

Study of transmission pattern in sarcoptic mange
of raccoon dogs (*Nyctereutes procyonoides*) in Japan

Summary of Doctoral Thesis

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Sarcoptic mange is a parasitic skin disease caused by the mite, *Sarcoptes scabiei*. In Japan, sarcoptic mange outbreaks have been observed in wild raccoon dogs (*Nyctereutes procyonoides*). Furthermore, *S. scabiei* have reportedly demonstrated close genetic relationships beyond the host-taxon relationships of wild mammals and domestic dogs in Japan, due to which sarcoptic mange epizootic in raccoon dogs may affect domestic/wild mammals.

Considering ecology of raccoon dogs, previous studies suspected that one of the primary factors associated with the sarcoptic mange epidemic in raccoon dogs was the increasing raccoon dog population densities and/or direct contact transmission between parent and offspring. It is imperative to fully elucidate the ecology of the host animals and parasites in cases of wildlife diseases. Therefore, there is a need to verify that the genetic structure of raccoon dogs and *S. scabiei* remains clear, and to examine the transmission pattern of *S. scabiei* in the same.

This study aimed to confirm factors associated with the epidemic of sarcoptic mange in raccoon dogs and transmission pattern of *S. scabiei*. The study area included Takasaki City, Gunma Prefecture, and Miura Peninsula, Kanagawa Prefecture; furthermore, the relationship between the raccoon dogs and sarcoptic mange was monitored using a camera trapping survey, and the genetic structure of raccoon dogs and *S. scabiei* was assessed via genetic analysis.

1. Monitoring the relationship between sarcoptic mange outbreak and the raccoon dog population densities, along with the associated breeding pairs via a camera trapping survey in Gunma Prefecture (Chapter 2, 3, 5)

The objective was the long-term monitoring of outbreak of sarcoptic mange and raccoon dog populations, and to identify the correlation between the former and the densities of raccoon dog population using the camera trapping survey method. Clinical signs of mange-compatible lesions were used, from camera trapping data, to estimate the population densities of the raccoon dogs using a Random Encounter Model. Additionally,

this study also aimed to investigate and examine *S. scabiei* transmission between breeding pairs of raccoon dogs. Therefore, I compared the number of sarcoptic mange outbreaks with that of raccoon dogs recorded at a given time.

A high relative population density of raccoon dogs is one of the factors resulting in an epidemic of sarcoptic mange in raccoon dogs. A three-fold increase in the normal raccoon dog population density significantly increased the possibility of causing a sarcoptic mange epidemic in raccoon dogs. It has previously been suggested that raccoon dog populations maintain a sarcoptic mange infestation over a period of 5 years; this has subsequently influenced the raccoon dog population dynamics in the long term.

There was no significant degree of correlation between a sarcoptic mange outbreak and number of raccoon dogs recorded at a given time; furthermore, mange-compatible individuals were typically recorded alone and not in pairs. Despite the fact that the *S. scabiei* transmission pattern in a breeding pair has previously been recognized, it is not considered as an important factor for sarcoptic mange epizootic in raccoon dogs.

2. Influence of kinship and habitat location on sarcoptic mange epizootic in raccoon dogs (Chapter 4)

This chapter aimed to examine the fact that *S. scabiei* transmission between kin via direct physical contact is an important factor for sarcoptic mange epizootic in raccoon dogs. Furthermore, we analyzed the genetic relationship between raccoon dogs, along with the effects of habitat on epizootic sarcoptic mange and the relationship between the habitats of raccoon dogs under investigation and *S. scabiei* infection

Genetic analysis revealed that the number of transmission between kin cases did not significantly influence *S. scabiei* infection rates. Rather, non-infested individuals demonstrated numerous kinship relationships. Therefore, direct contact *S. scabiei* transmission between kin may possibly be significantly unusual and may not be an important parameter with regard to sarcoptic mange epizootic in raccoon dogs.

However, the analysis of *S. scabiei* infection rates and regions of the village sections under investigation suggested that they were significantly correlated, and sarcoptic mange epizootic in raccoon dogs was considerably localized. Furthermore, direct contact between closely-related individuals in a habitat is an important factor in the transmission of sarcoptic mange.

3. Multiple paternity among raccoon dog examined via genetic analysis (Chapter 5)

This chapter aimed to examine whether the mating season facilitated direct physical contact among a large number of raccoon dogs; furthermore, we analyzed genotypes of three pregnant females and their respective litters using microsatellite markers to investigate the possibility of multiple paternity in wild raccoon dogs.

Among the three pregnant females, the fetuses of two demonstrated a certain number of loci with three or four paternity alleles. This outcome suggested that these fetuses had several different male parents. Microsatellites typically demonstrate higher mutation rates than other genetic markers. However, if mutation rates from 10^{-3} per locus per generation occurred one out of 17 loci, this probability is less than 0.1%. Therefore, there is a high probability that multiple paternity of wild raccoon dogs have occurred. Female wild raccoon dogs probably mate with several males during one estrus, and mating season is important for *S. scabiei* transmission.

