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Morphological and molecular comparison of host-derived populations of parasitic *Psoroptes* mites

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Abstract. Infestation by parasitic *Psoroptes* mites (Acari: Psoroptidae) is an important cause of economic loss and welfare problems in livestock in many areas of the world. At least five species within this genus have been recognized, based on the host infested, the infestation site and differences in length of the opisthosomal setae of adult male mites. Here the integrity of these species is considered by subjecting populations of mites from a range of host species and geographical locations to simultaneous morphological and molecular genetic analyses. Morphological analysis showed that there were significant differences in shape and size between mite populations from different hosts, and that length of the outer opisthosomal setae in males and the homologous seta in females were the most important distinguishing character in adults. However, considerable variation in outer opisthosomal seta length was evident within and between populations of mites, and differences were not clearly related to host-species or geographical origin and did not support the accepted species differences. Molecular characterization using sequence data from the mitochondrial second internal transcribed spacer (ITS-2) region and microsatellite markers found little or no consistent host-related variation between the mite population samples. The results suggest that there is no case for considering the *Psoroptes* mites from the different hosts examined as separate species and that the morphological variation observed therefore may represent phenotypic adaptation to the local microenvironment on particular species of host.

Key words. *Psoroptes*, mange, microsatellites, mites, morphology, second internal transcribed spacer region, species integrity.

Introduction

Psoroptes mites are superficial skin parasites of a range of mammalian hosts. Mites are believed to feed on a lipid emulsion of skin cells, bacteria and lymph, produced on the host skin as a result of a hypersensitivity reaction to the presence of antigenic mite faecal material (Blake *et al*., 1978; Sinclair & Kirkwood, 1983; van den Broek *et al*., 2003) Infestation may be chronic or even subclinical and localized, often in the ear of the host, or it may be acute and more generalized over the entire body, when it is described as psoroptic mange (Bates, 1999). Psoroptic mange is an important disease of livestock, particularly sheep, worldwide (van den Broek & Huntley, 2003; Colebrook & Wall, 2004), resulting in severe irritation to the host, making it restless and scratch at the infested areas (Corke & Broom, 1999; Berriatua *et al*., 2001; Bisdorff *et al*., 2006). This self-trauma results in hair loss, skin damage and weight loss and, if left untreated, can lead to death due to dehydration, pneumonia or bacterial septicaemia (Roberts *et al*., 1971; Tarry, 1974).

The genus *Psoroptes* is distinguished by the presence of a terminal sucker on a relatively long, jointed pre-tarsus (Hirst, 1922; Babcock & Black, 1933; Sweatman, 1958; Sanders *et al*., 2000). Following the initial description by Hering (1838), up to nine species of *Psoroptes* were proposed, each distinguished from the others mainly by the different mammalian hosts they infested, with varietal names such as *ovis* or *bovis* appended, depending on the

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host species from which they were obtained (Stockman & Berry, 1913; Shilston, 1915). In 1958, Sweatman carried out a detailed study in an attempt to find a stable morphological character which could be used to separate putative species of the genus *Psoroptes* (Sweatman 1958). He proposed that the outer opisthosomal setae of adult males could be used to distinguish five true species. These included *Psoroptes cuniculi* and *P. cervinus* from the ears of rabbits and bighorn sheep, respectively, and *P. equi*, *P. ovis* and *P. natalensis* which are described as primarily body mites of horses, sheep/cattle and buffalo, respectively.

This classification has achieved general acceptance in the literature (Strong & Halliday, 1992). However, use of this character has proved difficult. A high degree of variance in setae lengths within populations from any individual host and populations from different hosts have been reported (Lange *et al*., 1980; Wright *et al*., 1984; Bates & Sayers, 2002). The concept of host-specific species of *Psoroptes* has also been challenged by cross-host transmission studies, which have successfully transferred mites between rabbits, goats, sheep and cattle (Meleney, 1967; Wright, 1982). Successful cross-mating of *P. ovis* and *P. cuniculi* in the ears of rabbits to produce viable offspring has been claimed (Wright *et al*., 1983); however, to date this latter study has not been successfully repeated or confirmed.

Boyce & Brown (1991) found only minor antigenic differences between mites from cattle, rabbits and bighorn sheep, and found mites from mule deer to be antigenically identical to those from bighorn sheep. Host transfer experiments of mites between rabbits and sheep have also shown that these mites are antigenically cross-reactive (Siegfried *et al*., 2004). A number of molecular studies have generated sequence data for the second internal transcribed spacer region (ITS-2) of the ribosomal DNA. This rDNA sequence lies between the regions coding for the 5.8S and 28S ribosomal subunits and has been extensively used in species status studies in related groups of mites and ticks (Zahler *et al*., 1995; Essig *et al*., 1999; Lohse *et al*., 2002). However, using this sequence, only low levels of variation in samples of *Psoroptes* from a variety of host species have been reported, with as little as a single base difference observed (Zahler *et al*., 1998; Ochs *et al*., 1999). Interestingly, however, opposite conclusions have been drawn from this similar pattern of variation, with one group believing that the presence of any variation supports separate species status (Ochs *et al*., 1999), whereas the other group concluded that the variation was insufficient to separate putative species (Zahler *et al*., 1998). A study of the first internal transcribed spacer (ITS-1) in *Psoroptes* mites from the United States found similarly minimal levels of variation between samples (Ramey *et al*., 2000), which again bore no relationship to putative mite species.

The question of whether the genus *Psoroptes* is composed of one or several host-specific species has important practical epidemiological implications for the potential for cross-transmission and control of this disease. The aim of the work presented here therefore was to undertake a detailed re-examination of the morphology of *Psoroptes* mites derived from a range of hosts and to complement this with a simultaneous high resolution molecular analysis of specimens from the same samples, using a combination of ITS-2 sequence analysis and *Psoroptes*-specific microsatellite markers.

