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Phylogeny and Host Specificity of Psoroptic Mange Mites (Acarina: Psoroptidae) as Indicated by ITS Sequence Data

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ABSTRACT We used molecular phylogenetic techniques to study the systematic relationships and host specificity of *Psoroptes* mange mites, which are pests of numerous domestic and wild ungulates. Phylogenetic analysis of DNA sequence data from the internal transcribed spacer region 1 (ITS1) of nuclear ribosomal DNA indicated that populations of *Psoroptes* are not host specific. Furthermore, the currently used taxonomy of *Psoroptes* is not concordant with the phylogeny derived from ITS1. During the course of the study, we discovered apparent paralogous ITS sequences within individual mites as a result of varying polymerase chain reaction reaction conditions. This finding concords with other studies of ITS and suggests a cautious approach when interpreting data from ITS sequences. Host DNA contamination was also found to be a significant problem in data collection, and we report on the development of methods to overcome the problems of contamination in parasitic mites.

KEY WORDS *Psoroptes*, internal transcriber spacer region, bighorn sheep, phylogeny, parasitism

PSOROPTIC MITES (ACARINA: Psoroptidae) are deadly pests of numerous wild and domestic ungulate species worldwide. Infestations have been reported on domestic sheep, cattle, and goats, as well as wild populations of mountain sheep (*Ovis canadensis*), mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and bison (*Bison bison*) (Seton 1929, Murie 1951, Sweatman 1958, Strickland et al. 1970, Lange 1980). Psoroptic mites infest the ears or body of host species, and feed on lymph fluids by abrading the epidermis. Infestations on the body may cause dermatitis, lesions, and the formation of scabs (Rafferty and Gray 1987), whereas ear infestations cause damage to the eardrum leading to auditory impairment (Rollor et al. 1978, Clark and Jessup 1992). In severe cases, mortality results from dehydration, secondary infection, and predation caused by the effects of hearing loss (Stromberg et al. 1986, Clark and Jessup 1992). Although there are many examples of mites causing severe morbidity and mortality of their hosts, there are also examples of relatively benign mite infestations (Decker 1970, Welsh and Bunch 1983, Friel and Greiner 1988).

Psoroptic mange has apparently been eradicated from domestic sheep in North America, but it is still a persistent problem for some wildlife populations. In the San Andres Mountains of New Mexico, a severe scabies epizootic reduced the mountain sheep population from >250 animals to a single individual during 18 yr (Sandavol 1980, Hoban 1990; D. Holderman, U.S. Army, White Sands Missile Range, NM, personal communication). Similarly, during the 1970s, psoroptic mites killed ≈80% of the white-tailed deer populations in Georgia (Stromberg et al. 1986).

The evolutionary origins of psoroptic scabies mites in North America and the causes for the virulence of some mite populations are unknown. Several authors have suggested that *Psoroptes* were introduced into North America from Asia, Europe, or Africa with domestic livestock (sheep or cattle) and that the susceptibility of some indigenous North America host species is a consequence of their being exposed to novel mite antigens for which the hosts are not adapted (Stromberg et al. 1986; Boyce et al. 1991a, 1991b). However, other hypotheses are also consistent with the observations. For example, *Psoroptes* may be indigenous to North America and the evolution of virulence may be a result of recent genetic changes in some mite populations, allowing them to produce novel antigens that cause severe allergic reactions in the host.

Morphometric work by Boyce et al. (1990) suggested that several of the previously recognized species of *Psoroptes* may be conspecific, and therefore some species of mites may be less host-specific than previously thought. However, the fact that Boyce et al. (1990) were sometimes able to distinguish among

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Specimens were collected by the USDA, NPS, Arizona Fish and Game, and California Fish and Game as per their respective protocols. Collecting permits are on file and can be obtained upon request.

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mites sampled from different host species supports the hypothesis that mites found on different host species may be host races with varying degrees of host specificity. However, this work and previous morphological work has largely relied on a single morphological character (outer opisthosmal setae length). Results from cross-transmission experiments have also proven equivocal with some experiments finding host specificity whereas others have shown little such specificity (Sweatman 1958, Kinzer et al. 1983, Wright and Fisher 1984).

