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Skin-scale genetic structure of *Sarcoptes scabiei* populations from individual hosts: empirical evidence from Iberian ibex-derived mites

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Abstract

The objective of the present study was to examine the extent of genetic diversity among *Sarcoptes scabiei* individuals belonging to different skin subunits of the body from individual many hosts. Ten microsatellite primers were applied on 44 individual *S. scabiei* mites from three many Iberian ibexes from Sierra Nevada Mountain in Spain. Dendrograms of the mites from the individual Iberian ibexes, showing the proportion of shared alleles between pairs of individual mites representing three skin subpopulations (head, back, and abdomen subunits), allowed the clustering of some mite samples up to their skin subunits. This genetic diversity of *S. scabiei* at skin-scale did not have the same pattern in all considered hosts: for the first Iberian ibex (Cp1), only mites from the head subunit were grouped together; in the second individual (Cp2), the clustering was detected only for mites from the abdomen subunit; and for the third one (Cp3), only mites from the back subunit were clustered together. Our results suggest that the local colonization dynamics of *S. scabiei* would have influenced the nonrandom distribution of this ectoparasite, after a single infestation. Another presumable explanation to this skin-scale genetic structure could be the repeated infestations. To our knowledge, this is the first documentation of genetic structuring among *S. scabiei* at individual host skin-scale. Further studies are warranted to highlight determining factors of such trend, but the pattern underlined in the present study should be taken into account in diagnosis and monitoring protocols for studying the population genetic structure and life cycle of this neglected but important ectoparasite.

Introduction

The understanding of the relationship between landscape structure and species biology is the basis of landscape ecology (Manel et al. 2003; Storfer et al. 2007). It can provide information about population functioning which may be valuable cues for management and conservation decisions (Moritz 1994; Coulon et al. 2006). The classical population genetic view of a species range is of a network of randomly mating populations (Wright 1978). However population and behavioral ecologists working at finer scales have long realized that populations usually consist of demes or subgroups of varying degrees of relatedness or co-ancestry as a result of incomplete or sex-biased dispersal (Sugg et al.

1996). Detailed analyses of genetic structure within animal populations have mostly been conducted in species with well-developed social systems (Hughes 1998; Ross 2001a; Coltman et al. 2003). On another scale, ectoparasites are often site specific on the host (e.g., Choe and Kim 1988; Clayton 1991), but such phenomena have not been studied satisfactorily (Crompton 1997).

Historically, genetic studies on *Sarcoptes scabiei* has been extremely limited, primarily due to the difficulty in obtaining sufficient numbers of individual mites because of the low parasite burden (in ordinary scabies, less than ten organisms can be identified per host; Mellanby 1944) and the lack of an in vitro culture system (e.g., Brimer et al.

1993; Walton et al. 2004), and secondly because of the difficulty in getting adequate amounts of genetic material (due to the high rate of failed reactions; e.g., Walton et al. 1997; Berrilli et al. 2002). Almost all genetic studies on *S. scabiei* have been to answer the question that has been the subject of an ongoing debate for many years: whether *Sarcoptes* mites represent different species or whether they are, in fact, monospecific. Other questions that remain unclearly answered include: are *S. scabiei* from different geographical locations genetically different? Or could they be considered as a single panmictic population? (Burgess 1994; Ochs et al. 1999; Walton et al. 1999; Zahler et al. 1999; Berrilli et al. 2002; Soglia et al. 2007; Gu and Yang 2008).

Usually, infestation by *S. scabiei* is generated when mites (founder group) are in contact with their host animals (Pence and Ueckermann 2002). If the different genotypes expand with the same efficacy in

the different host skin subunits, alleles from the founder group should be distributed randomly in the different subunits of their host animal. Alternatively, if mites disperse unevenly, due to competition or avoidance processes, genetic mosaic should be created, resulting in unrandom genetic structure (Ross 2001b; Kanno and Harris 2002).

Recent theoretical and empirical studies have demonstrated that disease spread is often more complex than predicted by simple diffusion models (see Hastings et al. 2005 for a review). Nevertheless, to the best of our knowledge, no study was done to investigate the extent of genetic structure in *S. scabiei* subpopulations regarding the different subunits of the mangy skin from individual hosts (skin-scale). Therefore, the objective of the present study was to examine the genetic structure of *S. scabiei* populations at host skin- scale to test the hypothesis of the random genetic distribution of *Sarcoptes* mite.