Materials and methods

Morphological analysis

Samples of *Psoroptes* mites were collected, or were provided, from various host species and from various geographical locations worldwide (Table 1). Adult mites were cleared in Nesbitt's fluid (chloral hydrate 40 g, distilled water 25 mL, HCl conc. 2.5 mL) for approximately 3 days before mounting, dorsal side uppermost, in Hoyer's medium (chloral hydrate 200 g, distilled water 50 mL, gum arabic 30 g, glycerine 20 mL). The preparation was then dried at approximately 25°C for 3 days and the edge of the glass coverslip sealed with clear nail varnish.

Specimens were examined under a binocular light microscope and digital photographs and measurement of morphological features were captured by computer (Qwin, Leica Imaging Systems Ltd, Cambridge, U.K.). Five morphological features in males and six in females were found in preliminary assessments to be informative and not highly correlated with each other. For males these were: outer opisthosomal seta length, width of body at base of leg III, gnathosoma length, length of ambulacrum of leg I and propodosomal seta length. In females these were: width of body at base of leg III, gnathosoma length, length of ambulacrum of leg I, propodosomal seta length (pair below vulva), leg III posterior seta length and outer opisthosomal seta length. The outer opisthosomal setae in female mites were the outermost of the four setae found at the posterior of the ventral opisthosoma and were considered to be homologous to those found in adult males. Although no significant differences between left and right-hand measurements of paired characters were detected, both sides were nevertheless measured and the mean value used for analysis.

Discriminant analysis (SPSS 12.0, SPSS Inc. Chicago, U.S.A.), with host species as the grouping variable, was used to determine linear combinations of morphological characters that could be used to distinguish between mites collected from the various host species. This analysis was carried out for both adult male and female mites, first by pooling samples from the same species of host and then separately for samples from each individual host. For characters identified by the discriminant analysis as most highly informative, normality was confirmed using a 1-sample K-S-test and homogeneity of variances by Levene's test. Differences between character lengths in the samples obtained from different hosts were then considered using one-way ANOVA and Tukey HSD multiple range tests.

Table 1. Host, origin and, where known, site on the host from where the sample was obtained. The table also indicates which samples were included in the molecular and morphological analyses and, when included in the morphological analysis, in parenthesis the number of male and female mites examined (+, included; –, not included). ITS2, second internal transcribed spacer.

			Molecular analysis		Morphological analysis	
Host species	Origin	Site	ITS2	Microsatellites	No. males	No. females
Domestic rabbit	Bristol, U.K. (University of Bristol isolate)	Ear	+	+	+ (48)	+ (45)
	Bristol U.K. (Veterinary surgery)	Ear	+	+	+ (9)	+ (4)
	Argentina	Ear	+	+	–	–
Sheep	Yorkshire, U.K.	Body	+	+	+ (9)	+ (10)
	Scotland, U.K.	Body	+	+	+ (3)	+ (5)
	Gloucestershire, U.K.	Body	+	+	+ (2)	–
	Scotland, U.K.	Body	+	+	+ (6)	–
	Lancashire, U.K.	Body	+	+	+ (6)	+ (3)
	Wales, U.K.	Body	+	+	–	+ (12)
	Lancashire × Cornwall, U.K. (VLA Strain)	Body	–	+	+ (3)	+ (10)
	York, U.K.	Body	+	+	–	+ (5)
	Devon, U.K.	Body	+	+	–	+ (1)
	Cornwall, U.K.	Body	+	+	+ (4)	+ (3)
	Cumbria, U.K.	Body	+	+	–	+ (1)
	Scotland, U.K.	Body	–	+	+ (1)	+ (3)
	Yorkshire, U.K.	Body	–	+	+ (2)	+ (1)
	Dublin, Ireland (1988 strain – isolated since 1986)	Body	+	+	+ (1)	+ (1)
	Chester, U.K.	Body	–	–	+ (1)	+ (6)
	Wales, U.K.	Body	–	–	–	+ (5)
	Powys, Wales, U.K.	Body	–	–	+ (1)	+ (4)
	Gwynedd, Wales, U.K.	Body	–	–	+ (1)	+ (3)
	Powys, Wales, U.K.	Body	–	–	–	+ (1)
	Wales, U.K.	Body	–	–	+ (1)	+ (6)
	SW England, U.K.	Body	–	–	+ (3)	+ (4)
	Bloemfontein, South Africa	Body	–	–	+ (8)	+ (18)
	Northumberland, U.K.	Body	+	+	–	–
	Argentina	Body	–	+	–	–
Alpaca	Chile, South America	Ear	+	+	+ (1)	+ (1)
	Animal imported into U.K. from Chile; source of infestation unknown.	Ear	–	+	+ (8)	+ (7)
	Chile, South America	Ear	+	+	–	–
Goat	Bristol, U.K. (VLA)	Ear	–	–	+ (2)	+ (1)
	Georgia, U.S.A.	Not known	–	+	+ (6)	–
Cow	Texas, U.S.A.	Not known	–	+	+ (14)	+ (18)
	Belgium	Body	–	–	+ (7)	+ (29)
Big horn sheep	New Mexico, U.S.A.	Not known	–	+	+ (7)	+ (6)
	New Mexico, U.S.A.	Not known	–	+	+ (5)	+ (8)
	Utah, U.S.A.	Not known	–	+	+ (1)	–
	North America	Not known	–	+	–	–
Mule deer	New Mexico, U.S.A.	Not known	+	+	+ (6)	+ (6)
	New Mexico, U.S.A.	Not known	+	+	–	–
	New Mexico, U.S.A.	Not known	–	+	–	–
	New Mexico, U.S.A.	Not known	–	+	–	–
Elk	Idaho, U.S.A.	Not known	–	+	+ (6)	+ (1)
White tailed deer	U.S.A.	Not known	–	+	+ (5)	+ (5)

Second internal transcribed spacer region fragment amplification and sequencing

Prior to analysis, mite samples were stored in 100% ethanol at 5°C. DNA was extracted from individual mites by homogenizing the whole body in 40 μL of 50 mM NaOH in individual 1.5-mL microfuge tubes using a domestic power drill attached to a 'Pellet Pestle' (Kimble Kontes Inc, New Jersey, U.S.A.). The homogenate was incubated at 95°C for 15 min before neutralizing with 6 μL of 1 M Tris-HCl (pH 8.0). The identity of mite samples used for these molecular analyses is detailed in Table 1. Two pairs of primers were used amplifying different length sections of the ITS-2 region, Rib-4 and Rib-3 (Zahler *et al*., 1998) and Huo-3 and Huo-4 (Ochs *et al*., 1999).

