Host taxon-derived *Sarcoptes* mite in European wild animals revealed by microsatellite markers

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Ten markers specific to *Sarcoptes* mites were used in applying microsatellite genotyping to individual *Sarcoptes* mites collected in three European countries from 15 wild mammal populations belonging to 10 host species. The results showed that geographical separation had real biological significance for the definition of mite sub-populations, and that the degree of genetic exchange occurring between mites from different localities was apparently related to the geographical distance between locations. Wild host-derived mite populations were found to be clustered into three main groups: herbivore-, carnivore- and omnivore-derived *Sarcoptes* populations, with the omnivore-derived group located halfway between the herbivore- and carnivore-derived *Sarcoptes* populations. The separation between these three mite groups was better supported than the geographical separations; nevertheless, a kind of sub-clustering was detected within each of these three groups that separates mite populations into their geographical localities (countries). The lack of gene flow between *Sarcoptes* populations may have improved parasitic adaptations and led to what we refer to as a host-taxon-derived (carnivore host-, herbivore host- and omnivore host-derived) *Sarcoptes* mite found on European wild animals. Our results demonstrate that *Sarcoptes* is not a single panmictic population, even within each geographical location. This finding will have important ramifications for the study of the genetic structure of populations, life cycles, diagnosis and the monitoring protocols of the ubiquitous *Sarcoptes* mite, and could thus contribute to a better understanding of its associated epidemiology, which is of pivotal interest for wildlife biological conservation.

1. Introduction

Predicting the spread of a disease in wild animals is vital if we are to identify populations at risk, target surveillance and design proactive management programmes (Mathews et al., 2006; Blanchong et al., 2008). Recently, there has been an increased interest in disease in free-living animals and the ecological role of disease in populations, particularly in connection with its ability to regulate animal abundance (Scott, 1988; Lyles and Dobson, 1993; Robinson, 1996; Daszak et al., 2000). There is also an awareness that free-living species act as reservoirs of diseases that may affect man and domestic animals (Robinson, 1996; Daszak et al., 2000).

Although neglected as a pathogen, the ectoparasite *Sarcoptes scabiei* continues to affect humans and a wide range of mammalian

hosts worldwide (Bornstein et al., 2001; Pence and Ueckermann, 2002; Walton et al., 2004a). The introduction of infected domestic animals and the success of the *Sarcoptes* mite in adapting to new highly susceptible and receptive wild hosts have been proposed as the origin of sarcoptic mange epizootics in previously mange-free wildlife populations (Arlian, 1989).

In several European wild mammal populations, *Sarcoptes* mite infections are endemic and cause devastating mortality, as has been reported in the Alpine and Pyrenean chamois, Iberian ibex, aoudad and red fox (Fandos, 1991; Mörner, 1992; Pérez et al., 1997; León-Vizcaíno et al., 1999; González-Candela et al., 2004; Rossi et al., 2007). Nonetheless, only a few cases have ever been reported in other sympatric host species such as stone marten, badger, lynx and roe deer (Ryser-Degiorgis et al., 2002; Oleaga et al., 2008).

Morphological studies have failed to identify any significant differences between mite populations (Fain, 1978) and the experimental cross-contamination of *Sarcoptes* mites between hosts of different species commonly fails (Arlian et al., 1984; Arlian,

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Table 1
Countries, geographical locations and host species used in this study, together with the number of host animals and *Sarcoptes* mite samples.

Code	Countries	Geographical locations	Host taxon	Host species	No. of animals	No. of mites
ItNERr	Italy	North-east	Herbivore	Chamois (<i>Rupicapra rupicapra</i>)	20	63
ItNECi	Italy	North-east	Herbivore	Alpine ibex (<i>Capra ibex</i>)	10	25
ItNECe	Italy	North-east	Herbivore	Red deer (<i>Cervus elaphus</i>)	1	1
ItNEOam	Italy	North-east	Herbivore	European moufflon (<i>Ovis aries musimon</i>)	1	2
ItNEVv	Italy	North-east	Carnivore	Red fox (<i>Vulpes vulpes</i>)	7	23
ItNEMm	Italy	North-east	Carnivore	Pine marten (<i>Martes martes</i>)	1	3
ItNWVv	Italy	North-west	Carnivore	Red fox (<i>Vulpes vulpes</i>)	11	30
ItNWMf	Italy	North-west	Carnivore	Stone marten (<i>Martes foina</i>)	1	2
ItNWSs	Italy	North-west	Omnivore	Wild boar (<i>Sus scrofa</i>)	1	3
FrNESS	France	North-east	Omnivore	Wild boar (<i>Sus scrofa</i>)	4	5
SpNEVv	Spain	North-east	Carnivore	Red fox (<i>Vulpes vulpes</i>)	1	4
SpNWRp	Spain	North-west	Herbivore	Pyrenean chamois (<i>Rupicapra pyrenaica</i>)	9	26
SpSECp	Spain	South-east	Herbivore	Iberian ibex (<i>Capra pyrenaica</i>)	21	33
SpSWCp	Spain	South-west	Herbivore	Iberian ibex (<i>Capra pyrenaica</i>)	11	30
SpEMf	Spain	West	Carnivore	Stone marten (<i>Martes foina</i>)	1	1

1989). Apparently no epidemiological relationship exists in Europe between mange foci affecting wild ruminants, wild boars and carnivores (Berrilli et al., 2002).

