Microsatellites as markers for comparison among different populations of *Sarcoptes scabiei*

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ABSTRACT: The aim of the present investigation was to analyse genetic variation and relationships of epizootic mange mites from sympatric Alpine chamois and red fox populations. The results of multi-locus genotyping using microsatellite marker loci support the hypothesis that gene flow between mite varieties on sympatric Alpine chamois and red fox is absent or extremely rare. Although the number of samples analysed until now is very small, the transmission of parasites seem to be more frequent when phylogenetically related host species are involved.

Key words: *Sarcoptes*, Chamois, Red fox, Microsatellites.

INTRODUCTION – *Sarcoptes scabiei*, causative agent of human scabies and animal epizootic mange, is considered as a monospecific taxon with morphologically similar varieties or subspecies that show high degree of host specificity and reduced cross infectivity. Analyses using microsatellite loci as genetic markers showed that gene flow between mite populations in sympatric humans and dogs is rare or absent in Australia and that the control programs for human scabies must only focus on man-to-man transmission (Walton et al., 2004). Investigations based on molecular markers have been limited up until recently due to difficulties in obtaining sufficient numbers of mites as well as adequate DNA quantities from individual mites. The Alpine chamois (*Rupicapra rupicapra rupicapra*) is suitable as a natural model to study the host-parasite co-evolution since it suffers more frequently than other mammals from high mortality during epizootic events. In this respect, the aim of the present investigation was to assess the genetic relationships among mites isolated from both sympatric Alpine chamois-red fox populations and geographically different populations of these two hosts.

MATERIAL AND METHODS – Mites were obtained from 21 chamois and 15 red foxes. About 2-5 mites from each host were collected. Overall, 41 mites from 13 Alpine chamois, *R. r. rupicapra*, North-East Italy (NEIC), 21 from 8 Southern chamois, *R. pyrenaica pyrenaica*, Spain (SC), 16 from 4 red foxes, North-East Italy (NEIF), 29 from 10 red foxes, North-West Italy (NWIF), and 4 from a rex fox, Spain (SF) were analysed. NEIC and NEIF were sympatric. Some more mites were isolated from other host species in order to refine the picture of host-specific varieties, that is 1 from a red deer (*Cervus elaphus*), 3 from an Alpine ibex (*Capra ibex*), 1 from an European mouflon (*Ovis gmelini*), 2 from a pine marten (*Martes martes*), and 2 from a stone marten (*Martes foina*); the first 4 hosts originated from North-East Italy, the last from North-West Italy. In addition, 5 mites were isolated on 4 wild boars (*Sus scrofa*) from North-East France and used as an outgroup. DNA extraction was performed from individual mites using the NucleoSpin Tissue kit procedure (Macherey-Nagel) in combination with some freezing steps without integument disruption. From the panel proposed by Walton et al. (2004) 10 microsatellites (SAMS 33, 34, 35, 36, 37, 38, 40, 41, 44, and 45) were selected on the basis of good scoring performance on genetic analyser. Analyses were performed with one multiplex PCR. PCR fragments were separated using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The data set was prepared with the Microsoft® Excel 2000 software. The CONVERT 1.31 software (Glaubitz, 2004) was used to reformat files for the statistical analyses. Multi-locus proportion of shared alleles (Dps) was computed between all possible mite pairs using the MICSAT software (Minch, 1997) ignoring preliminary clustering information; 1000 data sets were generated by resampling the input data (bootstrapping). The Neighbor-Joining algorithm (NJ) was used as implemented by the PHYLIP v. 3.6
package (Felsenstein, 1989) and a consensus dendrogram was obtained. The dendrogram was visualised in the 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 implemented by the GENECLASS2 software (Piry et al., 2004). The probability of the multi-locus genotype of any chamois or fox-derived mite to be encountered in each of the reference populations NEIC, SC, NEIF, NWIF, and SF was computed. The individual mites were assigned to that population for which the highest probability was obtained. The mites from the other host species were assigned using the same reference populations. Because assignment methods allow us to draw inferences about where individuals may come from, they have the potential to provide direct estimates of real-time dispersal of the parasites.