Polymerase chain reaction (PCR) was carried out in a Thermo Hybaid Express™ thermal cycler in 15-μL volumes containing 0.75 μL of whole *Psoroptes* mite homogenate, 500 nM of each primer, 0.25 U of AGS*Gold*™ DNA polymerase (Hybaid, Ashford, U.K.) in the manufacturer's buffer [75 mM Tris/HCl (pH 9.0), 20 mM $(NH4)_2SO_4$, 0.01% Tween-20], with 200 μM of each dNTP and 1.6 mM $MgCl_2$. The reaction profile was 94°C for 3 min, then 35 cycles of 94°C for 30 s, T_a°C for 60 s and 72°C for 60 s, and finally 72°C for 5 min to allow extension to complete. T_a°C was the tested optimal temperature for each pair of primers and found to be 59°C for Rib-3 and Rib-4 and 64°C for Huo-3 and Huo-4. Following some difficulty in amplifying directly from homogenized mite extract, bovine serum albumin (fraction V) and Tween 20 were also added to the PCR at a final concentration of 1 mg/mL and 1%, respectively. The primer pair that produced the longest sequence read, Rib-3 and Rib-4, was used to generate sequence for as many samples as possible; however, some samples proved difficult to sequence and hence a smaller product was generated using the Huo-3 and Huo-4 primers. It was possible to achieve a shorter but stronger amplification with this arrangement so that these difficult samples could be included, albeit with a shorter sequence.

PCR products were purified from agarose gel using the MinElute™. Gel Extraction Kit (Qiagen, Crawley, U.K.), and sequenced (Lark Technologies Inc, Essex, U.K.) in both the 5′ and 3′ direction allowing the production of consensus sequences.

These consensus sequences were put through a BLASTN search (Altschul *et al.*, 1997) to check their identity against other sequences registered in the database. Two published ITS-2 sequences from *P. ovis* (EMBL accession numbers AF123079 and AF123080, Ochs *et al.* 1999) were included as positive controls to confirm the status of the new sequences as genuine *Psoroptes* ITS-2 sequence, and one from *Otodectes cynotis* (EMBL accession number AF367703) was included as an outgroup.

Sequences were then aligned using ClustalX (Thompson *et al.*, 1997), prior to performing maximum parsimony analysis using PAUP* 4.0b10 (Swofford, 2003). Parsimony analysis of the 26 mite ITS-2 sequences (Table 1) was performed with 100 bootstrap replicates. The default options of PAUP were used: heuristic search strategy, TBR (tree bisection-reconnection) branch swapping, zero length branches collapsed and 10 random addition sequences (bootstrap analyses used simple addition).

Microsatellite analysis

The same selection of *P. ovis* samples were PCR amplified with a suite of nine species-specific microsatellite markers (Evans *et al.*, 2003). The individuals genotyped in this way are detailed in Table 1. Amplification was carried out under the same conditions as the ITS-2 reactions using the same mite homogenates. PCR products were visualized on 6% polyacrylamide gels stained with silver (Promega, Southampton, U.K., Bassam *et al.* 1991). Allele sizes were identified by comparison to a 10 bp DNA ladder, 25 bp DNA ladder (both Invitrogen, Paisley, U.K.) and ϕX174 RF DNA/*Hae*III DNA marker (Abgene, Epsom, U.K.). The product sizes produced by this amplification were treated as independent characters and, as previously, were subjected to bootstrapped maximum parsimony analysis using PAUP*4.0b10 (Swofford, 2003).

Results

Morphology: adult males

Discriminant analysis, with host species as the grouping variable, identified five significant linear functions. Function 1, with an eigenvalue of 4.03, was most highly correlated with outer opisthosomal seta length with a correlation coefficient of 0.95. Function 1 alone explained 74.3% of the total variance and the first three functions combined explained 96.2% of the variance. Functions 2 and 3 were most highly correlated with body width and gnathosoma length and explained 12% and 9.9% of the variance, respectively. A plot of function 1 against 2 allows mites to be distinguished morphologically by host (Fig. 1). As outer opisthosomal length was found to be the main discriminatory feature, only this character will be considered in further analysis.

The difference between the outer opisthosomal seta lengths of adult male mites from different host species was highly significant ($F_{8,168} = 76.5$, $P < 0.001$), with the setae of mites from cattle being the longest and those from rabbits the shortest (Fig. 2). Very little variation was evident between the outer opisthosomal seta lengths of mites from elk, bighorn sheep, alpaca, mule deer, white-tailed deer, sheep and goat. The outer opisthosomal seta lengths of mites from rabbits were significantly shorter than the mites of all hosts other than goats. The outer opisthosomal seta lengths of mites from cattle were significantly longer than those of mites from all other host species.

When the outer opisthosomal seta lengths were compared for individual samples, a relatively large amount of variation between mites from the same host species was seen, making it difficult to assign mites to a particular host species based solely on their seta lengths (Fig. 3). For example, there was a significant difference between the two cattle isolates from U.S.A. and Belgium ($t_{19} = -4.23$, $P < 0.001$) and between the two rabbit isolates, both from Bristol ($t_{55} = 5.97$, $P < 0.001$). There was also a significant difference between outer opisthosomal seta lengths of males mites from different sheep isolates ($F_{15,36} = 3.10$, $P = 0.003$).