In general, knowledge of host specificity is crucial to understanding the evolution of virulence in infectious diseases and parasitic infestations (Brooks and McLennan 1993). For instance, the evolution of virulence is favored by high transmission rates (i.e., low host specificity) because there is essentially no "penalty" for being virulent (Bull et al. 1991, Herre 1993). If a parasite or pathogen can undergo frequent and radical host shifts, this greatly increases the number of "host" individuals available, and there is a concomitant increased in the potential for virulent parasitic populations to persist. Therefore, if *Psoroptes* mites found on North American wildlife are not host specific, but can be transmitted among alternative sympatric host species (e.g., between bighorn and deer in the San Andres Mountains), then lack of host specificity (i.e., host breadth) may be one of the ultimate causes for the persistence of virulence in mite populations. Furthermore, a large multi-species pool of host individuals can allow an epizootic to persist longer than if mites infested only a single species.

To determine both the host specificity and diet breadth of psoroptic mites, we used molecular systematic techniques to identify the number of distinct host-related lineages within *Psoroptes.* We sequenced DNA from the first internal transcribed spacer region 1 (ITS1) of nuclear ribosomal RNA from mites collected off a number of different host species in various locales (Table 1). With these data, we asked if related mites tended to be found on the same or related host species regardless of distribution. This approach also allowed us to ask how often virulence tended to evolve among various host populations.

Materials and Methods

Data Acquisition. Psoroptic mites were collected from a wide variety of hosts and locales, which are listed in Table 1. We collected specimens of *Psoroptes cervinus* Ward, *P. cuniculi* Delafond, *P. ovis* Hering, and an outgroup *Sarcoptes scabiei* L. Because of the mites minuscule size, DNA was initially extracted from batches of 100–200 mites ground between frosted glass microscope slides in sterile filtered Lifton extraction buffer (0.2 M sucrose, 0.05 M EDTA, 0.1 M Tris, 0.5% SDS, pH 9.0). The resulting homogenate was incubated at 55°C for 3 h with 10 mg/ml proteinase-K, extracted with phenol/chloroform, ethanol precipitated, and resuspended in 100 μl water. To obtain mite-specific primers, these samples were polymerase chain reaction (PCR) amplified with primers SP18s and SP28s from Wesson et al. (1992). These PCR products were cloned using the General Contractor Cloning Kit (5′-3′, Boulder, CO) and sequenced using standard M13 primers. Using this data, *Psoroptes*-specific primers were designed that overlapped the ITS1/18s and ITS2/28s junctions. These primers were 28sPsor: 5′-CGCTTGATCTGAGGTCGAAAGTTAAC-3′ and 18sPsor: 5′-CATTATCGGTGTTTGAGACTCTACG-3′.

Table 1. Specific epithet, host, and location of mites sequenced in this study

Species	Host	Location
P. cervinus Ward	Elk	Idaho
P. cuniculi	Rabbit	Northern California
P. cuniculi	Rabbit	Argentina
P. ovis	Bighorn sheep	Hells Canyon, ID
P. ovis	Bighorn sheep	Canyonlands National Park, UT
P. ovis	Bighorn sheep	Canyonlands National Park, UT
P. ovis	Bighorn sheep	Arizona
P. ovis	Bighorn sheep	Nevada
P. ovis	Bighorn sheep	San Andreas Mts., New Mexico
P. ovis	Cattle	Kerrville, TX
P. ovis	Domestic sheep	Argentina
P. ovis	Domestic sheep	Bangor, Wales
P. ovis	Mule deer	Bosque de Apache National Wildlife Refuge, New Mexico
P. ovis	Goat	Georgia
Sarcoptes scabiei	Red Fox	Hall County, GA

DNA extraction of subsequent samples used for phylogenetic analyses were prepared with the QIAGEN Tissue Extraction Kit (QIAGEN, Chatsworth, CA). Individual mites were ground between frosted glass microscope slides in QIAGEN ATL buffer and extracted using the protocols supplied with the kit. PCR amplifications were carried out using primers SP18s and SP28s under the following conditions: initial denaturing step of 94°C for 120 s, followed by 30 cycles of 96°C–15 s, 55°C–15 s, and 72°C for 80 s. This PCR product was purified using a QIAquick PCR Purification Kit and 1 μl of this product was reamplified using primers 18sPsor and 28sPsor under the following conditions: initial denaturing step of 94°C–90 s, and 30 cycles of 96°C–10 s, 60°C–15 s, 72°C for 80 s. Sequencing was conducted on an ABI 377 automated sequencer using ITS1/18s and an additional *Psoroptes*-specific primer located in 5.8 s. (5′-GATATCCAGTG GCTGCAATGTGCGTTC-3′). Because of the high secondary structure in ITS1 we used the following cycle sequencing conditions with an ABI dRhodamine Terminator Cycle Sequencing Kit (Foster City, CA): 96°C–10 s, 60°C–4 min for 25 cycles.