The question as to whether *Sarcoptes* mites may be divided into different species or whether they are in fact monospecific is the subject of ongoing debate (Zahler et al., 1999; Burgess, 1999; Berrilli et al., 2002; Gu and Yang, 2008; Alasaad et al., 2009c). Using the ITS-2 sequences as genetic markers, Zahler et al. (1999) and Berrilli et al. (2002) failed to find any clear-cut evidence of genetic separation between mite populations that could be related to host species or geographical location. However, in our previous study we showed that ITS-2 rDNA is probably not a suitable marker for examining genetic diversity existing between *Sarcoptes* mite populations from different wild host species and/or geographical localities (Alasaad et al., 2009c). In phylogenetic analyses bootstrapping support for the closest relationships may be relatively poor if not enough time has elapsed for informative changes in the sequences examined to be accumulated. However, further resolution can be achieved using faster-evolving hypervariable sequences such as nuclear polymorphic microsatellite loci (Walton et al., 2004b).

Walton et al. (1999, 2004b) used multi-locus genotyping applied to microsatellite markers to substantiate previous findings to the effect that gene flow between scabies mite populations in

human and dog hosts is extremely rare in northern Australia. Genetic differences were detected between geographically distinct populations, and even between different people in the same household. Microsatellite markers were used by Alasaad et al. (2008b) to describe a new phenomenon of genetic structuring among *S. scabiei* at individual host skin-scale.

Bearing in mind the above information, the aim of the present study was to test to what extent sympatric wild host-derived *Sarcoptes* mite populations are genetically related, and to study the influence of geographical isolation on the genetic structuring of this mite. The understanding of these factors are crucial if wildlife health management is to be able to comprehend the geographic variation between neighbouring mite populations, and to measure patterns of host-specific differences, especially in sympatric hosts.

2. Materials and methods

2.1. Collection of *S. scabiei*

Using Postponed Isolation method for frozen skins (Post-frozen Isolation; Alasaad et al., 2009b) and Direct Isolation method based on heating stimulation of the living *Sarcoptes* mite (Live Isolation;

Fig. 1. Map of Europe showing the approximate sites of sample collection, together with cluster structure. The colours within bars show separately the proportion of membership of each individual in the genetic clusters for each *Sarcoptes* population. The pie charts give the genetic membership per *Sarcoptes* population. 1 = SpNWRp, 2 = SpSWCp, 3 = SpSECp, 4 = ItNWSs, 5 = FrNESS, 6 = SpNEVv, 7 = ItNEVv, 8 = ItNWVv, 9 = ItNEMm, 10 = ItNWMf, 11 = SpEMf, 12 = ItNERr, 13 = ItNECi, 14 = ItNECe and 15 = ItNEOam. For site abbreviations see Table 1.

Alasaad et al., 2009a), 251 *Sarcoptes* mites were collected from the skin crust of 100 naturally dead or hunted animals belonging to 15 populations of 10 European wild mammal species (Table 1) from Italy, France and Spain (Fig. 1). *Rupicapra rupicapra*, *Cervus elaphus*, *Martes martes*, *Ovis musimon*, *Capra ibex* and *Vulpes vulpes* are sympatric in the north-east Italian Alps, while *V. vulpes*, *Martes foina* and *Sus scrofa* are sympatric in the north-west Italian Alps. Taking into account the topography of the Sierra Nevada (Spain) and the fact that the first case of *Sarcoptes* mite infection was reported from the Dilar valley (eastern Sierra Nevada) (Pérez et al., 1997), mites from the Sierra Nevada were divided into eastern and western populations. All mites were identified as *S. scabiei* on the basis of known morphological criteria (Fain, 1968).

In Table 1, the term 'Code' refers to all mites belonging to the same geographical and/or host-species-derived population, henceforth referred to as the 'component population' or, simply, population (Bush et al., 1997).

2.2. Preparation of *Sarcoptes* gDNA

The DNA of individual *Sarcoptes* mites was extracted using the NucleoSpin Tissue kit procedure (Macherey–Nagel, Düren, Germany), with some modifications proposed by Soglia et al. (2009), and the HotSHOT Plus ThermalSHOCK technique (Alasaad et al., 2008a).

2.3. Fluorescent-based polymerase chain reaction analysis of microsatellite DNA

From the panel described by Walton et al. (1997), ten microsatellites (Sarms 33–38, 40, 41, 44 and 45) were selected and analysed with one 10x multiplex PCR, with one primer from each set 5' labelled with 6-FAM, VIC, NED or PET[®] fluorescent dye tag (Applied Biosystems, Foster City, CA, USA). 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 10x 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) HotStar 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), before a final elongation of 7 min at 72 °C.

2.4. 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 analysed by ABI PRISM 310 Genetic Analyzer with pop4. Allele calling was performed using the GeneMapper v. 4.0 software (Applied Biosystems, Foster City, CA, USA). To track and minimize the amount of error associated with genotyping, the genetic data were collected twice, once by SA and once by DS.