Morphology: adult females

Discriminant analysis, using host species as the grouping variable (excluding goat and elk samples, each of

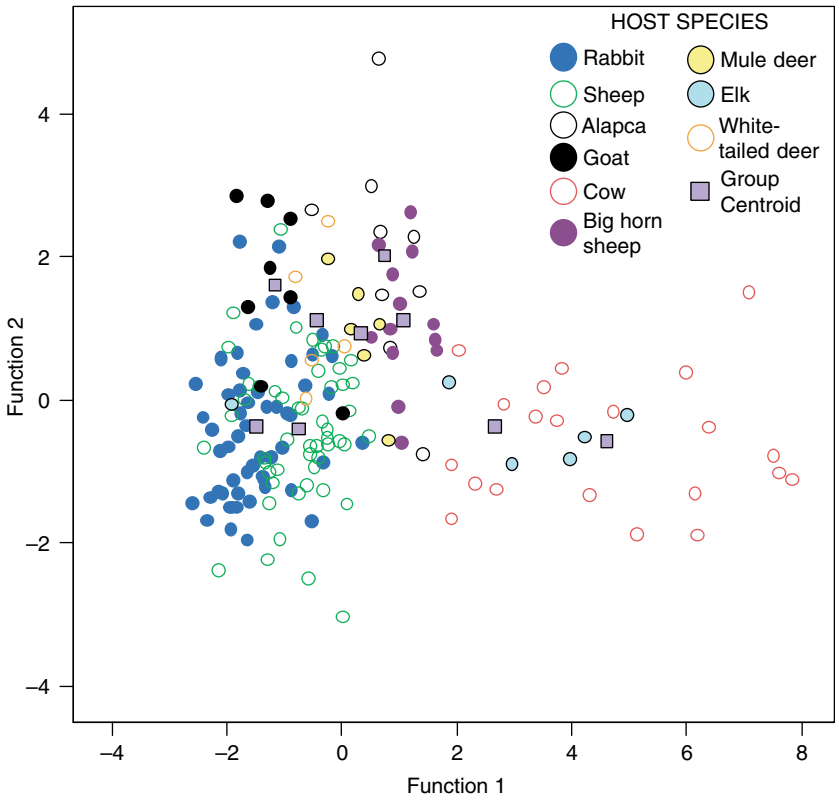

Fig. 1. Two linear functions of five morphological characters measured on 177 adult male mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opisthosomal seta length and body width, respectively.

which included only one female mite), identified four significant functions. Function 1 had an eigenvalue of 2.04 and, as for males, was most highly correlated with outer opisthosomal seta length with a correlation of 0.82 ($r^2 = 76.6\%$). Functions 2 and 3 were most highly correlated with gnathosoma length and leg 1 ambulacrum

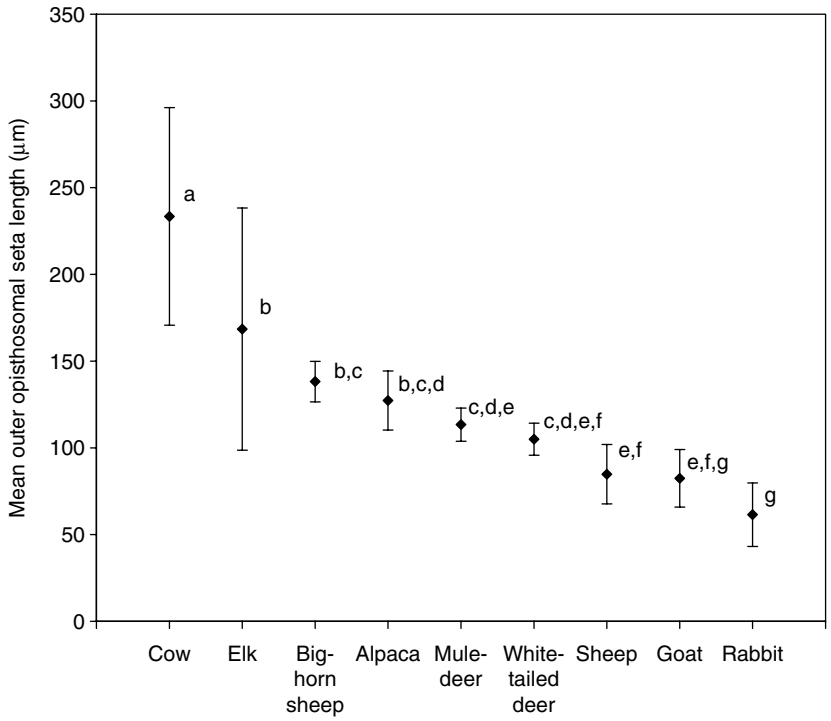

Fig. 2. Mean outer opisthosomal seta length (µm ± SD) of adult male *Psoroptes* mites collected from a range of host species. Letters indicate points between which there is no statistically significant difference ($F_{8,168} = 76.46$, $P < 0.001$).

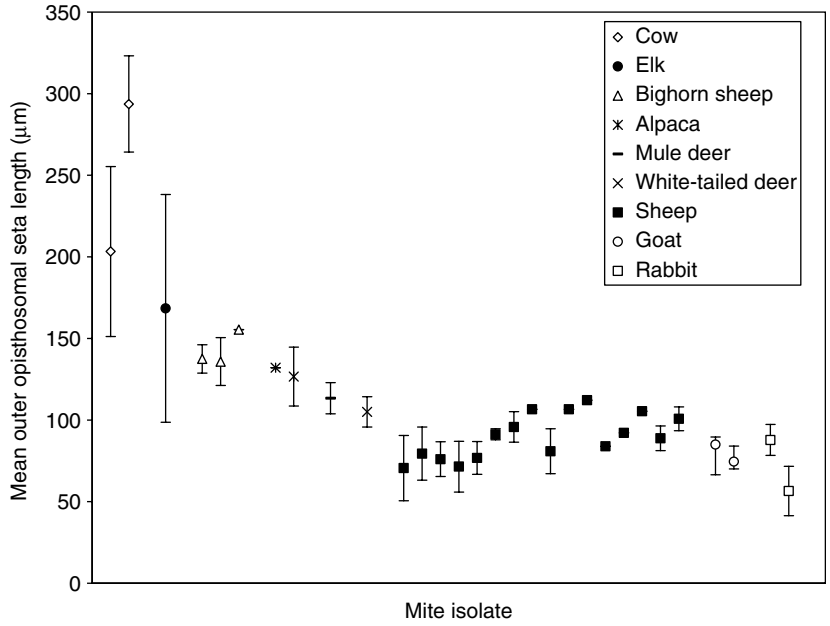

Fig. 3. Mean outer opisthosomal seta length (µm ± SD) of adult male *Psoroptes* mites of all isolates examined.

