S.F. & Kemp, D.J. (2003a). Normalisation of a cDNA library cloned in lambdaZAP by a long PCR and cDNA reassociation procedure. *Benchmarks 34*, 250-254.
Fischer, K., Holt, D.C., Harumal, P., Currie, B.J., Walton, S.F. & Kemp, D.J. (2003b). Generation and characterization of cDNA clones from *Sarcoptes scabiei* var. *hominis* for an expressed sequence tag library: identification of homologues of house dust mite allergens. *The American Journal of Tropical Medicine and Hygiene 68*, 61-64.
Fitzgerald, S.D., Cooley, T.M., Murphy, A., Cosgrove, M.K. & King, B.A. (2004). Sarcoptic mange in raccoons in Michigan. *Journal of Wildlife Diseases 40*, 347-350.
FlyBase: A database of th Drosophila *genome.* http://flybase.bio.indiana.edu (accessed 9-Apr-2005).
Folz, S.D., Kratzer, D.D., Kakuk, T.J. & Rector, D.L. (1984). Evaluation of a sponge-on therapy for canine scabies. *Journal of Veterinary Pharmacology and Therapeutics 7*, 29-34.
Franco, G.R., Adams, M.D., Soares, M.B., Simpson, A.J., Venter, J.C. & Pena, S.D. (1995). Identification of new *Schistosoma mansoni* genes by the EST strategy using a directional cDNA library. *Gene 152*, 141-147.
Funaki, B. & Elpern, D.J. (1987). Scabies epidemiology, Kauai, Hawaii, 1981-1985. *International Journal of Dermatology 26*, 590-592.
Fung, M.C., Lau, M.T. & Chen, X.G. (2002). Expressed sequence tag (EST) analysis of a *Schistosoma japonicum* cercariae cDNA library. *Acta Tropica 82*, 215-224.
GadFly: Genome annotation database of Drosophila. http://flybase.net/annot/ (accessed 9-Apr-2005).
Gish, W. & States, D.J. (1993). Identification of protein coding regions by database similarity search. *Nature Genetics 3*, 266-272.

Glantz, S.A. & Reinhardt, S. (1996). *Primer of biostatistics*. 3rd. McGraw-Hill, New York.

Green, M.S. (1989). Epidemiology of scabies. *Epidemiologic Reviews 11*, 126-150.

Haas, N., Wagemann, B., Hermes, B., Henz, B.M., Heile, C. & Schein, E. (2005). Crossreacting IgG antibodies against fox mite antigens in human scabies. *Archives of Dermatological Research 296*, 327-331.

Hannig, G.a.M., S.C (1998). Strategies for optimizing heterologous protein expression in *Escherichia coli*. *Trends in Biotechnology 16*, 54-60.

Harmsen, M.M., Cornelissen, J.B., Buijs, H.E., Boersma, W.J., Jeurissen, S.H. & van Milligen, F.J. (2004). Identification of a novel *Fasciola hepatica* cathepsin L protease containing protective epitopes within the propeptide. *International Journal for Parasitology 34*, 675-682.

Harumal, P., Morgan, M., Walton, S.F., Holt, D.C., Rode, J., Arlian, L.G., Currie, B.J. & Kemp, D.J. (2003). Identification of a homologue of a house dust mite allergen in a cDNA library from *Sarcoptes scabiei* var. *hominis* and evaluation of its vaccine potential in a rabbit/*S. scabiei* var. *canis* model. *The American Journal of Tropical Medicine and Hygiene 68*, 54-60.

Heilesen, B. (1946). Studies on *Acarus scabiei* and scabies. *Acta Dermato-Venereologica 26*, 1-370.

Heinonen, M., Bornstein, S., Kolhinen, R., Saloniemi, H. & Tuovinen, V. (2000). Eradication of porcine sarcoptic mange within a health declared production model. *Acta Veterinaria Scandinavica 41*, 41-50.

Hirata, H., Yokoyama, N., Xuan, X., Fujisaki, K., Suzuki, N. & Igarashi, I. (2005). Cloning of a Novel *Babesia equi* Gene Encoding a 158-Kilodalton Protein Useful for Serological Diagnosis. *Clinical and Diagnostic Laboratory Immunology 12*, 334-338.