Overcoming Host DNA Contamination and Low DNA Yields. Obtaining PCR amplified DNA sequences from *Psoroptes* mites that were free of host DNA contamination proved exceedingly difficult. This contamination resulted from fluids found in the gut of feeding *Psoroptes,* and from mites often being awash in host fluids at the surface of the skin. Even using an extensive selection of mitochondrial DNA primers (not mite specific) produced PCR amplification products that were either host DNA, a combination of both mite and host DNA, or anonymous DNA (no reliable match to any known genes in GenBank, http://nebi.nlm.nih.gov). Correspondence with researchers working on other parasitic mites (S. Little, University of Georgia, personal communication) revealed that this is a problem for them as well. However, despite these difficulties, we were able to obtain pure *Psoroptes* mite DNA sequences. This was accomplished by starving batches of mites to rid them of host fluids, extracting DNA from the starved mites, and then amplifying across internal transcribed subunit region (ITS) of nuclear ribosomal DNA (between 18s and 28s nuclear ribosomal genes). Starved mites were provided courtesy of W. Fisher (ARS Kipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, TX).

Because mites provided so little DNA, and because host contamination proved problematic early on in the study, we took a number of precautionary steps to avoid contamination of PCR reactions. (We present these in detail, because our experiences suggested that such precautions are necessary when working with such tiny parasites.) Laboratory equipment that was used in DNA extractions or PCR amplifications, (including tips, tubes, and glass) was sterilized with high intensity short-wave UV light. Filter barrier pipette tips were used for all procedures, and reagents (except *Taq*) were sterile filtered or UV irradiated before use. Negative controls were prepared using the same reagents, substituting HPLC purified H_2O for sample DNA, and negative controls were carried over in the reamplifications. These controls were performed in each round of PCR amplifications to ensure that amplified DNA products were not a result of reagent contamination. If there was any PCR product present in the negative controls, we immediately discarded all the samples and repeated the procedure with fresh reagents.

Sequence Analysis. Sequence data were entered, compiled, and edited for analysis using the Sequencher 3.0 program (Gene Codes, Ann Arbor, MI). To check for potential host, bacterial, and fungal DNA contamination, we screened all DNA sequences against the GenBank database with a BLAST search on the NCBI website (http://ncbi.nlm.nih.gov). Phylogenetic analyses of DNA sequence data from the ITS1 region from the *Psoroptes* populations were performed using the software package PAUP* (Swofford 1996). For the *Psoroptes* phylogeny, *Sarcoptes scabiei* was used as an outgroup because the Sarcoptidae is the presumed sister taxon to the Psoroptidae (O'Connor 1979, 1982). Maximum parsimony bootstrap analyses were performed using PAUP* (Swofford 1996). One thousand replicates were performed in all bootstrap analyses, with 10 random addition sequence heuristic searches performed in each replicate.

Results

We obtained ≈526 bases of DNA of the ribosomal ITS1 and 5.8s gene region from mites representing 19 populations on six different host species (Table 1). These sequences have been submitted to Genbank under accession numbers AF270805–AF270824. Phylogenetic analyses using these sequences revealed little phylogenetic structure among the various mite populations, and mites found on the same host species in different locales did not form monophyletic groups (Fig. 1). However, we discovered that some ITS1 sequences from individual mites formed highly divergent clades. For instance, the sequences from mites off goats from Georgia and off domestic sheep from England comprised a monophyletic group separated by a minimum of 21 mutational steps from the other sequences. We also found that the cloned ITS1 sequences formed a divergent and well-supported clade (Fig. 1). However, these clades were also not host-associated because they were composed of mites off multiple host species in a number of locales (Fig. 1; Table 1).