length, respectively, and functions 1 to 3 together accounted for 95.0% of the total variance. When functions 1 and 2 were plotted against each other, some separation of mites from different host species into groups was seen though there is considerable variation (Fig. 4). As outer opisthosomal length was found to be the main discriminatory feature, only this character will be considered in further analysis.

Comparison of the adult female outer opisthosomal seta lengths by host species showed that there is a significant difference ($F_{6,224} = 50.9$, $P < 0.001$). Outer opisthosomal setae were again longest in mites from cattle and shortest in mites from rabbits (Fig. 5). However there was no difference between the outer opisthosomal seta lengths of mites from alpaca, white-tailed deer, sheep, big-horn sheep or mule deer. There was no significant difference between

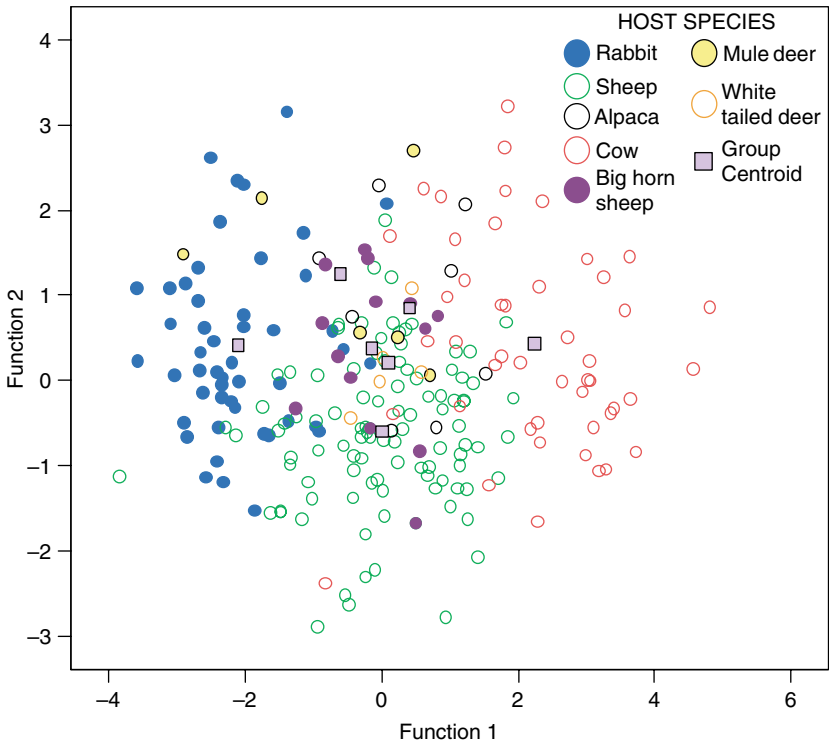

Fig. 4. Two linear functions of six morphological characters measured on 231 adult female mites as revealed by discriminant analysis. Functions 1 and 2 are most highly correlated with outer opsithosomal seta length and gnathosoma length, respectively.

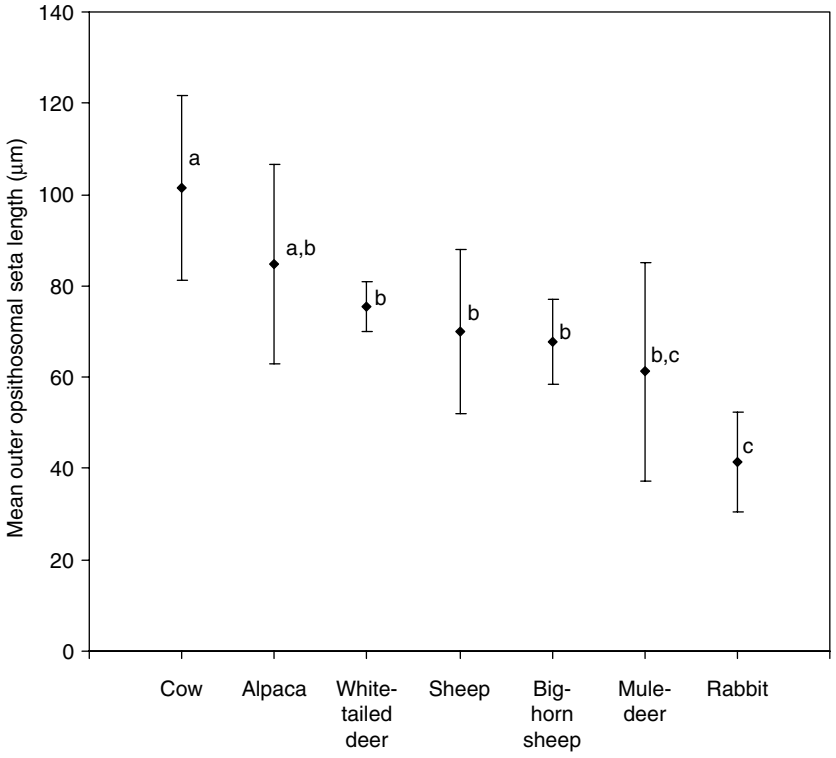

Fig. 5. Mean outer opisthosomal seta length (μm ± SD) of adult female mites collected from a range of host species. Letters indicate points between which there is no statistically significant difference ($F_{6,224} = 50.93$, $P < 0.001$).