Hollanders, W., Harbers, A.H., Huige, J.C., Monster, P., Rambags, P.G. & Hendrikx, W.M. (1995). Control of *Sarcoptes scabiei* var. *suis* with ivermectin: influence on scratching behaviour of fattening pigs and occurrence of dermatitis at slaughter. *Veterinary Parasitology 58*, 117-127.

Hollanders, W., Vercruysse, J., Raes, S. & Bornstein, S. (1997). Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of sarcoptic mange in swine. *Veterinary Parasitology 69*, 117-123.

Holness, D.L., DeKoven, J.G. & Nethercott, J.R. (1992). Scabies in chronic health care institutions. *Archives of Dermatology 128*, 1257-1260.

Holt, D.C., Fischer, K., Allen, G.E., Wilson, D., Wilson, P., Slade, R., Currie, B.J., Walton, S.F. & Kemp, D.J. (2003). Mechanisms for a novel immune evasion strategy in the scabies mite *Sarcoptes scabiei*: a multigene family of inactivated serine proteases. *The Journal of Investigative Dermatology 121*, 1419-1424.

Holt, D.C., Fischer, K., Pizzutto, S.J., Currie, B.J., Walton, S.F. & Kemp, D.J. (2004). A multigene family of inactivated cysteine proteases in *Sarcoptes scabie*i. *The Journal of Investigative Dermatology 123*, 240-241.

Jacobson, M., Bornstein, S. & Wallgren, P. (1999). The efficacy of simplified eradication strategies against sarcoptic mange mite infections in swine herds monitored by an ELISA. *Veterinary Parasitology 81*, 249-258.

Jensen, J.C., Nielsen, L.H., Arnason, T. & Cracknell, V. (2002). Elimination of mange mites *Sarcoptes scabiei* var. *suis* from two naturally infested Danish sow herds using a single injection regime with doramectin. *Acta Veterinaria Scandinavica 43*, 75-84.

Kapust, R.B. & Waugh, D.S. (1999). *Escherichia coli* maltose-binding protein is uncommonly effective at promoting the solubility of polypeptides to which it is fused. *Protein Science 8*, 1668-1674.

Kemp, D.J., Walton, S.F., Harumal, P. & Currie, B.J. (2002). The scourge of scabies. *Biologist 49*, 19-24.

Kent, N.A., Hill, M.R., Keen, J.N., Holland, P.W. & Hart, B.J. (1992). Molecular characterisation of group I allergen Eur m I from house dust mite *Euroglyphus maynei*. *International Archives of Allergy and Immunology 99*, 150-152.

Kenyon, F., Welsh, M., Parkinson, J., Whitton, C., Blaxter, M.L. & Knox, D.P. (2003). Expressed sequence tag survey of gene expression in the scab mite *Psoroptes ovis*--allergens, proteases and free-radical scavengers. *Parasitology 126*, 451-460.

Kimchi, N., Green, M.S. & Stone, D. (1989). Epidemiologic characteristics of scabies in the Israel Defense Force. *International Journal of Dermatology 28*, 180-182.

Krantz, G.W. (1978). *A manual of acarology*. 2nd edition. Oregon State University Book Stores, Corvallis. 509.

Krause, S., Sommer, A., Fischer, P., Brophy, P.M., Walter, R.D. & Liebau, E. (2001). Gene structure of the extracellular glutathione S-transferase from *Onchocerca volvulus* and its overexpression and promoter analysis in transgenic *Caenorhabditis elegans*. *Molecular and Biochemical Parasitology 117*, 145-154.

Kutzer, E. & Grunberg, W. (1969). Transmission of animal sarcoptic mange to human beings. *Berliner und Munchener Tierarztliche Wochenschrift 82*, 311-314.

Laclette, J.P., Landa, A., Arcos, L., Willms, K., Davis, A.E. & Shoemaker, C.B. (1991). Paramyosin is the *Schistosoma mansoni* (Trematoda) homologue of antigen B from *Taenia solium* (Cestoda). *Molecular and Biochemical Parasitology 44*, 287-295.

Lalli, P.N., Morgan, M.S. & Arlian, L.G. (2004). Skewed Th1/Th2 immune response to *Sarcoptes scabiei*. *The Journal of Parasitology 90*, 711-714.

Landa, A., Laclette, J.P., Nicholson-Weller, A. & Shoemaker, C.B. (1993). cDNA cloning and recombinant expression of collagen-binding and complement inhibitor activity of *Taenia solium* paramyosin (AgB). *Molecular and Biochemical Parasitology 60*, 343-347.