RESULTS AND CONCLUSIONS – Number of alleles/locus ranged on average from 1.2 (SD 0.4) as for SF to 2.2 (SD 0.9) as for NWIF. Average heterozygosity ranged from 0.017 (SD 0.028) as for NEIC to 0.111 (SD 0.199) as for NEIF. The number of monomorphic loci ranged from 3 (NWIF) to 9 (SC). This very low informative content may be due to the ascertainment bias because the markers were selected from a panel identified in different Sarcopes varieties. In spite of this small genetic variation, at all loci private alleles were observed at both host-specific and geographic population level.

Figure 1. Unrooted Dps consensus dendrogram. Numbers at the nodes are percentage values of 1000 bootstraps supporting the same branching structure.

As a consequence, the dendrogram showing the proportion of shared alleles between pairs of individuals (Figure 1) allowed us to clearly separate the chamois-derived mites, NEIC and SC, from the red fox-derived mites, NEIF, NWIF, and SF (0.986 bootstraps). In particular, the two sympatric populations NEIC and NEIF resulted to be different with no ambiguity. Moreover, mites isolated from R. r. rupicapra (NEIC) and R. p. pyrenaica (SC) occurred in different clusters, and mites isolated from red fox of the two different locations, Italy (NEIF and NWIF) and Spain (SF), clustered separately as well. On the other hand, differences between the two Italian red fox-derived groups were not very strongly supported. The addition of mites isolated from other host species did not alter this branching arrangement, but only reduced some bootstrap value (data not shown). The mites from Alpine chamois, red deer, Alpine ibex, and European mouflon grouped together whereas the mites from pine marten and stone marten clustered with the NEIF and NWIF parasites, respectively, that is with the sympatric red fox parasites. The wild boar parasites formed a distinct cluster, nevertheless its topography was poorly supported.

All mites from chamois and red fox were correctly assigned to their population of origin using the Bayesian test. Moreover, the mites isolated from the two chamois populations as well as from the single Spain red fox were only assigned to their populations of origin (zero probability to be assigned to other populations) (Table 1). Although only correct assignments were obtained, each of the two Italian fox-derived groups showed many mites with a minor non-zero probability to be assigned to the other Italian group. The characteristics of the northern Italy red fox-derived population must be analysed with a more complete and widespread sampling. Red deer, Alpine ibex, and European mouflon parasites were assigned to the Alpine chamois cluster whereas pine marten and stone marten parasites pertained to the two sympatric red fox-derived groups. On the contrary, the wild boar mites showed dif-
ferent genetic characteristics and were not assigned at all. The results of multi-locus genotyping using microsatellites support the hypothesis that gene flow between mite populations on sympatric Alpine chamois and red fox is absent or extremely rare. Although the number of samples analysed until now is very small, the transmission seem to be more frequent when phylogenetically related species are involved. The wild boar mites form a distinct cluster, but this could be due to the geographical isolation of the analysed sample. Our results on the Italy and Spain populations show the existence of differences between mites derived from the same host species but different geographical locations.

In conclusion, microsatellites show genetic distance between mites from the two sympatric populations, namely those deriving from chamois and red foxes collected in North-Eastern Italy. Our study supports the hypothesis of a very low degree of cross infectivity among sympatric but different mite varieties. The adaptive evolution of the Sarcoptes varieties seem to be strictly related to the phylogenetic similarity of the host species. Finally, although not strongly supported for the red fox groups living in Northern Italy, geographic differences were observed within the same host-specific variety.

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| Table 1. Average probabilities to assign an individual mite belonging to a population (row on the top) to each of the 5 reference populations (column on the left). |
|---------------------------------|----|----|----|----|----|
| NEIC                           | 0.402 ± 0.277 | 0   | 0   | 0   | 0   |
| SC                             | 0   | 0.872 ± 0.232 | 0   | 0   | 0   |
| NWIF                           | 0   | 0   | 0.598 ± 0.360 | 0.019 ± 0.024 | 0   |
| NEIF                           | 0   | 0   | 0.036 ± 0.064 | 0.793 ± 0.295 | 0   |
| SF                             | 0   | 0   | 0   | 0   | 0.764 ± 0.164 |