mites from rabbit and mule deer and no significant difference between mites from cattle or alpaca. When the outer opisthosomal seta lengths were considered for all individual mite samples, again a large amount of variation between mites from the same host species was evident (Fig. 6). As was the case for the male mites, there was a significant difference in seta length between the cattle isolates from Belgium and the U.S.A. ($t_{45} = -2.502$, $P = 0.016$). There was a significant difference between outer opisthosomal seta length from different sheep isolates ($F_{14,82} = 2.41$,

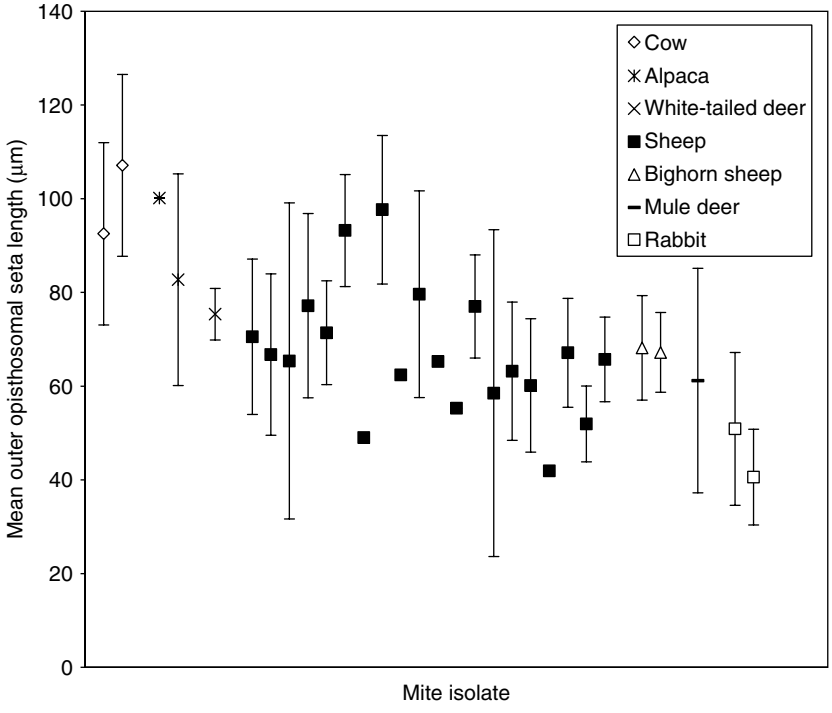

Fig. 6. Mean outer opisthosomal seta length (μm ± SD) of adult female *Psoroptes* mites of all isolates examined.

$P = 0.007$). However, there were no significant differences between isolates from the other host species.

Second internal transcribed spacer region sequencing analysis

Full sequences of the ITS-2 fragment were achieved for 26 of the 41 samples for which amplification was attempted. The remaining 15 samples either failed to amplify with either set of ITS-2 primers, or the amplification was too weak to enable sequencing to be carried out. All 26 *Psoroptes* ITS-2 sequences were aligned with the three published sequences. The ITS sequences amplified were between 325 bp (HUO primers) and 425 bp (RIB primers) long, with a total of 16 polymorphic sites (= 3.75–4.9% polymorphism).

A parsimony-based phylogram was then generated using available mite samples from ITS-2 sequencing data (Fig. 7). Only three pairs of samples clustered separately from the others. These were mites originating from infected sheep in