2.5. Descriptive statistics and cluster analysis

CONVERT 1.31 software (Glaubitz, 2004) was used to reformat files for the statistical software. Descriptive statistics and diversity analyses were carried out with GenAlEx v. 6.2 (Peakall and Smouse, 2006), Genepop v. 4.0 (Raymond and Rousset, 1995), Fstat v. 2.9.3 (Goudet, 1995) and Arlequin v. 3.1 (Excoffier et al., 2005) software to determine allelic richness (R), the number of private alleles, al-

lele frequencies and unbiased expected (He) and observed (Ho) heterozygosity, and also to test for Hardy–Weinberg (HWE) and linkage equilibriums (LE), and F statistics. All pairs of the component populations were compared for the homogeneity of genetic variation using Wilcoxon's matched-pairs signed-rank test (Graph-Pad InStat software).

The analysis of the structure and relationships between host-specific mite populations were studied using two different approaches:

- (i) The multilocus proportion of shared alleles (Dps) was computed between all possible pairs of individual mites using Microsat software (Minch, 1997), ignoring any preliminary information regarding the origins of the parasites. One thousand datasets were generated by resampling the input data (bootstrapping); the Neighbor-Joining algorithm was implemented by the Phylip v. 3.6 package (Felsenstein, 1989) to obtain a consensus dendrogram. The dendrogram was visualized using the Dendroscope v. 2.2.2 software (Huson et al., 2007).
- (ii) The analysis of relationships between mites was then improved by a Bayesian assignment test using the Structure v. 2.2 software (Pritchard et al., 2000). We performed 50,000 MCMC (Markov chain Monte Carlo) replicates following a burn-in period of 10,000 steps. This parameter set was run 20 times for each different number (K) of the genetic clusters of the multilocus genotypes; all values of K from 1 to 20 were tested. The probability of the multilocus genotype of any individual mite occurring in each of the K clusters was computed. We used the admixture model (each mite drew some fraction of its multilocus genotype from each of the K clusters), thereby allowing the allele frequencies to be correlated between clusters. This configuration has been described as the best in cases with subtle population structures (Falush et al., 2003). We used the height of the modal value of the distribution of DK to estimate the uppermost number of clusters capturing the overall mite sample structure, as suggested by Evanno et al. (2005). We then associated all individual mites with the cluster that corresponded to its greatest membership (q), that is, the fraction of its multilocus genotype; a threshold value q > 0.9 was used. Finally, each of the inferred clusters was associated with the component populations of its mites. If a cluster was labelled with multiple mite populations, an additional substructure analysis for K values from 1 to 5 was performed testing only the mites assigned to that cluster.

3. Results

3.1. Descriptive statistics

Ten marker loci were analysed from 251 mites originating from 15 populations of 10 European wild mammals from Italy, France and Spain; 101 alleles were detected. The allele count for each of the 10 loci ranged from 6 (Sarms 37) to 15 (Sarms 34). The proportion of missing genotypes was as low as 0.04% and did not affect single loci or populations. Forty-two private alleles were detected in 11 wild host-derived mite populations, ranging from 1 (ItNWMf, SpNEVv and SpNWRp) to 10 (FrNEss); in ItNECe, SpEMf, ItNEOam and ItNEMm no private alleles were identified (Table 2). The highest within-population genetic variability was observed in the French *S. scrofa* mite population, while little variation was found in either ItNWWv, SpNWRp or ItNWSs (Table 3).

Allelic richness (R) and heterozygosity (He) were used as the most informative parameters for diversity. In particular, allelic

Table 2

Private alleles detected at the 10 microsatellite loci of the host-associated mite populations, together with their frequencies. Codes in this table correspond to the sample codes in Table 1 and Fig. 1.

Pop (N° mites)	Locus	Allele	Frequency
ItNERr(63)	ms33	224	0.008
	ms33	244	0.025
	ms34	170	0.016
	ms34	192	0.190
	ms41	214	0.008
	ms41	250	0.083
	ms38	290	0.008
ItNECi (25)	ms34	188	0.104
	ms34	208	0.042
	ms35	138	0.022
	ms37	176	0.045
	ms41	244	0.026
	ms38	223	0.043
SpSWCp(30)	ms35	160	0.333
	ms36	263	0.017
	ms36	273	0.017
	ms40	217	0.100
	ms40	225	0.067
SpSECp(33)	ms35	158	0.015
	ms36	277	0.015
	ms45	164	0.030
ItNEVv(23)	ms35	150	0.065
	ms41	232	0.022
ItNWVv (30)	ms35	146	0.200
	ms41	264	0.033
SpNEVv(23)	ms38	205	1.000
FrNESSs(5)	ms33	266	0.800
	ms33	268	0.100
	ms33	270	0.100
	ms34	182	0.200
	ms35	126	0.200
	ms35	128	0.300
	ms36	287	0.400
	ms37	178	0.900
	ms41	228	0.600
	ms44	274	0.700
ItNWSs(3)	ms33	274	1.000
	ms34	200	0.250
	ms40	235	1.000
	ms45	176	0.500
ItNWMf(2)	ms38	219	0.250
SpNWRp (26)	ms45	198	0.679

ItNECi(1); SpEMf(1); ItNEOam(2) and ItNEMm(3): no private allele detected.

richness provided a measure of the number of alleles – regardless of the sample size – and hence allowed for comparisons to be made between different populations. The level of genetic diversity varied both across loci and between populations. Wilcoxon's test showed that *C. ibex* mites have more variability ($R = 1.3$, $H_e = 0.339$) than mites from the Spanish *R. rupicapra*; French *S. scrofa* mites were the most variable of all ($R = 1.5$, $H_e = 0.545$) ($P < 0.001$).