Most of the other sequences were highly similar, having diverged only a few mutational steps (Fig. 1). A maximum parsimony bootstrap analysis found these sequences formed an unresolved polytomy (Fig. 1). Because we suspected that the highly divergent clades were paralogous sequences, we performed a second phylogenetic analysis excluding these sequences. In this analysis, we again found little phylogenetic structure within the closely related ITS1 sequences, and there was no clear association of related mites using the same hosts or within the same locales (Fig. 2). In fact, we discovered that sequences from individual mites identified previously as different species, *Psoroptes ovis* and *P. cuniculi,* did not form clear monophyletic groups (Fig. 2).

Discussion

Phylogenetic analyses of the sequence data from ITS1 found no evidence for host specificity among populations of *Psoroptes* mites. Mites collected off the same host species in different locales were not more closely related than they were to mites collected off different host species (Fig. 1). This answer did not change when we excluded those sequences suspected of being paralogous (Fig. 2). Additionally, it appears that the currently accepted taxonomy of *Psoroptes* is not supported because *P. ovis* and *P. cuniculi* did not form monophyletic groups.

Although we cannot confirm that we obtained paralogous sequences, several observations support this conclusion for the cloned ITS1 sequences in particular. First, these sequences were obtained with different PCR reaction conditions and primers than all the other sequences. Second, divergent ITS1 sequences were obtained from the same population of mites sampled from the same host individual (Fig. 1). We also suspect that several of the more divergent sequences, including those from mites off goat and sheep from England, could be paralogous because they are highly divergent compared with the majority of the sequences (Fig. 1). Buckler et al. (1997) discovered that divergent paralogous sequences could be

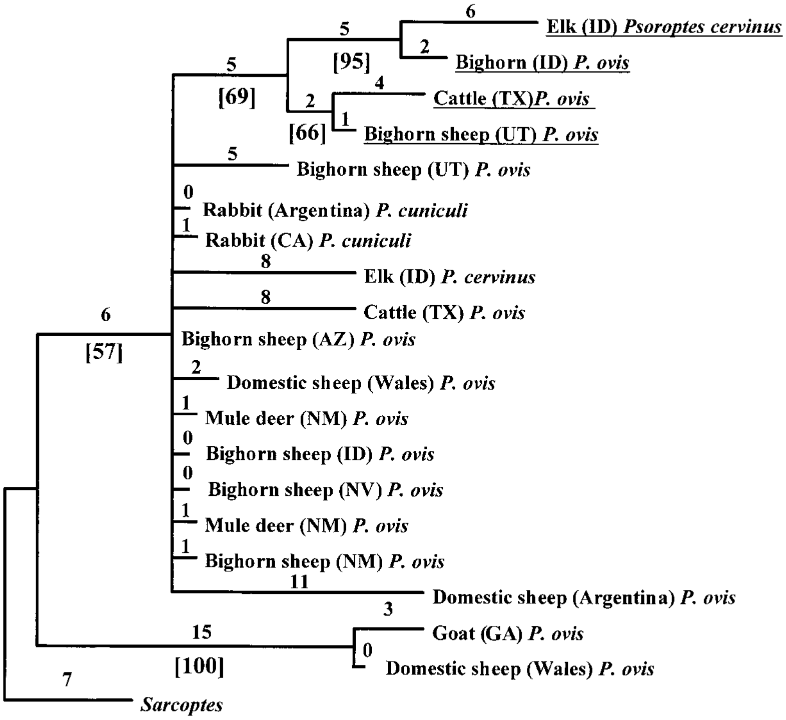

Fig. 1. Results of maximum parsimony bootstrap analysis of ITS1 sequences from *Psoroptes* mites using a sequence from *Sarcoptes* as an outgroup. Host, locale, and specific epithet are given for each of the samples at the branch tips. Cloned ITS sequences are underlined. Numbers above the branches indicate the mutational steps along that branch, and the numbers below the branches [in brackets] report the bootstrap values supporting that particular grouping. Only bootstrap proportions >50% are reported.

identified in maize by varying PCR reaction conditions, and Rich et al. (1997) found divergent ITS2 sequences within individuals ticks. Paralogous ITS sequences can exist because ribosomal RNA genes (including ITS1) are found in multiple, tandemly repeated copies per haploid genome. Most researchers who use ribosomal RNA in systematic studies assume that these repeats are homogenized through the process of concerted evolution or unequal crossing-over. However, divergent sequences within individuals can be the result the recent gene flow and recombination. Although the inclusion of nonhomologous sequences can be problematic for phylogenetic analyses, most of the ITS1 sequences we used were extremely similar and were almost certainly homologous (Fig. 2).

