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PRIMER NOTE

Isolation of *Psoroptes* scab mite microsatellite markers (Acari: Psoroptidae)

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Abstract

Nine microsatellite markers have been isolated from the scab mite, *Psoroptes ovis*. These markers have been tested for polymorphism in individual *Psoroptes* mites originating from two hosts, the European rabbit, *Oryctolagus cuniculus*, and sheep, *Ovis aries*. No definitive picture of *Psoroptes* species' status or interrelationships exists. This study provides the basis for a new molecular system to elucidate the systematics of groupings within the genus *Psoroptes*, allowing us to clarify the population dynamics and epidemiology of the mites causing sheep scab world wide.

Keywords: Acari, microsatellite, mite, *Psoroptes*, rabbit, sheep scab

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The exact classification of mites of the genus *Psoroptes* has always been the subject of much debate (Wright *et al*. 1983; Zahler *et al*. 1998; Sanders *et al*. 2000) and no definitive picture of the species' status or interrelationships exists. Nine microsatellite *Psoroptes* mite loci have been isolated which will be used to study gene flow between and within populations of *Psoroptes* mites, in particular the sheep scab mite, *Psoroptes ovis*. *Psoroptes ovis* has been widely studied with regard to its biology, pathology and epidemiology and is of significant economic importance in the sheep and livestock industry.

A laboratory colony (established in 1998) of *Psoroptes* mites (Grampian Pharmaceuticals strain) was maintained on the ears of a rabbit at Bristol University, UK. DNA was extracted from 1800 mites from this laboratory colony. Several precautions were taken in an attempt to limit contamination by rabbit DNA. This was particularly important due to the high frequency of microsatellites in mammalian genomes, in comparison to their much lower frequency in mite species (Hammond *et al*. 1998; Navajas *et al*. 1998). *Psoroptes* mites were removed from the rabbit scab material, placed on damp filter paper and left for 48–72 h to starve. This allowed time for most ingested rabbit tissue to clear the mites' guts before they died. The mites were then washed for 10 min with agitation in distilled water, 10% Tween 20 (Promega) and stored in 70% ethanol. After washing, the mites were micro-dissected to separate the cephalothorax from the abdomen and the legs in an attempt to limit the potential rabbit contamination. These three parts were then washed again, as above, before extraction to remove any remaining rabbit contamination. Following manual crushing, the mite tissue was digested with proteinase K, a phenol : chloroform extraction was performed and the DNA was precipitated with ethanol at –20 °C (Sambrook & Russell 2001). Approximately 43 ng of genomic DNA was obtained.

For the library preparation, *Psoroptes* mite genomic DNA was digested with *Mbo*I (ABgene). In order to limit the loss of DNA and since the majority of the fragment sizes produced by the digest were approximately of the size required (100–700 bp), *Mbo*I fragments were not size-selected. These *Mbo*I DNA fragments were purified by ethanol precipitation, and 40 ng was ligated to 400 ng of pUC18-*Bam*HI/BAP (Amersham Pharmacia Biotech) and transformed into XL1 Blue competent cells (Stratagene). Seventeen thousand transformant clones were screened by hybridization to [α-^{32}P]dCTP radiolabelled sequences $(CA.GT)_n$ $(GA.CT)_n$ $(GCT.CGA)_n$ and $(TTG.AAC)_n$ (Amersham Pharmacia Biotech), which identified 93 positives.

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All 93 positively hybridizing clones were sequenced using Big Dye Terminators on an ABI 377 Sequencer (PE Applied Biosystems). Twenty-five sequences were suitable for primer design; 26 pairs of primers were designed with the assistance of PRIMER version 3 (Rozen & Skaletsky 2000). The sequences were confirmed unique using GENEJOCKEY Sequence Processor software (Biosoft Ltd) and submitted to the EMBL sequence database. After submission to the EMBL database, the sequences were double-checked unique using BLASTN 2.2.4 software (Altschul *et al*. 1997). Eleven clones partially matched other rabbit sequences in the EMBL database and 17 clones were later confirmed to be of rabbit origin by testing whether the primers designed would amplify rabbit DNA using the polymerase chain reaction (PCR). Nine unique loci were confirmed experimentally (see below) to be of *Psoroptes* mite origin and details of these nine characterized loci are provided in Table 1.