The LE test (Lewontin, 1964; Slatkin, 1994; Slatkin and Excoffier, 1996) was performed for all loci and significant linkage disequilibrium ($P < 0.05$) was observed for 18 pairs when all the

mite populations were pooled. Disequilibrium was never observed at the same loci in more than two individually analysed populations. HWE estimates were assessed from 90 loci-by-population comparisons, 43 (48%) of which showed significant heterozygosity deficiencies. Deviations from HWE did not indicate any locus in particular. All but the two *S. scrofa* mite populations deviated from HWE across loci after sequential Bonferroni correction ($P < 0.001$).

Population differentiation based on allele frequencies for all 15 populations gave an overall $F_{st} = 0.721$. Each locus significantly ($P < 0.001$) contributed to the distribution of variability between populations, with per-locus values ranging from 0.290 to 0.821. This very high estimate means that most of the global *Sarcoptes* genetic variability resided between rather than within component populations.

3.2. Structure and relationships between mite populations

- (i) Multilocus proportion of shared alleles (Dps) as a measure of genetic similarity between all pairs of mites.

Genetic variability between populations of *Sarcoptes* mites collected from the same host species in different localities.

The proportions of alleles shared between pairs of individual mites from the two *C. pyrenaica* mite populations in Spain (SpSWCp and SpSECp) were scattered randomly with no evidence of any distribution based on the geographical location of hosts. Individual *Sarcoptes* mites belonging to the three *V. vulpes* mite populations from the north-east and north-west Italian Alps, as well as from north-east Spain, showed clear clustering in their original populations. The *V. vulpes* mite population from Spain was the most different, and was supported by 980/1000 bootstraps. In comparison, the *V. vulpes* mites from the north-east and north-west Italian Alps resembled each other much more, and their distribution across two distinct clusters was only very poorly supported (169/1000 bootstraps) (Fig. 2). The mites from the *S. scrofa* populations from the north-west Italian Alps were well separated from those from north-east France (1000/1000 bootstraps, data not shown).

Genetic variability between populations of *Sarcoptes* mites collected from sympatric host species.

The dendrogram of individual mites from six sympatric host-derived populations from the north-east Italian Alps and from three sympatric host-derived populations from the north-west Italian Alps reveals a clustering of mites into three groups. The first group consisted of all carnivore-derived mites from the eastern and western Italian Alps, mainly from *V. vulpes*, but also from *M. foina* and *M. martes*, as well as one from *C. ibex*. The second group included herbivore-derived mite populations from the north-east Italian Alps, mainly from *C. ibex* and *R. rupicapra*, and is scattered across the cluster. In addition, this cluster included mites from *O. aries musimon* and *C. elaphus*. The separation of herbivore- and carnivore-derived mites is thus quite clear cut (470/1000 bootstraps). The *S. scrofa* mites from the north-west Italian Alps were similar to the carnivore-derived mites, but nevertheless still distinct (572/1000 bootstraps) (Fig. 3).

Genetic variability between *Sarcoptes* populations distributed according to both host species and geographical localities.

Table 3

Descriptive statistics for the mite populations (R = allelic richness; H_e = expected heterozygosity; H_o = observed heterozygosity). Codes in this figure correspond to the sample codes in Table 1 and Fig. 1.

	ItNERr	ItNECi	ItNEMm	ItNEVv	ItNWVv	SpNWRp	SpSWCp	SpSECp	SpNEVv	FrNESSs	ItNWSs
R	1.2	1.3	1.5	1.2	1.1	1.1	1.2	1.2	1.2	1.5	1.1
H_e	0.219	0.339	0.283	0.232	0.119	0.119	0.217	0.216	0.117	0.545	0.167
H_o	0.048	0.097	0.250	0.126	0.020	0.051	0.077	0.103	0.075	0.460	0.117

Fig. 4. Unrooted Dps consensus dendrogram for individual *Sarcophages* from the 15 wild host-derived populations (Table 1) using a similarity matrix based on the proportion of shared alleles. Thick branches represent all carnivore-derived mites, thin branches all herbivore-derived mites, and grey branches *S. scrofa*-derived mites. Cluster I (SpNWRp, SpSWCp and SpSECp), Clusters IIa and IIb (ItNWSs and FrNESs), Cluster III (ItNEMm, ItNWMf, ItNWVv and ItNEVv) and Cluster IV (ItNERr, ItNECi, ItNECe and ItNEOam). Codes in this figure correspond to the sample codes in Table 1 and Fig. 1.

with the information from the mites. For each cluster the average membership and number of mites assigned with the greatest membership were computed (Fig. 1). Cluster I shared all mites with Cluster I in Fig. 4, i.e. nearly all the mites of the Spanish ruminants (SpNWRp, SpSECp and SpSWCp) and one ItNECi. High proportions of membership were always obtained ($q > 0.97$). Cluster II grouped all the *S. scrofa* mites (ItNWSs and FrNESs) (see Fig. 4, Clusters IIa and IIb) with a membership fraction of $q > 0.98$. Two ItNECi (for one, see Fig. 4, Cluster IIb) and three SpNEVv mites were also added, but did not have high membership in this cluster ($q < 0.70$). Cluster III shared most mites with Cluster III in Fig. 4, grouping together all mites from Italian *V. vulpes* (ItNEVv and ItNWVv), *M. martes* (ItNEMm) and *M. foina* (ItNWMf and SpEMf) with very robust membership ($q > 0.95$ for all mites except SpEMf parasite with $q = 0.86$). One SpNEVv mite showed some similarity to this cluster, although with poor membership ($q = 0.56$). Cluster IV grouped the majority of the mites from Italian ruminants (ItNERr, ItNECi, ItNECe, and ItNEOam) and one SpNWRp with very high membership ($q > 0.97$). Its members corresponded to those of Cluster IV in Fig. 4.