Leon-Vizcaino, L., Ruiz de Ybanez, M.R., Cubero, M.J., Ortiz, J.M., Espinosa, J., Perez, L., Simon, M.A. & Alonso, F. (1999). Sarcoptic mange in Spanish ibex from Spain. *Journal of Wildlife Diseases 35*, 647-659.

Li, L., Brunk, B.P., Kissinger, J.C., Pape, D., Tang, K., Cole, R.H., Martin, J., Wylie, T., Dante, M., Fogarty, S.J., Howe, D.K., Liberator, P., Diaz, C.,

Anderson, J., White, M., Jerome, M.E., Johnson, E.A., Radke, J.A., Stoeckert, C.J., Jr., Waterston, R.H., Clifton, S.W., Roos, D.S. & Sibley, L.D. (2003). Gene discovery in the apicomplexa as revealed by EST sequencing and assembly of a comparative gene database. *Genome Research 13*, 443-454.

Li, L., Crabtree, J., Fischer, S., Pinney, D., Stoeckert, C.J., Jr., Sibley, L.D. & Roos, D.S. (2004). ApiEST-DB: analyzing clustered EST data of the apicomplexan parasites. *Nucleic acids research 32*, D326-328.

Limberger, R.J. & McReynolds, L.A. (1990). Filarial paramyosin: cDNA sequences from *Dirofilaria immitis* and *Onchocerca volvulus*. *Molecular and Biochemical Parasitology 38*, 271-280.

Lizotte-Waniewski, M., Tawe, W., Guiliano, D.B., Lu, W., Liu, J., Williams, S.A. & Lustigman, S. (2000). Identification of potential vaccine and drug target candidates by expressed sequence tag analysis and immunoscreening of *Onchocerca volvulus* larval cDNA libraries. *Infection and Immunity 68*, 3491-3501.

Lowenstein, M., Kahlbacher, H. & Peschke, R. (2004). On the substantial variation in serological responses in pigs to *Sarcoptes scabiei* var. *suis u*sing different commercially available indirect enzyme-linked immunosorbent assays. *Parasitology Research 94*, 24-30.

Lower, K.S., Medleau, L.M., Hnilica, K. & Bigler, B. (2001). Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of sarcoptic mange in dogs. *Veterinary Dermatology 12*, 315-320.

Makrides, S.C. (1996). Strategies for achieving high-level expression of genes in *Escherichia coli*. *Microbiological Reviews 60*, 512-538.

Marchler-Bauer, A., Anderson, J.B., DeWeese-Scott, C., Fedorova, N.D., Geer, L.Y., He, S., Hurwitz, D.I., Jackson, J.D., Jacobs, A.R., Lanczycki, C.J., Liebert, C.A., Liu, C., Madej, T., Marchler, G.H., Mazumder, R., Nikolskaya, A.N., Panchenko, A.R., Rao, B.S., Shoemaker, B.A., Simonyan, V., Song, J.S., Thiessen, P.A., Vasudevan, S., Wang, Y., Yamashita, R.A., Yin, J.J. & Bryant, S.H. (2003). CDD: a curated Entrez database of conserved domain alignments. *Nucleic acids research 31*, 383-387.

Martin, R.W., Handasyde, K.A. & Skerratt, L.F. (1998). Current distribution of sarcoptic mange in wombats. *Australian Veterinary Journal 76*, 411-414.

Mattsson, J.G., Bergström, K. & Näslund, K. (1999). Molecular cloning and expression of a major antigen from *Sarcoptes scabiei*. 17th International Conference of the World Association for the Advancement of Veterinary Parasitology. Denmark, 15-19 August 1999.

McGoldrick, S., O'Sullivan, S.M. & Sheehan, D. (2005). Glutathione transferase-like proteins encoded in genomes of yeasts and fungi: insights into evolution of a multifunctional protein superfamily. *FEMS Microbiology Letters 242*, 1-12.

Meijer, P. & van Voorst Vader, P.C. (1990). Canine scabies in man. *Nederlands Tijdschrift voor Geneeskunde 134*, 2491-2493.