Materials and methods

Iberian ibex-derived *S. scabiei*

Iberian ibex (*Capra pyrenaica*) is a natural host for *S. scabiei* (Pérez et al. 1997, 2002), with high prevalence (up to 50%) of mangy animals (Alasaad et al. 2008a). For this study, we used three mangy males of Iberian ibex, culled for management purpose in Sierra Nevada Mountain in Southern Spain. The three animals were coded as Cp1, Cp2, and Cp3.

Collection of *S. scabiei* samples

A total of 44 *S. scabiei* mites were collected from the skin of three culled Iberian ibexes: 14 from Cp1, 16 from Cp2, and 14 from Cp3 (Table 1 and Fig. 1). Mites from each individual skin were divided into three subpopulations, namely head subunit (H), back subunit (B), and abdomen subunit (A). The reason behind that was because these three skin subunits: (1) differed according to the timing of the parasitic progression in the host (Pence and Ueckermann

2002); (2) could be the first to contract the ectoparasite (León-Vizcaíno et al. 1999); and (3) because of the large distance between each other, which could facilitate the detection of the expected skin-scale genetic diversity. Back and abdomen subunits were circular areas of approximately 400 cm² each, while all head surface formed the head subunits.

Mites were collected by the wet scraping of the mangy skin using distilled water. The scrapings were made by a scalpel or similar bladelike tool on Petri plates. This step was followed by mite collection using dissecting micro- scope. Mites were identified as *S. scabiei* according to the morphological criteria of Fain (1968).

Preparation of *S. scabiei* genomic DNA

As described previously (Alasaad et al. 2008b), HotSHOT Plus ThermalSHOCK technique was applied to prepare the DNA for all single *S. scabiei* mites in this study. Aiming to study the possible intrapopulation polymorphisms, all of the DNA samples were prepared from individual *S. scabiei* mites.

Fluorescent-based polymerase chain reaction analysis of microsatellite DNA

From the panel described by Walton et al. (1997), ten microsatellites (Sarms 33, 34, 35, 36, 37, 38, 40, 41, 44, and 45) were selected and applied with one multiplex 10× polymerase chain reaction (PCR) as reported by Soglia et al. (2007). Each 15 µl PCR reaction mixture consisted of 3 µl of the single mite DNA, together with the PCR mixture containing all primer pairs (ranged from 0.04 to 0.1 µM per primer), 200 µM of each dATP, dCTP, dGTP, and dTTP, 1.5 µl of 10× PCR buffer (200 mM KCl and 100 mM Tris- HCl, pH 8.0), 1.5 mM MgCl₂, and 0.15 µl (0.5 U/reaction) HotStartar Taq (QIAGEN, Milano, Italy). Samples were subjected to the following

thermal profile for amplification in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA): 15 min at 95°C (initial denaturing), followed by 37 cycles of three steps of 30 s at 94°C (denaturation), 45 s at 55°C (annealing) and 1.5 min at 72°C (extension), and a final elongation of 7 min at 72°C.

Microsatellite analysis

Using 96-well plates, aliquots of 12 µL of formamide with Size Standard 500 Liz (Applied Biosystems, Foster City, CA, USA) and 2 µL PCR product were prepared. Then, the plates were heated for 2 min at 95°C and chilled to 4°C. Fluorescent PCR amplification products were analyzed by ABI PRISM 310 Genetic Analyzer with pop4 (Applied Biosystems, Foster City, CA, USA).

Molecular analysis

The CONVERT 1.31 software (Glaubitz 2004) was used to reformat files for the statistical analyses. Multilocus proportion of shared alleles (Dps) was computed between all possible mite pairs using the MICROSAT software (Minch 1997) ignoring preliminary clustering information; 10,000 datasets were generated by resampling the input data (bootstrapping). The Neighbor-Joining algorithm was used as implemented by the PHYLIP v. 3.6 Package (Felsenstein 1989), and a consensus dendrogram was obtained. The dendrogram was visualized using TreeIllustrator v. 0.52 Beta software format (Trooskens et al. 2005). The analysis of relationships among the different mite populations was improved by a simulation-based Bayesian assignment test. The test was carried out by the GENECLASS2 software (Piry et al. 2004). The probability of the multilocus genotype of any Iberian ibex-derived mites to be encountered in each of the skin subunits H, B, and A of the same individual animal was computed. The individual mites were assigned to that population for which the highest probability was obtained.

