GENETICALLY DISTINCT DOG-DERIVED AND HUMAN-DERIVED SARCOPTES SCABIEI IN SCABIES-ENDEMIC COMMUNITIES IN NORTHERN AUSTRALIA

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Abstract. Overcrowding is a significant factor contributing to endemic infection with Sarcoptes scabiei in human and animal populations. However, since scabies mites from different host species are indistinguishable morphologically, it is unclear whether people can be infected from scabies-infested animals. Molecular fingerprinting was done using three S. scabiei-specific single locus hypervariable microsatellite markers, with a combined total of 70 known alleles. Multilocus analysis of 712 scabies mites from human and dog hosts in Ohio, Panama and Aboriginal communities in northern Australia now shows that genotypes of dog-derived and human-derived scabies cluster by host species rather than by geographic location. Because of the apparent genetic separation between human scabies and dog scabies, control programs for human scabies in endemic areas do not require resources directed against zoontotic infection from dogs.

When scabies is endemic in humans and dogs in the same community, it is unclear whether humans and dogs are potential hosts in a single transmission cycle or whether there are two separate transmission cycles. Morphologic studies cannot distinguish mites from dogs (Sarcoptes scabiei var. canis) from those of human origin (var. hominis), and it is not known whether var. canis is capable of infecting and completing a life cycle in humans.1-4

In Aboriginal communities in northern Australia, scabies-infested dogs and people live in close proximity. Concerns have been raised about the allocation of health resources for dog scabies programs on the assumption that this will help control human scabies5. Additionally, if dogs are reservoirs of infection, an understanding of gene flow between mite populations is important for the development of rational policies to minimize the spread of any developing scabicide resistance. The genetic comparison of mites from different global locations is also important to understand whether a regional epidemiologic situation can be generalized to the remainder of the world, as well as to measure patterns of host specific differences, and investigate geographic variation among populations.

We have developed microsatellite genotyping to study scabies mites using a novel fluorescent-based polymerase chain reaction (PCR) automated assay (Walton S and others, unpublished data) for 3 hypervariable S. scabiei specific microsatellites.6-8 The method has been used to explore whether mites of dog and human origin in overlapping and geographically isolated populations are genetically different, and to thus determine whether dog scabies can be a reservoir for infestation of humans.

MATERIALS AND METHODS

Collection of S. scabiei. Sarcoptes scabiei were collected from humans and dogs in Aboriginal households in remote communities in northern Australia, with 10–15 residents and 0–5 dogs per house. Houses for study were selected through cases with uncomplicated clinical scabies, or through patients with crusted scabies. Individuals with clinical symptoms had their skin scraped dry or following the application of mineral oil.9 Dogs with mange were dry-scraped, primarily around the ears and head. Skin scrapings and oil were transferred to a microscope slide under a coverslip, and viewed within 24 hr. Dry skin scrapings were collected into a petri dish and viewed within 24 hr after warming to 37°C for 30 min. Individual mites were placed in digestion buffer (50 mM Tris-HCl, 1 mM EDTA, 0.5% sodium dodecyl sulfate, pH 8.5) and kept at −20°C until they could be stored at −70°C.

Human-derived mites were also obtained from a scabies-endemic village in Panama, with mites extracted from the skin by skilled indigenous assistants using sterile sewing needles. Individual mites collected from each person were pooled and stored in 70% ethanol. Dog-derived mites from long-term, experimentally infested rabbits and dogs were obtained from Ohio. Mites were extracted from the skin scrapings of individual animals, pooled, and stored dry at −20°C. Finally wombat-derived mites were obtained from the skin crusts of a severely infested Australian native common wombat (Vombatus ursinus). Wombat-derived mites were pooled and stored dry at −70°C.

Approval to undertake this study was received from the Joint Institutional Ethics Committee of the Royal Darwin Hospital and Menzies School of Health Research and informed consent was obtained from all communities and patients.

Fluorescent PCR analysis of microsatellite DNA. Preparation of S. scabiei genomic DNA was as described9; we are unaware of any other studies on S. scabiei DNA or genetics. A fluorescent PCR analysis based on the Applied Biosystems (Foster City, CA) format was introduced (Walton S and others, unpublished data) using 3 dinucleotide repeat markers (AG) of variable length, as summarized previously.8 Baverock and Moritz suggest reliable estimates of summary statistics can be obtained with a minimum of 3 loci composed of multiple alleles at a reasonably high frequency.10 To date, 18, 30, and 22 alleles for Sarms 1, Sarms 15, and Sarms 20, respectively, have been recorded.