Meinking, T.L., Taplin, D., Hermida, J.L., Pardo, R. & Kerdel, F.A. (1995). The treatment of scabies with ivermectin. *The New England Journal of Medicine 333*, 26-30.
Mellanby, K. (1944). The development of symptoms, parasitic infection and immunity in human scabies. *Parasitology 35*, 197-206.
Mellanby, K. (1972). *Scabies*. 2nd. Classey, Hampton.
Menzano, A., Rambozzi, L. & Rossi, L. (2004). Outbreak of scabies in human beings, acquired from chamois (*Rupicapra rupicapra*). *The Veterinary Record 155*, 568.
Mimouni, D., Ankol, O.E., Davidovitch, N., Gdalevich, M., Zangvil, E. & Grotto, I. (2003). Seasonality trends of scabies in a young adult population: a 20-year follow-up. *The British Journal of Dermatology 149*, 157-159.
Montgomery, J. (1985). The aerobic bacteriology of infected skin lesions in children of the Eastern Highlands Province. *Papua and New Guinea Medical Journal 28*, 93-103.
Moriello, K.A. (2003). Zoonotic skin diseases of dogs and cats. *Animal Health Research Reviews 4*, 157-168.
Morner, T. (1992). Sarcoptic mange in Swedish wildlife. *Revue Scientifique et Technique 11*, 1115-1121.
Morris, D.O. & Dunstan, R.W. (1996). A histomorphological study of sarcoptic acariasis in the dog: 19 cases. *Journal of the American Animal Hospital Association 32*, 119-124.
Morsy, T.A., Kenawi, M.Z., Zohdy, H.A., Abdalla, K.F. & el Fakahany, A.F. (1993). Serum immunoglobulin and complement values in scabietic patients. *Journal of the Egyptian Society of Parasitology 23*, 221-229.
Mueller, R.S., Bettenay, S.V. & Shipstone, M. (2001). Value of the pinnal-pedal reflex in the diagnosis of canine scabies. *The Veterinary Record 148*, 621-623.
Nisbet, A.J. & Billingsley, P.F. (2000). A comparative survey of the hydrolytic enzymes of ectoparasitic and free-living mites. *International Journal for Parasitology 30*, 19-27.
Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H. & Kanehisa, M. (1999). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic acids research 27*, 29-34.
Orkin, M. (1993). Scabies in AIDS. *Seminars in Dermatology 12*, 9-14.
Orkin, M. & Maibach, H.I. (1985). Modern aspects of scabies. *Current Problems in Dermatology 13*, 109-127.
Otero, L., Varela, J.A., Espinosa, E., Sanchez, C., Junquera, M.L., del Valle, A. & Vazquez, F. (2004). *Sarcoptes scabiei* in a sexually transmitted infections unit: a 15-year study. *Sexually Transmitted Diseases 31*, 761-765.
Ouaissi, A., Ouaissi, M. & Sereno, D. (2002). Glutathione S-transferases and related proteins from pathogenic human parasites behave as immunomodulatory factors. *Immunology Letters 81*, 159-164.
Parish, L.C., Witkowski, J.A. & Millikan, L.E. (1991). Scabies in the extended care facility. Revisited. *International Journal of Dermatology 30*, 703-706.
Pence, D.B. & Ueckermann, E. (2002). Sarcoptic manage in wildlife. *Revue Scientifique et Technique 21*, 385-398.

Porcel, B.M., Tran, A.N., Tammi, M., Nyarady, Z., Rydaker, M., Urmenyi, T.P., Rondinelli, E., Pettersson, U., Andersson, B. & Aslund, L. (2000). Gene survey of the pathogenic protozoan *Trypanosoma cruzi*. *Genome Research 10*, 1103-1107.

Prins, C., Stucki, L., French, L., Saurat, J.H. & Braun, R.P. (2004). Dermoscopy for the in vivo detection of *Sarcoptes scabiei*. *Dermatology 208*, 241-243.

Pryor, K.D. & Leiting, B. (1997). High-level expression of soluble protein in *Escherichia* coli using a His6-tag and maltose-binding-protein double-affinity fusion system. *Protein Expression and Purification 10*, 309-319.

Renard, G., Garcia, J.F., Cardoso, F.C., Richter, M.F., Sakanari, J.A., Ozaki, L.S., Termignoni, C. & Masuda, A. (2000). Cloning and functional expression of a *Boophilus microplus* cathepsin L- like enzyme. *Insect Biochemistry and Molecular Biology 30*, 1017-1026.