The computation of the statistic DK was repeated separately for four subsets of samples consisting of the main geographical and host-specific mite groups, i.e. mites belonging to *S. scrofa* (ItNWSs and FrNESs), Italian ruminants (ItNERr, ItNECi, ItNECe and ItNEOam), *V. vulpes* (ItNEVv, ItNWVv and SpNEVv) and Spanish ruminants (SpNWRp, SpSECp and SpSWCp). No evidence of substructure was detected in any case.

Spanish *V. vulpes* mites were ambiguously assigned as in case of mixed ancestry. However, they were collected from a single host animal and lacked a substantial component population as a reference. Two ItNECi were misplaced in the *S. scrofa* mite cluster with low membership. One of them was also assigned to the sympatric *V. vulpes* mite cluster (Fig. 3) or to the allopatric Spanish ruminant mite cluster (Fig. 4, Cluster I), depending on which populations was used for the comparison. These individual parasites seemed to be randomly assigned since they carried multilocus genotypes that are infrequent in their populations and thus the algorithm was not able to recognize their ancestry.

In synthesis, the patterns generated by the use of the proportion of shared alleles as a similarity measure between mites and by a structure assessment using the Bayesian method agreed with each other. When the full data set was used, four distinct genetic clusters of mites were inferred: omnivore-, Italian herbivore-, carnivore- and Spanish herbivore-derived parasites.

4. Discussion

Besides being very difficult and time-consuming, the differentiation of host-specific mites using morphological traits proved to be impossible when mites of the same host-specific variant but from different geographical component populations had to be compared (Arlian et al., 1984; Arlian, 1989). Short fragments of mitochondrial or ribosomal DNA spacer regions have been shown to be unsuitable markers for examining genetic diversity between *Sarcophages* mite populations (e.g. Alasaad et al., 2009c; Skerratt et al., 2002). Better resolution is obtained from faster-evolving hypervariable sequences such as nuclear polymorphic microsatellite loci. Microsatellites have previously been shown to provide strong support for geographically discrete populations and congruence with evolutionary patterns at population level, as well as reported genetic differentiation at the skin-scale of individual mangy hosts (Bowcock et al., 1994; Walton et al., 2004b; Alasaad et al., 2008b). Walton et al. (1999) using multi-locus genotyping, applying microsatellite markers, substantiated previous data that gene flow between scabies mite populations on human and dog hosts is extremely rare in northern Australia.

Most component populations were seriously deficient in heterozygosity for all loci and mites belonging to the same component populations were more scattered through the same cluster than they were subdivided across individual host animals.

Sarcophages mites lack free-living stages, and individual hosts, depending on their susceptibility and behaviour, are essentially ephemeral habitats providing patchy environments that hamper random mating (Price, 1980; Criscione et al., 2005). All mites on an individual host may in fact form an 'infrapopulation' (Bush

et al., 1997) that has a number of recurrent generations. The number of generations is influenced by the short generation interval in this parasite (about 3 weeks), as well as by the infected host's life expectancy and susceptibility.

In our data set, reduced gene pools made mites resemble each other and hid any possible equilibrium existing between dispersive processes and gene flow within infrapopulations. This may be due to the rapid diffusion of a few genotypes such as occurs in an epidemic population structure (Oura et al., 2005).

Another evident feature of our results is the lack of homogeneity in the genetic diversity across populations. French *S. scrofa* mites were the most variable of all and did not have the heterozygosity deficiency that appeared in the other populations. Wild boar populations are widespread and growing, and generally have greater resistance to parasites than other mammalian species (Rodrigues and Hiraoka, 1996; Nejsun et al., 2009). Consequently, a single host can be affected by repeated infestation events caused by mites from other infrapopulations or even from other component populations. This hypothesis must be confirmed by analysing more *S. scrofa* mites from both France and Italy.

The other major determinants of gene flow between mites are the degree of host specificity and geographical structure of host populations. Previous research has shown that individual clustering approaches provide an appropriate characterization of population structure at high *F_{st}* values (Rosenberg et al., 2001; Manel et al., 2002; Latch et al., 2006). In the case of very different taxa, only a few loci are needed to achieve high performances, regardless of sample sizes (Manel et al., 2002; Tadano et al., 2008). In fact, the ideal marker locus for our purposes would have to be monomorphic within the taxon but polymorphic between different taxa (Reed, 1973).

In our data set, the unusually high value of *F_{st}* and the high number of private alleles in most populations indicated that the mite component populations differed greatly. All the 10 loci provided a significant component of between populations diversity. Consequently, our marker panel provided a very accurate analysis of the genetic characteristics of *Sarcoptes* populations.

S. scabiei (a) from different host species from different geographical localities, (b) from the same host species from different geographical localities, and (c) from closely related host species from different geographical localities clustered to their original populations. Clear genetic diversity between mite populations from different geographical localities exists. It seems that the differences increase as the geographical separation between host populations grows. In the case of small geographical separations (eastern and western Sierra Nevada) mites from *C. pyrenaica* scattered randomly in the dendrogram and no clear separation was detected. The differentiation between *V. vulpes* mites from Spain and Italy was highly supported, whereas the genetic separation between *V. vulpes* mites from the eastern and the western Italian Alps was poorly supported. This finding suggests that gene flow occurring between mites from different localities is apparently related to geographical distances.