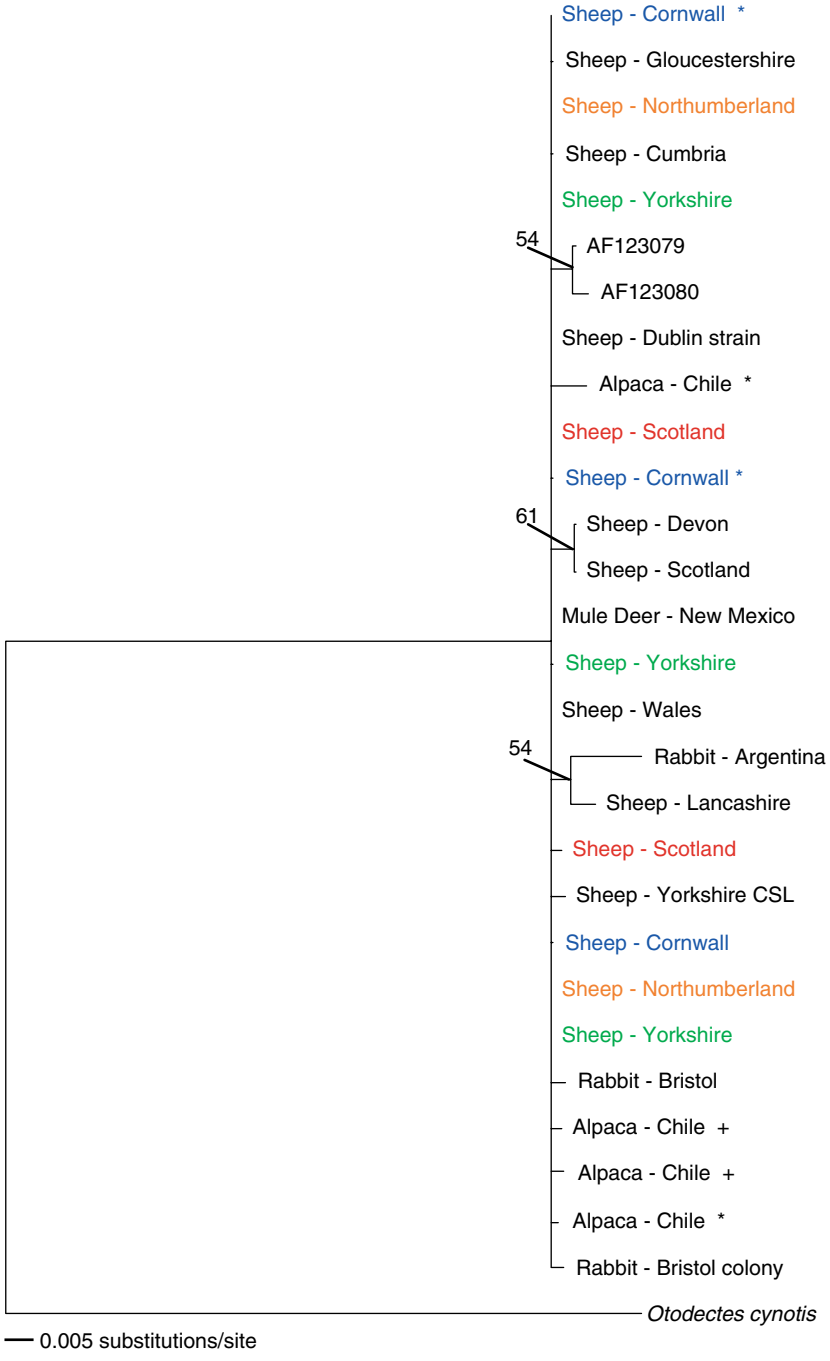

Fig. 7. Phylogram of second internal transcribed spacer (ITS-2) sequences from *Psoroptes* samples, rooted to *Otodectes cynotis*. Bootstrapped consensus tree, bootstrap values > 50% shown on supported branches. Samples from animals within a single herd or wildlife reserve are highlighted with a matching colour. Sequences from the same mite using different primers are marked with matching symbols. AF123079 and AF123080 are published sequences included for sequence identification purposes.

Scotland and Devon, mites from an infected sheep in Lancashire and a rabbit in Argentina, and the two previously published *P. ovis* sequences downloaded from the EMBL database. The sequence alignment is available from JRS (j.r.stevens@ex.ac.uk).

Microsatellite analysis

Amplification was achieved for all 41 samples for seven of the markers, *Psor02, Psor04, Psor05*, *Psor07*, *Psor11, Psor14* and *Psor16*. The two remaining markers, *Psor01* and *Psor13*, failed to amplify most of the new world samples. The phylogram generated from these data us shown in Fig. 8. The phylogram shows several consistent clusters of samples. Samples from two flocks, one in Northumberland and one in Cornwall, group together, and some of the samples from the San Andres Wildlife Refuge, New Mexico group together. Similarly, the two samples from Chilean alpacas cluster together. Two other groupings also remain consistent after bootstrapping; two sheep samples, from South Uist and the Dublin laboratory strain, and a group of sheep samples from a variety of British flocks.

Discussion

The exoskeleton of astigmatid *Psoroptes* mites is largely unsclerotized and, as a result, one potential problem in studies of morphology is the effect of fixation and mounting media on the mites. The effects of fixation on 13 morphological characters of *Psoroptes* mites was considered by Reese *et al.* (1996). Fixation in alcohol was found to result in significant reductions in body size measurements, size decreasing with increasing time of fixation, but the concentration of alcohol had no effect. Mounting in Hoyer's medium had the opposite effect on body size. Changes in

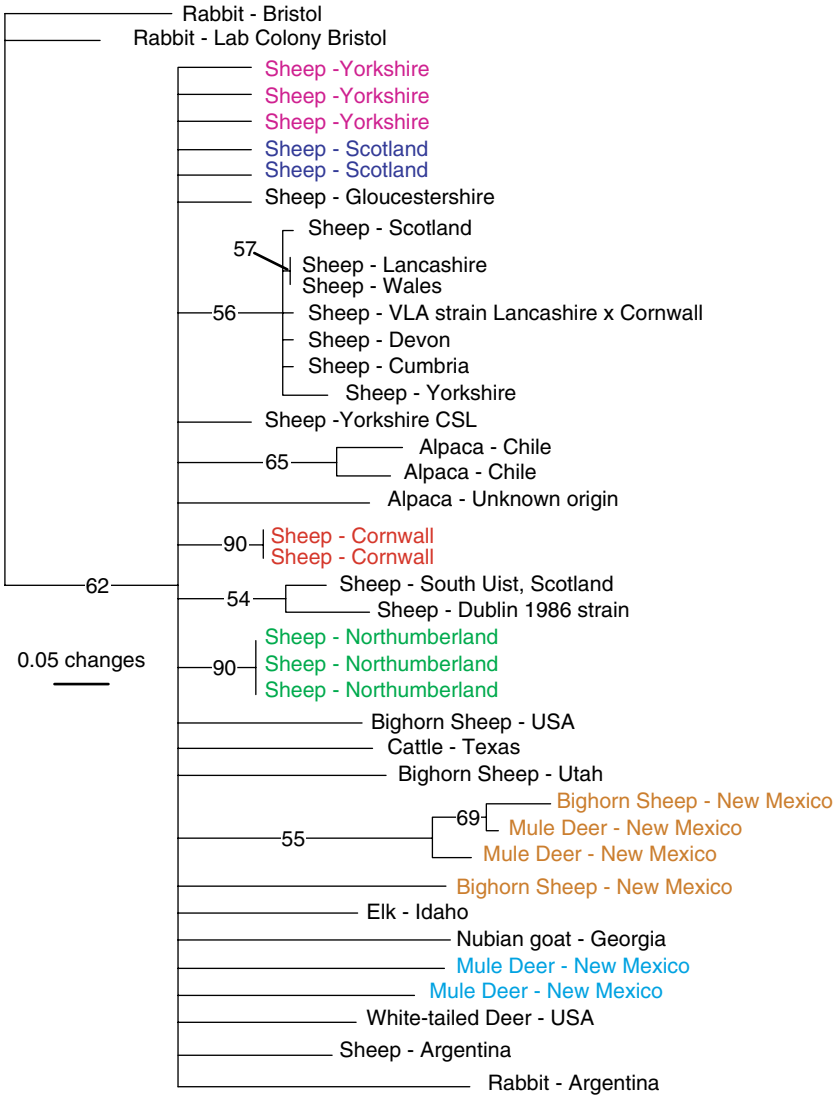

Fig. 8. Phylogram of *Psoroptes ovis* samples created from microsatellite data. Bootstrapped consensus tree, bootstrap values $>50\%$ are shown on supported branches. Samples from animals within a single herd or wildlife reserve are marked with a matching symbol.

measurements as a result of fixation were most apparent in characters that were determined the most by internal tissues, such as body width or length. However, in characters including outer opisthosomal seta length, there was no effect of fixation. Consistent methodology was advocated in morphometric studies by Reese *et al*. (1996) and this was adopted in the present investigation.