4. Genetic structure analysis of raccoon dogs and *S. scabiei* in Gunma Prefecture (Chapter 7, 8)

The objective of this chapter was to estimate the genetic population structure of raccoon dogs to understand ecological factors such as their sedentary habits, and to evaluate the transmission patterns of sarcoptic mange by comparing the same with the genetic population structure of *S. scabiei*. I analyzed the genetic population structure of

raccoon dogs and *S. scabiei* from raccoon dogs and a red fox using mitochondrial DNA (mtDNA) and microsatellite loci.

Six mtDNA haplotypes were identified in raccoon dogs. Genetic population structure analysis of microsatellite loci revealed that the raccoon dogs could be categorized into three groups; F_{ST} values suggested that these groups were genetically differentiated. Each group was distributed and subsequently gathered, following which these outcomes indicated that there was little genetic exchange among these groups. Therefore, raccoon dogs in this study area were generally sedentary; thus it is possible that the presence of such ecological characteristics may have facilitated the occurrence of local epizootic sarcoptic mange.

Furthermore, mtDNA analysis of *S. scabiei* suggested that the occurrence of maternity sarcoptic mange in both raccoon dogs and red foxes were relatively identical. Genetic population structure analysis of microsatellite loci categorized *S. scabiei* into two groups; additionally, there were cases demonstrated the occurrence of both groups in the same host animal. Moreover, since the two genetic groups were mixed between raccoon dogs and red foxes, cross transmission of *S. scabiei* among these host animals may have been possible. Therefore, *S. scabiei* transmission may be a considerably complex process in raccoon dogs and several other animals.

5. Genetic structure analysis of raccoon dogs and *S. scabiei* in Kanagawa Prefecture (Chapter 9)

This chapter aimed to verify whether the results recorded in the Gunma Prefecture were universal, and to understand the transmission pattern of *S. scabiei* in urban areas. Here, I analyzed the genetic population structure of raccoon dogs rescued from sarcoptic mange and that of *S. scabiei* in the Miura Peninsula, Kanagawa Prefecture.

Nine mtDNA haplotypes were found in raccoon dogs, of which several were not genetically related. Genetic population structure analysis of microsatellite loci revealed that raccoon dogs could be categorized into three groups. These groups demonstrated

minimal genetic differences, suggesting that these three groups were evenly distributed in the entire region.

Three haplotypes were identified in the mtDNA analysis of *S. scabiei*. Three maternity sarcoptic mange cases were identified in raccoon dogs in the Kanagawa Prefecture. Genetic population structure analysis of microsatellite loci suggested that the *S. scabiei* could be categorized into two groups, with each demonstrating minor genetic differences. Both mtDNA analysis and microsatellite analysis revealed the presence of multiple genotypes in same host animals. There is a high degree of possibility of repeated *S. scabiei* infestation in raccoon dogs in the Kanagawa Prefecture. Therefore, this indicated that the infection opportunity of *S. scabiei* in raccoon dogs increases in the presence of a high raccoon dog population density caused by urbanization and direct physical contact between the hosts.

6. Major histocompatibility complex (MHC) variation in raccoon dogs (Chapter 10)

This chapter aimed to examine the relationship between sarcoptic mange epizootic in raccoon dogs and their immune system. Here, I analyzed the genotypes of MHC class II-linked microsatellites loci.

There were no significant differences between the recognized values of expected and observed heterozygosity, since the MHC gene diversity in raccoon dogs population is not particularly low. In Kanagawa Prefecture, several infested individuals demonstrated specific genotypes of MHC-linked microsatellites loci. Therefore, specific genotypes of the MHC gene may have been influenced the occurrence of severe sarcoptic mange infestation by eliciting an excessive immune response.

7. Conclusion (Chapter 11)

This study demonstrated that one of the main factors associated with the occurrence of sarcoptic mange epizootic in raccoon dogs was an increase in the their

population densities. A three-fold increase in the normal raccoon dog population densities resulted in the a high possibility of demonstrating sarcoptic mange. In the genetic analysis of pregnant females and fetus it was evident that the occurrence of multiple paternity in wild raccoon dogs was a distinct possibility. This suggested the presence of direct physical contact between multiple raccoon dogs during the mating season. An increase in the raccoon dog population densities during the mating season was associated with an increased frequency of *S. scabiei* transmission.

Previous studies suspected that the main factor associated with sarcoptic mange that was epizootic in raccoon dogs was is transmission via direct physical contact between the parent and offspring. However, these results suggested that the raccoon dogs were highly sedentary; furthermore, direct physical contact between closely related individuals in a habitat, without a familial bond, was an important factor in the local transmission of sarcoptic mange. Moreover, the distribution of genetic population structure of *S. scabiei* was not associated with the genetic population of raccoon dogs and demonstrated the presence of multiple genotypes within a single host animal. Additionally, two genetic groups were evenly distributed between raccoon dogs and red foxes, suggesting that cross transmission of *S. scabiei* among these animals were possible. Therefore, it is possible that *S. scabiei* transmission is relatively complex and occurs between raccoon dogs and several other animals. Additionally, there is a high possibility that numerous host animals demonstrated *S. scabiei* transmission in raccoon dogs. Therefore, to prevent the transmission of sarcoptic mange to human beings and domestic animals, it is necessary to avoid improvident contact with all wildlife, and not just raccoon dogs.