The individual mites belonged to six sympatric host-derived mite populations from the eastern Italian Alps and three sympatric host-derived mite populations from the western Italian Alps clustered into three main groups (Figs. 1, 3 and 4): herbivore-derived mites (ItNECi, ItNERr, ItNEOm and ItNECe), carnivore-derived mites (ItNEVv, ItNEMm, ItNWMf and ItNWVv) and omnivore-derived mites (ItNWSs). In particular, mites from *S. scrofa* were distinct from both herbivore- and carnivore-derived mites and did not cluster with the sympatric north-west Italian population. In other words, the host-specific separation between the three clusters was stronger than that of the geographical separation between the eastern and western Italian Alps.

Similar results were obtained when the mite samples of our investigation were analysed as a whole (Figs. 1 and 4). For example, Cluster III contained all the carnivore-derived mites regardless of their geographical origins in the different European countries under study.

Our results from the sympatric wild animals in Italy and from the general analysis of all mite populations show unambiguously that there has been a lack of gene flow or recent admixture between carnivore-, herbivore-, and omnivore-derived *Sarcoptes* populations. Mite transmission may occur within each herbivore-, carnivore-, and omnivore-derived mite cluster, but it seems to be extremely rare or absent between them. Genetic separation of host-associated mites, emerged in this study, may reasonably mirror selection of adaptive albeit putative physiological differences between mite strains. This might improve adaptations in these parasites and lead to what we have called host-taxon-derived (carnivore, herbivore, and omnivore host-derived) *Sarcoptes* mite populations.

The population structure of *Sarcoptes* is probably that of a species subdivided into genetically small populations with restricted gene flow between local demes (Price, 1980; Martínez et al., 1999; Nadler and Hafner, 1990). Strong specialisation could be the result of a host-taxon-derived shift and, even if two host-taxon-derived species are sympatric for their host species, they should be considered as allopatric if the parasites have no possibility of host choice. In other words, host sympatry is not the same as parasite sympatry.

The probability of disease transfer between sympatric host-taxon-derived species could be reduced by the evolution of intrinsic mechanisms such as behaviour that are selected to impede crosses between individuals from two different host-taxon-derived species, and this could represent the first step towards sympatric speciation (McCoy, 2003). As such, the host-taxon-derived effect would seem to be stronger than the geographical separation in the definition of speciation events.

The existence of host-taxon-derived *Sarcoptes* mites could explain why mange-free populations can live in sympatry with many animals, as is the case of the mange-free *C. ibex* and *R. rupicapra* of the western Italian Alps that live in sympatry with the endemically mangy population of *V. vulpes*. This effect could also be the reason why cross-transmission/infection occurs in some *Sarcoptes* varieties (for example, *S. scabiei* var *vulpes/canis*) and readily infects dogs and other canids, as well as felids including domestic European cats, all of which possess the same host-taxon-derived mite (Bornstein, 1995).

We have no clear explanation of this taxonomic affiliation. Further studies on the dispersal capability of host animals and their readiness to interact, host behaviour and parasite adaptation are still needed if we are to understand host-taxon-derived *Sarcoptes*. The characterization of both host and mite population genetic structures would contribute further valuable knowledge.

The new phenomenon that we describe in this study will have important implications in *Sarcoptes* diagnostic test development, the emergence and monitoring of drug resistance, as well as in health surveillance in wildlife reintroduction programmes.