The results of this study show that both male and female adult *Psoroptes* mite populations from some different host species can be distinguished by their morphological characters, with outer opisthosomal seta length being the most important character, as suggested by Sweatman (1958). However, when mites are examined from a wide range of host species, considerable variation in character measurements is apparent. In this study, mites from elk and cattle, which would be identified as *P. cervinus* and *P. ovis*, respectively, using Sweatman's key, here appear almost identical in terms of outer opisthosomal seta length. Outer opisthosomal seta lengths of body mites from cattle and sheep were found to be significantly different from each other in both male and female mites, although according to Sweatman's key they should both be described as *P. ovis*. However, significant differences were also found between mites from the same host species, but this was not necessarily a result of differing geographical locations. Differences were seen in male and female mites isolated from cattle from the U.S.A. and Belgium, and also in male mites from two rabbit samples, both from Bristol, U.K. Few morphological differences were found between adult male mites from sheep from all over the U.K. and Ireland but differences were found in the female mites, although this did not appear to correspond to host location.

From these measurements, although there appears to be some broad underlying relationship between morphology and host species, clearly these relationships are weak and for most hosts, interspecific differences are not greater than the morphological variation seen between samples from the same host species, supporting the conclusions of Boyce *et al*. (1990). As a result, it would seem most plausible that the character measurements recorded here are not fixed, but are phenotypically plastic, representing the conditions under which mites have developed and related perhaps to the location on the host, temperature, the age of the infection and possibly the immune status of the host, as suggested by Bates (1999). Host-related phenotypic differences are well known in parasitic mites. For example, the mites *Unionicola poundsi* and *U. lasellai*, which were originally identified as a single species, are found on different freshwater mussel hosts, and adult mites are separated by the shape of particular tarsal claws and setae. Host transfer experiments showed that these morphological characters are dependent on the host species on which the mites moult from the nymphal to adult stages (Downes, 1990). Phenotypically host-adapted mite populations have also been described classically in *Sarcoptes* (Fain, 1994).

The molecular analysis looked initially at the ITS-2 spacer region. This region appears well suited to the phylogenetic study of species groups (McLain *et al*., 1995; Fukunaga *et al*., 2000), as it is a non-coding transcribed portion of DNA and hence has been shown to be evolving at a higher rate than the coding regions of the genes on either side (Cruickshank, 2002). Additionally, its location between two highly conserved genes results in a high level of cross-species utility of the primers designed to amplify the region. The ITS-2 sequencing phylogram (Fig. 7) shows very little structure within the Psoroptidae mites; only three pairs of samples group together and of these, bootstrap support is at best only weak (54–61%). These were mites originating from infected sheep in Scotland and Devon, mites from an infected sheep in Lancashire and a rabbit in Argentina, and the two previously published *P. ovis* samples from the EMBL database. The last two samples originate from a sheep host, but information about their geographical location was unavailable. The reasons for the other consistent groupings are unclear. Given that other samples in the collection came from the same host species and similar geographical locations, there is no obvious pattern to the groupings seen in the phylogram. As alluded to previously (Zahler *et al*., 1998; Ramey *et al*., 2000), the paralogous nature of this multicopy rRNA gene could possibly account for the lack of meaningful patterns observed within *Psoroptes* ITS-2 data, but, in common with these previous authors, lack of any correlation of sequence-based clades with host or parasite geographical origins do not appear to offer any support for cryptic species within these *Psoroptes* specimens. Overall, this lack of apparently meaningful variation suggests that this kind of sequence data, although excellent for highlighting differences and relationships between genera, is not ideal for investigating the potential differences within genera, or for epidemiological tracking of mite populations.

The microsatellite analysis shows a greater degree of resolution between samples. Several groupings are supported by bootstrap analysis, most grouping individuals from the same host population. Similarly, mites from diverse host animals within a shared geographical range, such as those from the San Andres National Wildlife Refuge, New Mexico, are consistently grouped together. This implies an isolated local population of mites being maintained on the various wildlife species in the Refuge. The extent of this isolation is questioned by the presence of one sample from the Refuge that does not cluster with the others. However, the presence of mites from different host species seems to confirm the cross-infectivity of *P. ovis* as suggested by Ramey *et al*. (2000). In contrast, many of the British mite samples show no pattern of grouping at all, suggesting a countrywide population of genetically indistinguishable mites. The only interesting exception to this is a cluster of two samples, one from South Uist in Scotland and one from the Department of Agriculture, Food and Fishery, Dublin. These samples have been isolated from other sources of sheep scab for 14 and 19 years, respectively. This chronological isolation may mean that the Scottish island sample may be a genetic remnant of the mites that brought sheep scab back to Britain

from Ireland in the mid-1970s, thus explaining their similarity.

Overall, the data presented here suggest that morphological variation, most notably in the length of the outer opisthosomal setae, has only a very approximate relationship with host species, and the variation between mite populations within a host type may be as great as the variation between mites from different host types. The data suggest that Sweatman's (1958) putative five species are not sufficiently different or genetically isolated to support the claim that they should be classified as distinct. Morphological differences appear more likely to be the result of adaptation to the local microenvironment. The one exception, however, may be *P. natalensis*. *Psoroptes natalensis* from South Africa was first described from mites found on cattle and has opisthosomal setae that are quite different to other populations, flattened and blade-like at the distal end (Hirst, 1922; Bates & Sayers, 2002). Samples of *P. natalensis* could not be obtained for the present study.

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