Results and discussion

Aiming to detect the possible *S. scabiei* skin-scale genetic diversity among three skin subpopulations (head, back, and abdomen subunits), 44 *S. scabiei* mites were collected from the skin of three culled Iberian ibexes. Due to amplification and/or sequencing failure, the final analysis was based on 27 mites, nine from each animal. Of the nine ectoparasites, three were from each subunit H, B, and A (Table 1 and Fig. 1).

For all *S. scabiei* mites in this study, the number of alleles or locus on average was 3.20 ranging from 1 (Sarms 44) to 6 (Sarms 36), the mean observed heterozygosity was 0.102 ranging from 0.038 (Sarms 33 and Sarms 37) to 0.25 (Sarms 36), and the mean expected heterozygosity was 0.284 ranging between 0.038 (Sarms 37) and 0.599 (Sarms 34).

The dendrogram, showing the proportion of shared alleles between pairs of individual mites from Iberian ibex Cp1, separated the mites of head subunit from mites of back and abdomen subunits (5607/10,000 bootstraps), while no clear separation was detected between mites from back and abdomen subpopulations (Fig. 2). Clear separation was detected in Iberian ibex Cp2, where mites from abdomen subunit were unambiguously clustered together (7911/10000 bootstraps), while the *S. scabiei* from head and back subpopulations were randomly distributed between two clusters (Fig. 3). Regarding *Sarcoptes* mites from *C. pyrenaica* Cp3, mites from back subpopulation were grouped in single cluster (5061/10,000 bootstraps), while the remaining ones from head and abdomen subunits were clustered randomly into two groups (Fig. 4).

Consequently, the dendrograms, showing the proportion of shared alleles between pairs of individual mites representing the three skin subpopulations (head, back, and abdomen subunits) from the individual Iberian ibexes, allowed the clustering of some mite samples up to their skin subunits. This skin-scale *S. scabiei* genetic structure did not have the same pattern in all the individual animals since mites were grouped together for the head in Cp1, for the abdomen in Cp2, and for the back in Cp3,

which suggests interindividual differences in the colonization of the host skin by *S. scabiei*. The skin-scale genetic structure would be explained by local colonization dynamics of *S. scabiei* after a single infestation due to, e.g., competition or avoidance processes (Kanno and Harris 2002). Another presumable explanation could be that this skin-scale genetic structure would be the result of repeated infestations, especially taking into account that active infestation, residual skin reaction, and reinfestation are yet considered difficult to distinguish (Walton and Currie 2007). Further studies will, hence, be needed to test these hypotheses.

To our knowledge, this is the first record of skin-scale genetic structure among *S. scabiei* from individual mangy animals. Despite the fact that the determining factors of this phenomenon remain to be explored, the observed skin-scale genetic structure has implications for studies on the population genetic diversity, life cycle, as well as for diagnosis and monitoring protocols of the ubiquitous *Sarcoptes* mite.

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Table 1 Host animal and skin-subunits used in this study, together with the total/useful number of *Sarcoptes* mite samples

Codes	Animal and code	Skin subunit and code	Total no. of parasites	No. of useful parasites	
Cp1H	Capra pyrenaica	Cp1	Head subunit H	4	3
Cp1B			Back subunit B	5	3
Cp1A			Abdomen subunit A	5	3
Cp2H	Capra pyrenaica	Cp2	Head subunit H	5	3
Cp2B			Back subunit B	6	3
Cp2A			Abdomen subunit A	5	3
Cp3H	Capra pyrenaica	Cp3	Head subunit H	4	3
Cp3B			Back subunit B	6	3
Cp3A			Abdomen subunit A	4	3

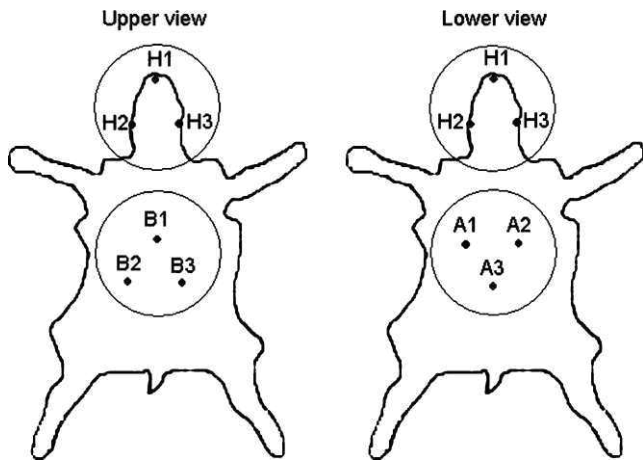


Fig. 1 Drawing showing approximate sites for *Sarcoptes* mite collection from three skin subunits of Iberian ibex. Codes next to the drawings represent the sample codes in Table 1