The lack of phylogenetic structure in the ITS1 data set is consistent with the hypothesis that gene flow or multiple host shifts have occurred in Psoroptic mites. Although it is possible that the lack of phylogenetic structure in the data set is the result of ITS being a poor genetic marker in this group, the biogeography of Psoroptic mite infestations (Boyce and Zarnke 1996) suggest that Psoroptic mites are not native to North America. Instead, they probably were imported into North America along with domestic livestock from Europe. As evidence of this scenario, Boyce and Zarnke (1996) found no serological evidence of *Psoroptes* exposure in 403 Dall sheep from Alaska, and found no records of *Psoroptes* infestations in Dall sheep or potential wild hosts species from Siberia. Because ancestral populations of bighorn sheep, elk, and deer colonized North America over the Bering land bridge during the Pleistocene, they concluded that *Psoroptes* was not introduced into North America on ancestral populations of wild sheep. Our findings support this conclusion, and suggest that the susceptibility of bighorn sheep to Psoroptic mite infestations can be seen as a consequence of their being exposed to novel mite antigens for which they are not adapted. Additionally, the extensive worldwide trade in livestock could have facilitated the gene flow among divergent Psoroptic mite populations and favored the evolution of virulence in mite populations.

Our finding that host DNA contamination can be a serious problem when using PCR-based DNA sequences suggests that caution be used when exploring the genetic relatedness of parasitic mites (and possibly other parasites or symbionts). We strongly urge all researchers to compare putative mite DNA with host sequences. Because of serious issues of host contamination, techniques that do not allow the investigator to perform the proper controls, such as randomly amplified polymorphic DNA (RAPD), cannot distinguish reliably between genetic information derived from the host and that derived from the parasite. Because we found divergent ITS sequences within

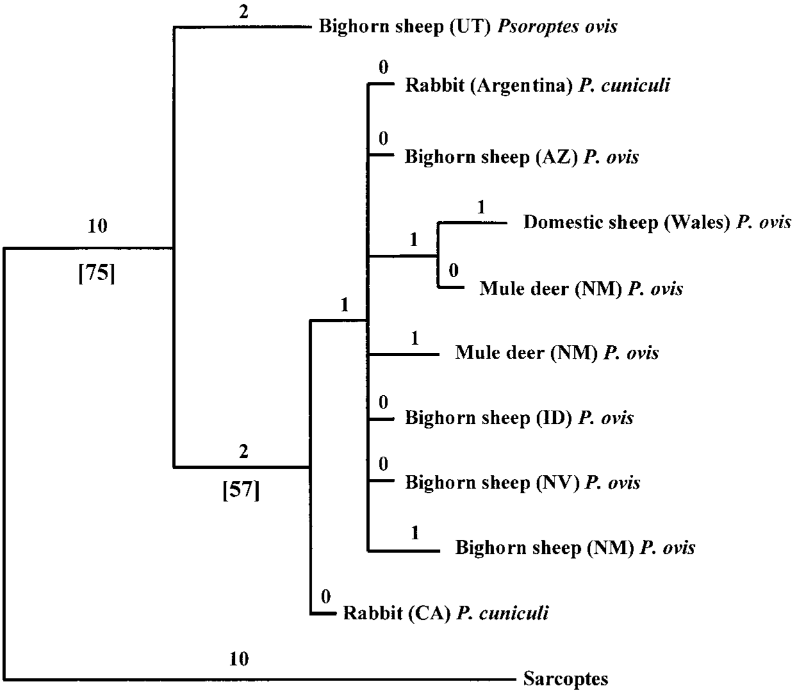

Fig. 2. Maximum parsimony bootstrap analysis of *Psoroptes* ITS1 sequences excluding suspected paralogous sequences (see *Results*). Host, locale, and specific epithet are indicated at the branch tips. Numbers above the branches indicate the mutational steps along that branch, and the numbers below the branches [in brackets] report the bootstrap values (>50%) supporting that particular grouping.

mite populations, we urge other researchers to exercise caution in the interpretation ITS sequence data from mites.

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