Allele sizes were determined using the Prism® Gene Scan® version 2.2 software (Applied Biosystems) and the Genotyper DNA version 1.1 analysis software (Applied BioSystems, Foster City, CA). Allele names were designated based on total length in basepairs. Electrophoretic gel vari-
alleles were normalized using an internal lane standard (Applied Biosystems).

Statistical analysis. Because of null alleles at all loci,\(^4\) it was impossible to estimate unbiased allele frequencies without also assuming Hardy-Weinberg Equilibrium (HWE). Accordingly, a non-parametric exact test of genotypic frequencies was used.\(^1\) The GENEPOP software was used to compute exact tests for HWE in populations exhibiting no null alleles at specific loci, and calculate gene frequencies for genotypic population differentiation and for genotypic disequilibrium among pairs of loci.\(^2\) Genetic distance based on the proportion of shared alleles was used to measure the relationship between multilocus genotypes of human-derived and dog-derived mite populations.\(^3\) The average proportion, \(P_s\), of alleles shared over all loci gives the distance measure as \(1 - P_s\). Matrices were constructed from pairwise population distances using MICROSAT (Stanford University, Stanford, CA), with dendrograms based on hierarchical clustering.\(^4,5\) Two distance matrices were compared, one assuming all single alleles were homozygous, the other assuming all single alleles were heterozygous nulls.

RESULTS

Collection of \(S.\) scabiei. One hundred thirty-two people with scabies and 84 dogs with mange were examined over a 1-year period in 6 Aboriginal communities (Table 1). Two hundred fifty-two mites of human origin were obtained from 200 people with uncomplicated scabies and 84 dogs with mange were examined over a 1-year period in 6 Aboriginal communities (Table 1).

<table>
<thead>
<tr>
<th>Community</th>
<th>Host species</th>
<th>Number of mites</th>
<th>Number of hosts*</th>
<th>Number of houses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human</td>
<td>20</td>
<td>7 (104)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>175</td>
<td>6 (51)</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Human</td>
<td>170</td>
<td>5 (7)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>32</td>
<td>1 (10)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Human</td>
<td>82</td>
<td>3 (3)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Human</td>
<td>0</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>149</td>
<td>9 (20)</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Human</td>
<td>1</td>
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</tr>
<tr>
<td></td>
<td>Canine</td>
<td>1</td>
<td>1 (2)</td>
<td>1</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate the total number of hosts examined.

Genetics of \(S.\) scabiei populations in Aboriginal communities. Variability within a single host. Genotypes were determined for 128 \(S.\) scabiei mites from 5 body sites from a patient with crusted scabies and 64 mites from 5 body sites from a dog with mange. Due to amplification failures, the final analysis was based on 109 human-derived and 53 dog-derived mites. The human-derived subpopulation had a total of 10 alleles at each of the 3 loci, with only minor deviations from HWE at 2 body sites. The dog-derived subpopulation exhibited a total of 5, 11, and 8 alleles at Sarms 1, 15, and 20 respectively, again with only marginal evidence for non-random segregation at 1 body site. There was no evidence of significant linkage disequilibrium between alleles at Sarms 1, 15, or 20 loci for mites from either host species. Allele frequencies of the human-derived mites at the Sarms 15 locus are shown in Figure 1A. Genotypic differentiation using a G-like exact test, assuming independent loci, suggested there were no significant differences between samples collected from different body sites on an individual (Table 2, A and B). These results suggest that individual hosts harbor genetically restricted populations of mites with no significant subdivision by body site.

Variability between mites from different hosts of the same species in the same community. A total of 112 human-derived mites from 4 crusted scabies patients living in separate households in Community 2 were studied, with genotypes available for 102. Exact tests for genotypic differentiation showed highly significant differences between individual hosts, both overall and at individual loci (Table 2, C). Thirty-two mites were studied from each of 2 dogs from Community 1, with typings available for 55 mites, showing a high proportion of null homoyzogotes for Sarms 20 (69\% and 96\%, respectively). There was highly significant genotypic differentiation between the two dogs, both overall and at individual loci, with a combined \(P < 5 \times 10^{-9}\).