Due to difficulties in obtaining mite samples from natural infections, only 11 unrelated and geographically distinct individual *Psoroptes* mites [one from a European rabbit (*Oryctolagus cuniculus*) and 10 mites from sheep (*Ovis aries*)], plus mites from the Bristol laboratory colony, were available for testing for polymorphism. The restrictions imposed by the Foot and Mouth Disease outbreak in Europe prevented further samples being included. The 10 mites originating from sheep were from individual hosts within nine naturally infected British flocks. The rabbit mite came from a naturally infected pet rabbit in Bristol, UK.

DNA from individual mites to be used for PCR was extracted by a different method to that used with the mites used in the library preparation. Single mites were homogenized in 40 μL of 50 mM NaOH in individual 1.5-mL microfuge tubes using a hand-held power drill and a 'Pellet Pestle' (Kimble Kontes Inc.). This homogenate was then incubated at 95 °C for 15 min and neutralized with 6 μL 1 M Tris-HCl (pH 8.0). A 0.75-μL aliquot of the cooled homogenate was then used directly as template in each microsatellite PCR. The quality of individual mite DNA extractions for PCR was tested by amplifying an aliquot of the homogenate with *Psoroptes*-specific primers for the second internal transcribed spacer (ITS-2) region of the ribosomal DNA (Zahler *et al*. 1998) as a positive control.

In addition to the template DNA, each 15 μL microsatellite PCR reaction contained 0.5 μM of each primer, a final concentration of 2.6 mM $MgCl_2$, 0.1 mM of each dNTP and 0.25 U of Red *Taq* polymerase (Sigma) in the manufacturer's buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl and 0.01% gelatin). In addition to these components, it was found that the addition of 1% Tween-20 and 1.0 mg/mL Bovine Serum Albumin (fraction V) (Sigma) helped to produced clean and reproducible PCR products. PCR amplification was performed in a Hybaid PCR Express thermal cycler. The reaction profile was 94 °C for 2 min, then 94 °C for 30 s, T_a °C for 1 min, 72 °C for 1 min for 35 cycles, and finally 72 °C for 5 min, where T_a °C was the optimal annealing temperature for each primer pair (Table 1).

Ten individual mites from the original laboratory colony of *Psoroptes* used to make the microsatellite library were used to check for initial PCR amplification of the nine loci. Those primers that amplified successfully were then tested with DNA from the 10 mites collected from sheep. Positive amplification in mites from sheep was taken as a confirmation of a mite origin, as it was assumed that any loci of rabbit origin would not amplify in the sheep-derived mites. To double-check this, the primers were also tested for amplification with rabbit DNA and a negative PCR water control; any loci found to amplify rabbit DNA were eliminated from the study.

Of the 26 primer sets tested, nine were confirmed to be of mite origin (Table 1) and 17 were of rabbit origin (EMBL Accession nos AJ507618, AJ507621, AJ507623, AJ507625, AJ507627, AJ507630 (includes two loci), AJ549294, AJ549295, AJ549296, AJ549297, AJ549298, AJ549299, AJ564263, AJ507624, AJ493327 and AJ549300). Several of the rabbit loci are currently being tested for polymorphism in wild rabbit and hare *Lepus europaeus* individuals (D. A. Dawson, unpublished data) and mapped in the rabbit genome (R. Korstanje, unpublished data). Polymorphism was assessed on 6% polyacrylamide gels stained with silver (Promega, Bassam *et al*. 1991) and allele sizes were assigned by comparison to a 10 bp DNA ladder, 25 bp DNA ladder (both from Invitrogen Ltd) and φ X174 RF DNA/*Hae*III DNA marker (ABgene).