Renard, G., Lara, F.A., De Cardoso, F.C., Miguens, F.C., Dansa-Petretski, M., Termignoni, C. & Masuda, A. (2002). Expression and immunolocalization of a *Boophilus microplus* cathepsin L- like enzyme. *Insect Molecular Biology 11*, 325-328.

Reunala, T., Ranki, A., Rantanen, T. & Salo, O.P. (1984). Inflammatory cells in skin lesions of scabies. *Clinical and Experimental Dermatology 9*, 70-77.

Roncalli, R.A. (1987). The history of scabies in veterinary and human medicine from biblical to modern times. *Veterinary Parasitology 25*, 193-198.

Ryser-Degiorgis, M.P., Ryser, A., Bacciarini, L.N., Angst, C., Gottstein, B., Janovsky, M. & Breitenmoser, U. (2002). Notoedric and sarcoptic mange in free-ranging lynx from Switzerland. *Journal of Wildlife Diseases 38*, 228-232.

Sajid, M. & McKerrow, J.H. (2002). Cysteine proteases of parasitic organisms. *Molecular and Biochemical Parasitology 120*, 1-21.

Santos, T.M., Johnston, D.A., Azevedo, V., Ridgers, I.L., Martinez, M.F., Marotta, G.B., Santos, R.L., Fonseca, S.J., Ortega, J.M., Rabelo, E.M., Saber, M., Ahmed, H.M., Romeih, M.H., Franco, G.R., Rollinson, D. & Pena, S.D. (1999). Analysis of the gene expression profile of *Schistosoma mansoni* cercariae using the expressed sequence tag approach. *Molecular and Biochemical Parasitology 103*, 79-97.

Scott, D.W. & Horn, R.T., Jr. (1987). Zoonotic dermatoses of dogs and cats. *The Veterinary Clinics of North America. Small Animal Practice 17*, 117-144.

Shakib, F. & Gough, L. (2000). The proteolytic activity of Der p 1 selectively enhances IgE synthesis: a link between allergenicity and cysteine protease activity. *Clinical and Experimental Allergy : Journal of the British Society for Allergy and Clinical Immunology 30*, 751-752.

Shakib, F., Schulz, O. & Sewell, H. (1998). A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. *Immunology Today 19*, 313-316.

Sharp, P.J., Smith, D.R., Bach, W., Wagland, B.M. & Cobon, G.S. (1991). Purified glutathione S-transferases from parasites as candidate protective antigens. *International Journal for Parasitology 21*, 839-846.

Shrank, A.B. & Alexander, S.L. (1967). Scabies: another epidemic? *British Medical Journal 1*, 669-671.

Skerratt, L.F., Campbell, N.J., Murrell, A., Walton, S., Kemp, D. & Barker, S.C. (2002). The mitochondrial 12S gene is a suitable marker of populations of *Sarcoptes scabiei* from wombats, dogs and humans in Australia. *Parasitology Research 88*, 376-379.

Smets, K., Neirynck, W. & Vercruysse, J. (1999). Eradication of sarcoptic mange from a Belgian pig breeding farm with a combination of injectable and in-feed ivermectin. *The Veterinary Record 145*, 721-724.

Smith, E.B. & Claypoole, T.F. (1967). Canine scabies in dogs and in humans. *JAMA : the Journal of the American Medical Association 199*, 59-64.

Sreter, T., Szell, Z. & Varga, I. (2003). Ectoparasite infestations of red foxes (*Vulpes vulpes*) in Hungary. *Veterinary Parasitology 115*, 349-354.

Stemmer, B.L., Arlian, L.G., Morgan, M.S., Rapp, C.M. & Moore, P.F. (1996). Characterization of antigen presenting cells and T-cells in progressing scabietic skin lesions. *Veterinary Parasitology 67*, 247-258.

Taplin, D. & Meinking, T.L. (1990). Pyrethrins and pyrethroids in dermatology. *Archives of Dermatology 126*, 213-221.

Taplin, D. & Meinking, T.L. (1997). Treatment of HIV-related scabies with emphasis on the efficacy of ivermectin. *Seminars in Cutaneous Medicine and Surgery 16*, 235-240.

Taplin, D., Rivera, A., Walker, J.G., Roth, W.I., Reno, D. & Meinking, T. (1983). A comparative trial of three treatment schedules for the eradication of scabies. *Journal of the American Academy of Dermatology 9*, 550-554.