In contrast, for three dogs less than 1 year of age within a single household in Community 1, analysis of 15 mites revealed 4, 9, and 3 alleles at Sarms 1, 15, and 20, respectively. The G-based exact test revealed only minor deviations (combined \(P = 0.04\)) from the null hypothesis of genetic identity between dogs. Another comparison of 10 mites from a litter of 3 puppies (< 3 months old) in Community 4 showed only 2 alleles at Sarms 1, 5 alleles at Sarms 15, and 2 alleles at Sarms 20, with no significant genetic differences between mites from the 3 puppies.

These results suggest that population substructure does exist within a community, demonstrated here as highly significant genotypic differences between subpopulations of \(S.\) scabiei from different households. However, as shown by the \(P\) values associated with the analysis of dog-derived mites collected from a single household, there is evidence that some genetic exchange between mite subpopulations found within a host family may be occurring.

Variability between subpopulations of \(S.\) scabiei from humans and dogs in the same house. In Community 1, genotypes of 13 \(S.\) scabiei mites from 3 young dogs were compared with 9 genotypes of mites from a 4-week-old baby in the same house. Six alleles were observed for Sarms 1, 12 alleles for Sarms 15, and 6 alleles for Sarms 20. The frequency of Sarms 20 null-homozygous in the dog-derived population was 77\% compared with 0\% in human-derived mites. Allele frequencies at the Sarms 15 locus are shown...
Figure 1. Allele frequencies at the Sarms 15 locus of Sarcoptes scabiei. Alleles are designated according to their length in basepairs (bp).

A, 109 human-derived mites collected from 5 body sites from a person with crusted scabies (abdomen, buttocks, left arm, right arm, and mixed sites).

B, nine human-derived (C5) and 13 dog-derived mites (C5.dog) collected from the same household.

in Figure 1B. Indeed, in this analysis, genotypes at Sarms 15 and Sarms 20 loci did not overlap in dog and human mites, with highly significant P values estimated by exact tests for genotypic differentiation (Table 2, D). Despite collection difficulties and small numbers, these results suggest that household dogs were not the source of infection in the young baby and that dog-derived mites and human-derived mites are involved in separate transmission cycles.

Genetic variability between populations of mites collected from different host species within a community. All 102 human-derived S. scabiei mites genotyped from 4 crusted scabies patients in Community 2 were pooled and compared with 28 mites from a single dog living in the same community. Highly significant differences were recorded ($P < 0.0001$), with virtually non-overlapping genotypes between dog-derived and human-derived mites at all loci (Table 2, E). A comparison of 15 mites from 4 people with 68 mites from 5 dogs in Community 1 showed similar genotypic differentiation ($P < 0.0001$).

Variability between mites from different communities
within Northern Australia. The genotypic distribution of 66 dog-derived mites from Community 4 was clearly different from that for 68 mites derived from 5 dogs in Community 1 ($P < 0.0001$). In a similar analysis, 157 human-derived mites from 4 people living in Community 2 were clearly different from 54 human-derived mites obtained from 3 people in Community 3 ($P < 0.0001$).

**Sarcoptes scabiei populations from different countries and host species.** Variability between subpopulations of mites collected from a village in Panama. Of 114 human-derived *S. scabiei* mites stored in 70% ethanol and sent from Panama for analysis, only 36 from 3 individuals amplified a product with one or more markers. Genotype distributions of the three subpopulations consisting of 7, 17, and 12 mites, revealed 52% null homozygotes for Sarms 1. There was highly significant genetic heterogeneity between the samples from the three different human hosts (combined $P < 0.0001$) (Table 2, F). There was heterogeneity of similar degree (see above) between *S. scabiei* collected from individual hosts within communities in northern Australia.

Genetic variability between subpopulations of mites collected from long-term experimentally infested hosts. In the only animal model of scabies, *S. scabiei* var. *canis* are repeatedly passed on rabbits and dogs. Mites were collected from 2 subpopulations of dog-derived mites obtained from experimentally infested dogs and rabbits in Ohio. Thirty-two dog-derived mites (ex-rabbit) were collected in 1992, and 32 dog-derived mites (ex-dog) were collected in 1997, and subsequently genotyped. From 59 typings, 5 Sarms 1 alleles, 14 Sarms 15 alleles, and 3 Sarms 20 alleles were observed. Both subpopulations revealed greater than 95% null homozygotes at Sarms 20 loci, with no significant differences between subpopulations in genotype for all 3 loci, affirming their descent from a common founder group (Table 2, G).