Ten individuals from the Bristol laboratory colony were tested for polymorphism at all nine loci; this was the only population for which it was possible to obtain more than one individual. Seven loci were monomorphic and two displayed two alleles (Table 1). No variation was detected between the 10 individuals with any of the nine markers; however, the value of this result, as a measure of the variability present in natural infections, is somewhat reduced by the long-standing and inbred nature of this rabbit-maintained laboratory colony. Overall, due to low genetic variation in these inbreeding organisms, and the difficulties in obtaining individual mite samples, population sizes were too small for meaningful comparisons of observed and expected heterozygosities.

Allele sizes were compared in single *Psoroptes* mites isolated from other typical hosts: sheep and a rabbit (Table 2). In a single mite from a naturally infected rabbit host, two loci (*Psor*02 and *Psor*14) displayed two alleles and seven loci displayed a monomorphic allele. For six loci, allele sizes from the naturally infected rabbit-derived mite matched those obtained from the laboratory colony mites. All nine *Psoroptes* loci were also tested for polymorphism in 10 unrelated mites derived from 10 different sheep. One locus displayed three alleles, four loci displayed two alleles and four loci displayed only one allele.

Table 1 Characterization of nine *Psoroptes* scab mite microsatellite loci

Locus	EMBL Accession no. and clone name	Repeat motif (5′–3′)*	Primer sequence (5′–3′)*	Designed T_a (°C) using PRIMER version 3	T_a used in PCR (°C)	$MgCl_2$ conc. (mM)	Expected PCR product size (bp)*	No. of alleles in 10 mites from the laboratory colony†	Allele sizes in 10 mites from the laboratory colony† (bp)
*Psor*01	AJ507616	$(CA)_{13}$	F: ACAATAGATATTTGTTTGATTGTGCC	60	52	2.6	94	1	102
	SCAB001E07		R: TCCAAAATTTTACAATTGTTTTCG	59					
*Psor*02	AJ507617	$(CA)_{12}$	F: TGAATTTAGGGTGTTTGAAGGGG	63	63	2.6	139	1	162
	SCAB005A09		R: CGATGTTAGTTTCACATTATCCACTCTC	62					
*Psor*04	AJ507619	$(CA)_{9}$	F: TTAGTTGTTGTTGATTGAAA	49	55	2.6	147	1	172
	SCAB034E01		R: TCAAGGATACATATTGTGTG	49					
*Psor*05	AJ507620	$(TG)_{11}$	F: TTAACACATTTACTATTGCTGTTCTTT	57	62	2.6	150	1	174
	SCAB038S08		R: TGCAAATGAAACGAATGAGG	60					
*Psor*07	AJ507622	$(GT)_{11}$	F: GATCATCATCATCGTAT	42	55	2.6	66	1	68
	SCAB045A05		R: CGTTGATTATTATCTAAACT	44					
*Psor*11	AJ507626	$(GCT)_2(GTT)_1(GCT)_4$	F: TTGTTGAATTGGATTTGTTGTTG	60	58	2.6	126	1	140
	SCAB100C09		R: AAATTAATCCTTTAAATCAGTGGAAA	58					
*Psor*13	AJ507628	$(GA)_{18}$	F: TCTATTTAAAAATGGAAAGAGAG	52	55	2.6	200	2	208
	SCAB129G04		R: ATGGAATGAAATACGACAAA	53	226				
*Psor*14	AJ507629	$(TTG)_5(CTG)_1(TTG)_3$	F: ATCCTAAAAGGCTTGCAT	52	51	2.6	109	2	114
	SCAB130D03		R: ATCACTGCTGTAGCTGTATC	50	120				
*Psor*16	AJ507631	$(GA)_{13}$	F: GCAGAGATATTTGCTGGCCTTG	63	68	2.6	387	1	397
	SCAB150B03		R: GATCAATTTGGGCGCCTTTG	65					

*From sequenced clone.

†Grampian Pharmaceuticals *Psoroptes* strain maintained on a rabbit.

T_a, annealing temperature.