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References

- Alasaad, S., Rossi, L., Maione, S., Sartore, S., Soriguer, R.C., Pérez, J.M., Rasero, R., Zhu, X.Q., Soglia, D., 2008a. HotSHOT Plus ThermalSHOCK, a new and efficient technique for preparation of PCR-quality Sarcopites mite genomic DNA. *Parasitology Research* 103, 1455–1457.
- Alasaad, S., Soglia, D., Sarasa, M., Soriguer, R.C., Pérez, J.M., Granados, J.E., Rasero, R., Zhu, X.Q., Rossi, L., 2008b. Skin-scale genetic structure of *Sarcopites scabiei* populations from individual hosts: empirical evidence from Iberian ibex-derived mites. *Parasitology Research* 104, 101–105.
- Alasaad, S., Rossi, L., Soriguer, R.C., Rambozzi, L., Soglia, D., Pérez, J.M., Zhu, X.Q., 2009a. *Sarcopites* mite from collection to DNA extraction: the lost realm of the neglected parasite. *Parasitology Research* 104, 723–732.
- Alasaad, S., Soglia, D., Maione, S., Sartore, S., Soriguer, R.C., Pérez, J.M., Rasero, R., Rossi, L., 2009b. Effectiveness of the postponed isolation (post-frozen isolation) method for PCR-quality *Sarcopites* mite gDNA. *Experimental and Applied Acarology* 47, 173–178.
- Alasaad, S., Soglia, D., Spalenza, V., Maione, S., Soriguer, R.C., Pérez, J.M., Rasero, R., Ryser Degiorgis, M.P., Nimmervoll, H., Zhu, X.Q., Rossi, L., 2009c. Is ITS-2 rDNA suitable marker for genetic characterization of *Sarcopites* mites from different wild animals in different geographic areas? *Veterinary Parasitology* 159, 181–185.
- Arlian, L.G., 1989. Biology, host relations and epidemiology of *Sarcopites scabiei*. *Annual Review Entomology* 34, 139–161.
- Arlian, L.G., Runyan, R.A., Estes, S.A., 1984. Cross infectivity of *Sarcopites scabiei*. *Journal of American Academy of Dermatology* 10, 979–986.
- Berilli, F., D'Amelio, S., Rossi, L., 2002. Ribosomal and mitochondrial DNA sequence variation in *Sarcopites* mites from different hosts and geographical regions. *Parasitology Research* 88, 772–777.
- Blanchong, J.A., Samuel, M.D., Scribner, K.T., Weckworth, B.V., Langenberg, J.A., Filcek, K.B., 2008. Landscape genetics and the spatial distribution of chronic wasting disease. *Biology Letters* 4, 130–133.
- Bornstein, S., 1995. *Sarcopites scabiei* infections of the domestic dog, red fox and pig: clinical and serodiagnostic studies. Doctoral thesis, Swedish University of Agricultural Sciences, Department of Veterinary Microbiology, Section of Parasitology, Uppsala, Sweden.
- Bornstein, S., Möhrner, T., Samuel, W.M., 2001. *Sarcopites scabiei* and sarcoptic mange. In: Samuel, W.M., Pybus, M.J., Kocan, A.A. (Eds.), *Parasitic Diseases of Wild Mammals*, 2nd ed. Iowa State University Press, Ames, pp. 107–119.
- Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J., Cavalli-Sforza, L.L., 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368, 455–457.
- Burgess, I., 1999. Biology and epidemiology of scabies. *Current Opinion in Infectious Diseases* 12, 177–180.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83, 575–583.
- Criscione, C.D., Poulin, R., Blouin, S., 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Molecular Ecology* 14, 2247–2257.
- Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. *Science* 287, 443–449.
- Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14, 2611–2620.
- Excoffier, L., Laval, G., Schneider, S., 2005. ARLEQUIN ver 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1, 47–50.
- Fain, A., 1968. Étude de la variabilité de *Sarcopites scabiei* avec une révision des Sarcopitidae. *Acta zoologica et pathologica Antverpiensia* 47, 1–196.
- Fain, A., 1978. Epidemiological problems of scabies. *International Journal of Dermatology* 17, 20–30.
- Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164, 1567–1587.
- Fandos, P., 1991. La cabra montés (*Capra pyrenaica*) en el Parque Natural de Las Sierras de Cazorla, Segura y Las Villas. ICONA-CSIC, Madrid.
- Felsenstein, J., 1989. PHYLIP – phylogeny inference package (version 3.2). *Cladistics* 5, 164–166.
- Glaubitz, J., 2004. CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes* 4, 309–310.
- González-Candela, M., León-Vizcaino, L., Cubero-Pablo, M.J., 2004. Population effects of sarcoptic mange in Barbary sheep (*Ammotragus lervia*) from Sierra Espuña Regional Park, Spain. *Journal of Wildlife Diseases* 40, 456–465.
- Goudet, J., 1995. FSTAT (v. 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86, 485–486.
- Gu, X.B., Yang, G.Y., 2008. A study on the genetic relationship of mites in the genus *Sarcopites* (Acari: Sarcopitidae) in China. *International Journal of Acarology* 32, 183–190.
- Huson, D.H., Dezulian, T., Franz, M., Rausch, C., Richter, D.C., Rupp, R., 2007. Dendroscope – an interactive tree drawer. *BMCB* 8, 460.
- Latch, E.K., Dharmarajan, G., Glaubitz, J.C., Rhodes Jr., O.L., 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Biology* 7, 295–302.
- León-Vizcaino, L., Ruiz de Ybáñez, M.R., Cubero, M.J., 1999. Sarcoptic mange in Spanish ibex from Spain. *Journal of Wildlife Diseases* 35, 647–659.
- Lewontin, R.C., 1964. The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics* 49, 49–67.
- Lyles, A.M., Dobson, A.P., 1993. Infectious disease and intensive management: population dynamics, threatened hosts, and their parasites. *Journal of Zoo and Wildlife Medicine* 24, 315–326.
- Manel, S., Berthier, P., Luikart, G., 2002. Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conservation Biology* 16, 650–659.
- Martínez, J.G., Soler, J.J., Soler, M., Möller, A.P., Burke, T., 1999. Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*) and its primary host, the magpie (*Pica pica*). *Evolution* 53, 269–278.
- Mathews, F., Moro, D., Strachan, R., Gelling, M., Buller, N., 2006. Health surveillance in wildlife reintroductions. *Biological Conservation* 131, 338–347.
- McCoy, K.D., 2003. Sympatric speciation in parasites – what is sympatry? *Trends in Parasitology* 19, 400–404.
- Minch, E., 1997. <<http://hpgl.stanford.edu/projects/microsat/>>.
- Mörner, T., 1992. Sarcoptic mange in Swedish wildlife. *Revue Scientifique et Technique de l'Office International des Epizooties* 11, 115–121.
- Nadler, S.A., Hafner, M.S., 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomyidae). *Evolution* 44, 942–951.
- Nejsum, P., Roepstorff, A., Jørgensen, C.B., Fredholm, M., Göring, H.H., Anderson, T.J., Thamsborg, S.M., 2009. High heritability for Ascaris and Trichuris infection levels in pigs. *Heredity* 102, 357–364.
- Oleaga, A., Balseiro, A., Gortázar, C., 2008. Sarcoptic mange in two roe deer (*Capreolus capreolus*) from northern Spain. *European Journal of Wildlife Research* 54, 134–137.
- Oura, C.A.L., Asimwe, B.B., Weir, W., Lubega, G.W., Tait, A., 2005. Population genetic analysis and sub-structuring of *Theileria parva* in Uganda. *Molecular and Biochemical Parasitology* 140, 229–239.
- Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288–295.
- Pence, D.B., Ueckermann, E., 2002. Sarcoptic mange in wildlife. *Revue Scientifique Et Technique* 21, 385–398.
- Pérez, J.M., Ruiz-Martínez, I., Granados, J.E., Soriguer, R.C., Paulino, F., 1997. The dynamics of sarcoptic mange in the ibex population of Sierra Nevada in Spain – influence of climatic factors. *Journal of Wildlife Research* 2, 86–89.
- Price, P.W., 1980. *Evolutionary Biology of Parasites*. Princeton University Press, Princeton NJ.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenism. *Journal of Heredity* 86, 248–249.
- Reed, T.E., 1973. Number of gene loci required for accurate estimation of ancestral population proportions in individual human hybrids. *Nature* 244, 575–576.
- Robinson, T., 1996. Wildlife – a reservoir for disease. *Microbiology Australia* 7, 30–32.
- Rodrigues, D.L., Hiraoka, M., 1996. Sus scrofa domestica endoparasitic resistance in the Amazonas. *Annals of the New York Academy of Sciences* 791, 473–477.
- Rosenberg, N.A., Burke, T., Eloe, K., Feldman, M.W., Freidlin, P.J., Groenen, M.A.M., Hillel, J., Mäki-Tanila, A., Tixier-Boichard, M., Vignal, A., Wimmers, K., Weigend, S., 2001. Empirical evaluation of genetic clustering methods using multilocus genotypes from 20 chicken breeds. *Genetics* 159, 699–713.
- Rossi, L., Fraquelli, C., Vesco, U., Permunian, R., Sommariva, G.M., Carmignola, G., Da Pozzo, M., Meneguz, P.G., 2007. Descriptive epidemiology of a scabies epidemic in chamois in the Dolomite Alps, Italy. *European Journal of Wildlife Research* 53, 131–141.
- Ryser-Degiorgis, M.P., Ryser, A., Bacciarini, L.N., Angst, C., Gottstein, B., Janovsky, M., Breitenmoser, U., 2002. Notoedric and sarcoptic mange in free-ranging lynx from Switzerland. *Journal of Wildlife Diseases* 38, 228–232.
- Scott, M.E., 1988. The impact of infection and disease on animal populations: implications for conservation biology. *Conservation Biology* 2, 40–56.
- Skerratt, L.F., Campbell, N.J.H., Murrell, A., Walton, S., Kemp, D., Barker, S.C., 2002. The mitochondrial 12S gene is a suitable marker of populations of *Sarcopites scabiei* from wombats, dogs and humans in Australia. *Parasitology Research* 88, 376–379.
- Slatkin, M., 1994. Linkage disequilibrium in growing and stable populations. *Genetics* 137, 331–336.