**Analyses of genetic dissimilarity between host-associated populations.** A total of 712 mites were partitioned into 27 subpopulations of mites from individual hosts. Mites collected from a wombat in Melbourne, Australia, geographically isolated and derived from a host species independent of the group under study, were included as an outgroup. Hierarchical clustering of subpopulations (see Materials and Methods) identified 2 host-associated clades (Figure 2). Figure 2A displays the dendrogram when all single alleles are assumed to be homozygous, whereas Figure 2B assumes all single alleles are heterozygous nulls. Neither assumption alters the overall clustering of the mite subpopulations, indicating that the influence of null alleles had minimal affect on the outcome and supporting the robustness of the separation of human-derived mites from dog-derived mites. It is of particular interest that dog-derived mites from Ohio cluster with dog mites from northern Australia and that human-derived mites from Panama cluster in their own group with the northern Australian human-derived mites. Hierarchical partitioning in the outer tree branches reflects the within- and between-host associations of mite subpopulations previously observed with the non-parametric exact tests. The analysis also shows that host-associated subpopulations of mites also cluster by geographic origin. For example, in Figure 2A, dd and six are mite subpopulations from two dogs from Community 4, and C5.dog, bla, and blu are mite subpopulations from three dogs from Community 1. The dendrogram also confirms the significant heterogeneity recorded between the dog-derived mites (C5.dog) and the human-derived mites (C5) collected from a single household. The two sympatric host-associated subpopulations are clearly dissimilar and differentiate into the two host-associated clades observed in the dendrogram.

**DISCUSSION**

Although scabies mites are genetically variable at the 3 microsatellite loci studied (Sarms1, Sarms 15, and Sarms 20), samples from individual hosts are close to HWE, with little evidence for genetic differentiation between different body sites in individual hosts. However, when mites were sampled from different individuals of the same host species in the same community, there was clear genetic differentiation; mites with like genotypes were more often found within the same host. This non-random distribution within individual hosts is characteristic of other parasite populations, presumably because of colonization by limited numbers of founders. 17,18

Dog-derived and human-derived mites were also geneti-
Multilocus clustering analyses of *Sarcoptes scabiei* populations using a similarity matrix based on the proportion of shared alleles.11-15 The study is based on 470 human-derived mites, 217 dog-derived mites, and 25 wombat-derived mites. NT = Northern Territory, Australia; USA = United States (Ohio). **A**, analysis assumes all single alleles are homozygous. **B**, analysis assumes all single alleles are heterozygous nulls.
cally different from each other when sampled from the same community, and even when sampled from the same household. The hierarchical clustering (Figure 2) provides an overall summary of the genetic relatedness between all genotyped mites from 27 individual human and animal hosts. Human-derived mites from northern Australia and the Americas are more closely related to each other than they are to dog-derived mites, even when the latter come from the same community or household as the human-derived mites. Single or double locus analysis was unable to reproduce this divergence indicating the influence of all three loci on the outcome. The clustering of *S. scabiei* mites into host-associated populations indicates that mites from dogs and humans have separate transmission cycles and that dogs are not contributing to human scabies in Aboriginal communities (or vice versa). Although no diagnostic microsatellite genotypes (or combinations of genotypes) could identify individual mites of human or dog origin, the frequency of null alleles for Sarsm 20 was 50–95% in dogs, much greater than in humans (approximately 36%).

This study provides the first genetic evidence of within host-family transmission of *S. scabiei* mites. On 3 separate occasions mites, collected from 2 or more members of the same family were not shown to be genotypically different. Evidence for intrafamilial homogeneity was found for nine dog-derived mites from a litter of 3 puppies, for 13 dog-derived mites from three dogs in the same household, and for 42 human-derived mites from two sisters in the same community. In endemic areas, intrafamilial aggregation of scabies is frequently reported. Our results confirm long-held beliefs that transmission is mediated by close personal contact with an infected person. This explanation is supported by the lack of genotypic clustering between mites from individuals living in separate households.

This study shows that dog-derived and human-derived *S. scabiei* are genetically distinct even when present in the same household. This suggests that they are different species. Further analyses using different DNA-based methods are needed to finally resolve this question. However, the current evidence shows that dog scabies are unlikely to be a significant source of infection for people in scabies-endemic communities in northern Australia. This is supported by the recent success of a community-based intervention involving treatment of humans in the absence of a concurrent dog scabies program. Control programs directed against human scabies in endemic areas can now focus on human-to-human transmission.

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REFERENCES