Table 2 Utility of nine *Psoroptes* scab mite microsatellite loci in mites from a rabbit and sheep

Locus	EMBL Accession no. and clone name	No. of alleles in single mite from naturally infected rabbit	Allele sizes in single mite from naturally infected rabbit (bp)	No. of alleles in 10 mites from 10 infected sheep	Allele sizes in 10 mites from 10 infected sheep (bp)
*Psor*01	AJ507616 SCAB001E07	1	102	1	104
*Psor*02	AJ507617 SCAB005A09	2	158 164	3	144 150 152
*Psor*04	AJ507619 SCAB034E01	1	172	1	168
*Psor*05	AJ507620 SCAB038S08	1	174	2	168 174
*Psor*07	AJ507622 SCAB045A05	1	72	1	68
*Psor*11	AJ507626 SCAB100C09	1	140	1	140
*Psor*13	AJ507628 SCAB129G04	1	226	2	248 244
*Psor*14	AJ507629 SCAB130D03	2	114 120	2	114 120
*Psor*16	AJ507631 SCAB150B03	1	397	2	391 397

When testing for variation between different mite populations from the same host species, two loci (*Psor*02 and *Psor*13) displayed polymorphism between the 10 mites from 10 different sheep (Table 2). Between two mites, each isolated from a different rabbit, three loci (*Psor*02, *Psor*07 and *Psor*13) displayed polymorphism, though it should be noted that only one mite was from a natural infection, the other being from the laboratory colony. To date, only one individual per sample has been tested and more polymorphism may be revealed after a larger number of individuals from each host are tested for variability. A single locus, *Psor*11, showed a monomorphic allele across our entire test panel of samples. However, preliminary tests with samples from other host species indicate that other alleles may exist, but are not displayed in the British sheep samples included here.

Overall, however, sufficient variation in allele size was present in seven loci (*Psor*02, 04, 05, 07, 13, 14 and 16; Table 2) to allow comparison between samples obtained from different host species. Initial analysis of the distribution of allele sizes (Table 2) suggests some differentiation between mites from different host species. Thus, these markers appear to provide the basis for an in-depth investigation of the population genetics and epidemiology of *Psoroptes* species.

All nine *Psoroptes* loci were also tested for amplification in the mite species *Sarcoptes scabiei* (Acari: Sarcoptidae); no amplification was observed with any of the loci.

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References

Altschul SF, Madden TL, Schäffer AA *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, **25**, 3389–3402.

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of polyacrylamide gels. *Analytical Biochemistry*, **196**, 80.

Hammond RL, Saccheri IJ, Ciofi C *et al.* (1998) Isolation of microsatellite markers in animals. In: *Molecular Tools for Screening Biodiversity* (eds Karp A, Isaac PG, Ingram DS), pp. 279–285. Chapman & Hall, London, UK.

Navajas MJ, Thistlewood HMA, Lagnel J, Hughes C (1998) Microsatellite sequences are under-represented in two mite genomes. *Insect Molecular Biology*, **7**, 249–256.

Rozen S, Skaletsky HJ (2000) PRIMER Version 3 on the web for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, NJ, USA. http://www-genome.wi.mit.edu/genome_software/other/primer3.html

Sambrook J, Russell DW (2001) *Molecular Cloning, a Laboratory Manual*, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Sanders A, Froggatt P, Wall R, Smith KE (2000) Life-cycle stage morphology of *Psoroptes* mange mites. *Medical and Veterinary Entomology*, **14**, 131–141.

Wright FC, Riner JC, Guillot FS (1983) Cross-mating studies with *Psoroptes ovis* (Hering) and *Psoroptes cuniculi* Delafond (Acarina, Psoroptidae). *Journal of Parasitology*, **69**, 696–700.

Zahler M, Essig A, Gothe R, Rinder H (1998) Genotypic evidence suggests that *Psoroptes* isolates of different phenotypes, hosts and geographical origins are conspecific. *International Journal for Parasitology*, **28**, 1713–1719.

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