- Slatkin, M., Excoffier, L., 1996. Testing for linkage disequilibrium in genotypic data using the EM algorithm. *Heredity* 76, 377–383.
- Soglia, D., Rambozzi, L., Maione, S., Spalenza, V., Sartore, S., Alasaad, S., Sacchi, P., Rossi, L., 2009. Two simple techniques for the safe *Sarcoptes* collection and individual mite DNA extraction. *Parasitology Research* 105, 1465–1468.
- Tadano, R., Nishibori, M., Tsudzuki, M., 2008. High accuracy of genetic discrimination among chicken lines obtained through an individual assignment test. *Animal Genetics* 39, 567–571.
- Walton, S.F., Currie, B.J., Kemp, D.J., 1997. A DNA fingerprinting system for the ectoparasite *Sarcoptes scabiei*. *Molecular and Biochemical Parasitology* 85, 187–196.
- Walton, S.F., Choy, J.L., Bonson, A., Valle, A., McBroom, J., Taplin, D., Arlian, L., Mathews, J.D., Currie, B., Kemp, D.J., 1999. Genetically distinct dog-derived and human-derived *Sarcoptes scabiei* in scabies-endemic communities in northern Australia. *American Journal of Tropical Medicine and Hygiene* 61, 542–547.
- Walton, S.F., Holt, D.C., Currie, B.J., Kemp, D.J., 2004a. Scabies: new future for a neglected disease. *Advances in Parasitology* 57, 309–376.
- Walton, S.F., Dougall, A., Pizzutto, S., Holt, D., Taplin, D., Arlian, L.G., Morgan, M., Currie, B.J., Kemp, D.J., 2004b. Genetic epidemiology of *Sarcoptes scabiei* (Acari: Sarcoptidae) in northern Australia. *International Journal for Parasitology* 34, 839–849.
- Zahler, M., Essig, A., Gothe, R., Rinder, H., 1999. Molecular analyses suggest monospecificity of the genus *Sarcoptes* (Acari: Sarcoptidae). *International Journal for Parasitology* 29, 759–766.