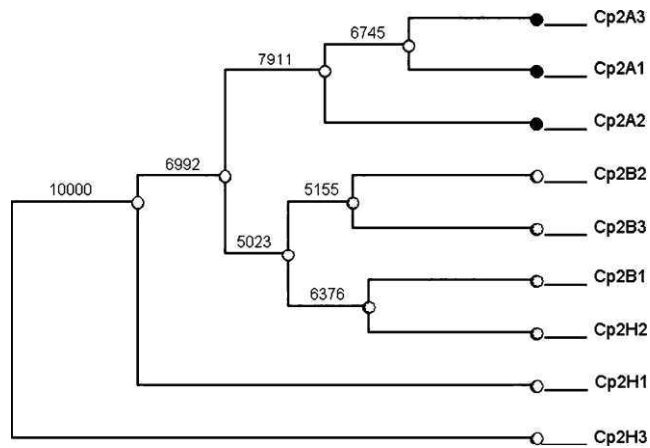


Fig. 3 Unrooted Dps consensus dendrogram for *Sarcoptes* mites from three skin subpopulations on the individual Cp2 of Iberian ibex. Numbers at the nodes are percentage values of 10,000 bootstraps supporting the same branching structure. Codes in this figure represent the sample codes in Table 1

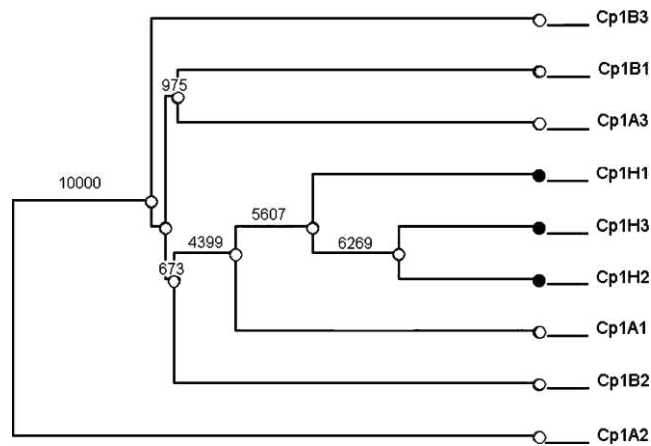


Fig. 2 Unrooted Dps consensus dendrogram for *Sarcoptes scabiei* from three skin subunits on the individual Cp1 of Iberian ibex. Numbers at the nodes are percentage values of 10,000 bootstraps supporting the same branching structure. Codes in this figure represent the sample codes in Table 1

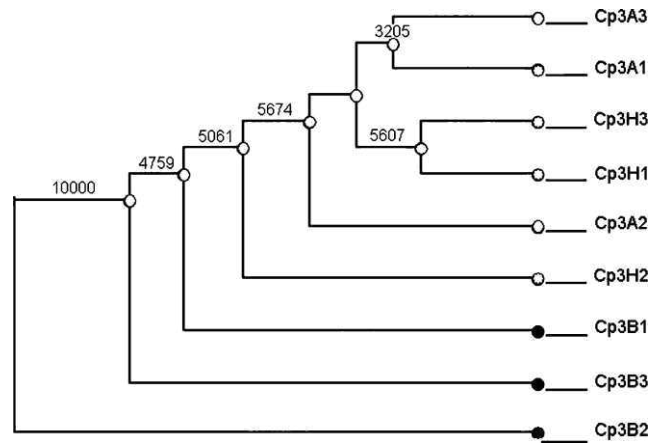


Fig. 4 Unrooted Dps consensus dendrogram for *Sarcoptes scabiei* from three skin subunits on the individual Cp3 of Iberian ibex. Numbers at the nodes are percentage values of 10,000 bootstraps supporting the same branching structure. Codes in this figure represent the sample codes in Table 1