Thappa, D.M. & Karthikeyan, K. (2002). Exaggerated scabies: a marker of HIV infection. *Indian Pediatrics 39*, 875-876.

Thomas, W.R. & Smith, W. (1998). House-dust-mite allergens. *Allergy 53*, 821-832.

Thyresson, N. (1994). [The remarkable debate during the beginning of the nineteenth century concerning the aetiology of scabies]. *Sydsven Medicinhist Sallsk Arsskr 31*, 79-90.

Wakhlu, I., Thakur, S., Govil, Y.C. & Rastogi, A.K. (1988). Clinical profile of scabies in north Indian children. *Indian Pediatrics 25*, 1050-1053.

Valks, R., Buezo, G.F. & Dauden, E. (1996). Scabies and leukocytoclastic vasculitis in an HIV-seropositive man. *International Journal of Dermatology 35*, 605-606.

Wallgren, P. & Bornstein, S. (1997). The spread of porcine sarcoptic mange during the fattening period revealed by development of antibodies to Sarcoptes scabiei. *Veterinary Parasitology 73*, 315-324.

Walton, S.F., Choy, J.L., Bonson, A., Valle, A., McBroom, J., Taplin, D., Arlian, L., Mathews, J.D., Currie, B. & Kemp, D.J. (1999). Genetically distinct dog-derived and human-derived *Sarcoptes scabiei* in scabies-endemic communities in northern Australia. *The American Journal of Tropical Medicine and Hygiene 61*, 542-547.

Walton, S.F., Currie, B.J. & Kemp, D.J. (1997). A DNA fingerprinting system for the ectoparasite *Sarcoptes scabiei*. *Molecular and Biochemical Parasitology 85*, 187-196.

Walton, S.F., Dougall, A., Pizzutto, S., Holt, D., Taplin, D., Arlian, L.G., Morgan, M., Currie, B.J. & Kemp, D.J. (2004a). Genetic epidemiology of

Sarcoptes scabiei (Acari: Sarcoptidae) in northern Australia. *International Journal for Parasitology 34*, 839-849.

Walton, S.F., Holt, D.C., Currie, B.J. & Kemp, D.J. (2004b). Scabies: new future for a neglected disease. *Advances in Parasitology 57*, 309-376.

Walton, S.F., Myerscough, M.R. & Currie, B.J. (2000). Studies in vitro on the relative efficacy of current acaricides for *Sarcoptes scabiei* var. *hominis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene 94*, 92-96.

Wan, K.L., Chong, S.P., Ng, S.T., Shirley, M.W., Tomley, F.M. & Jangi, M.S. (1999). A survey of genes in *Eimeria tenella* merozoites by EST sequencing. *International Journal for Parasitology 29*, 1885-1892.

Van Neste, D. (1981). Immunologic studies in scabies. *International Journal of Dermatology 20*, 264-269.

Van Neste, D.J. & Staquet, M.J. (1986). Similar epidermal changes in hyperkeratotic scabies of humans and pigs. *American Journal of Dermatopathology 8*, 267-273.

Whitton, C., Daub, J., Thompson, M. & Blaxter, M. (2004). Expressed sequence tags: medium-throughput protocols. *Methods in Molecular Biology 270*, 75-92.

Vieille, C. & Zeikus, G.J. (2001). Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. *Microbiology and molecular biology reviews 65*, 1-43.

Woodley, D. & Saurat, J.H. (1981). The Burrow Ink Test and the scabies mite. *Journal of the American Academy of Dermatology 4*, 715-722.

Yang, H.L., Zeng, Q.Y., Nie, L.J., Zhu, S.G. & Zhou, X.W. (2003). Purification and characterization of a novel glutathione S-transferase from *Atactodea striata*. *Biochemical and Biophysical Research Communications. 307*, 626-631.

Yasuhara, T., Takai, T., Yuuki, T., Okudaira, H. & Okumura, Y. (2001). Biologically active recombinant forms of a major house dust mite group 1 allergen Der f 1 with full activities of both cysteine protease and IgE binding. *Clinical and Experimental Allergy 31*, 116-124.

Zahler, M., Essig, A., Gothe, R. & Rinder, H. (1999). Molecular analyses suggest monospecificity of the genus Sarcoptes (Acari: Sarcoptidae). *International Journal for Parasitology 29*, 759-766.

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