

Principal Research Results

Development of a New Survey Method for Wild Animals Using DNA Information – Raccoon-dog Individual Identification and Unknown Animal Sample Species Identification –

Background

It is necessary for Ecological impact assessments to select an indicator species from the viewpoint of superiority, typicality, and specialty to predict an impact on ecosystems resulting from human business activities. However, it is difficult to obtain sufficient data for quantitative evaluation merely by conventional ecological surveys that consist mostly of observation and confirmation. On the other hand, behavior and identification data of subject animals that cannot be found by conventional methods are likely to be obtained efficiently and accurately using state-of-art technologies including DNA analysis and information technology. Our institute has shown that feed plants and populations can be assessed using DNA information of hare feces excreted outdoors. Introduction of DNA analysis to Ecological impact assessment can present researchers with a highly accurate and effective survey tool.

Objectives

DNA markers for individual and sex identification were developed using the raccoon-dog as the subject; it is likely to be chosen as an indicator species for typicality in Ecological impact assessments. In addition, applicability of species identification by DNA analysis was demonstrated for biological samples including feces and bone fragments, for which species were difficult to distinguish by their appearance.

Principal Results

1. Raccoon-dog individual identification

(1) Development of markers for DNA profiling.

Microsatellite DNA sequences were used for raccoon-dog individual identification. They are typically employed in DNA examinations of humans under criminal investigation, in parentage tests, and the like (Fig.1). Microsatellite DNA with four-nucleotide repeats was isolated from raccoon-dogs to prepare eight markers for individual identification. For sex identification, a marker was developed that can identify sex on the basis of differences in nucleotide sequences using genes on the X and the Y chromosomes (Fig.2). In all, 20 raccoon-dogs were analyzed using these DNA markers. All individuals were identified, including their sex.

(2) Individual identification by DNA analysis of feces.

Individual identification by DNA analyses of raccoon-dog feces which were collected in Abiko city, Chiba prefecture, was attempted using the DNA markers described above. For DNA analysis of feces, the freshness and the preservation state outdoors of the feces is the most important. Feces that were collected in December and January with low temperature showed no corruption or decomposition. They were identifiable to the specific individual that excreted them. Moreover, even feces that were collected in warm September weather produced good DNA analysis results in cases where the samples were excreted the previous night, demonstrating that individual identification is possible if the feces are fresh.

2. Species identification using unknown animal samples

Animal species are identifiable using genes on mitochondrial DNA. Therefore, DNA analyses were performed for feces of Mustelidae that were unidentifiable as those of a certain species by their appearance (Fig.3), feces of small birds, broken bone fragments, and pieces of flesh of dead animals on the road, resulting in accurate species identification for all the samples. Such species identification using DNA information was confirmed to be an effective method for accurate species identification even from field signs such as feces, hair, and tissues of a part of an animal, for which identification has been difficult in conventional ecological surveys of wild animals.

Future Developments

An examination method will be developed using the individual information obtained from raccoon-dog feces DNA to analyze the population in the test field, the range of activities, and the social structure, to present a highly accurate method for ecosystem survey.

Main Researcher: Rikyu Matsuki, Ph. D.,

Research Scientist, Biological Environment Sector, Environmental Science Research Laboratory

Reference

R. Matsuki, et.al., 2006, "Development of new wildlife research techniques using DNA analysis - DNA typing of raccoon dog and species identification of unknown animal samples - ", CRIEPI Report V05017 (in Japanese)

2. Environment - Coexistence with the environment

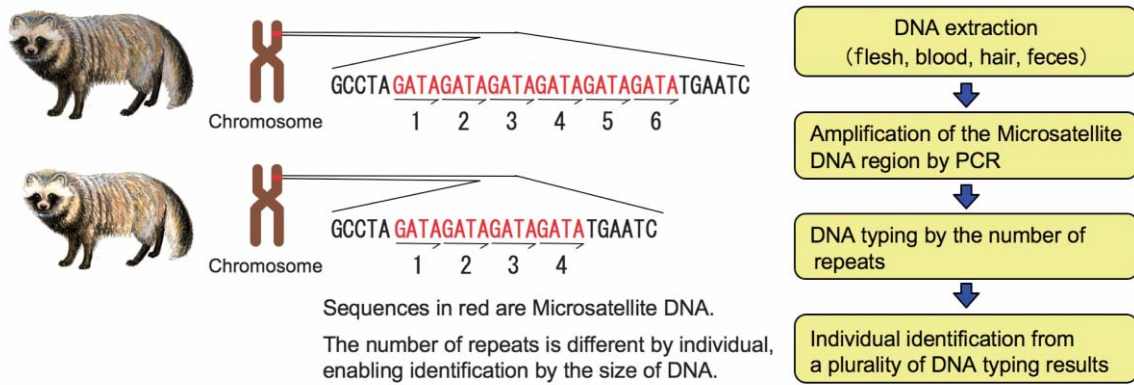


Fig.1 Individual identification using Microsatellite DNA

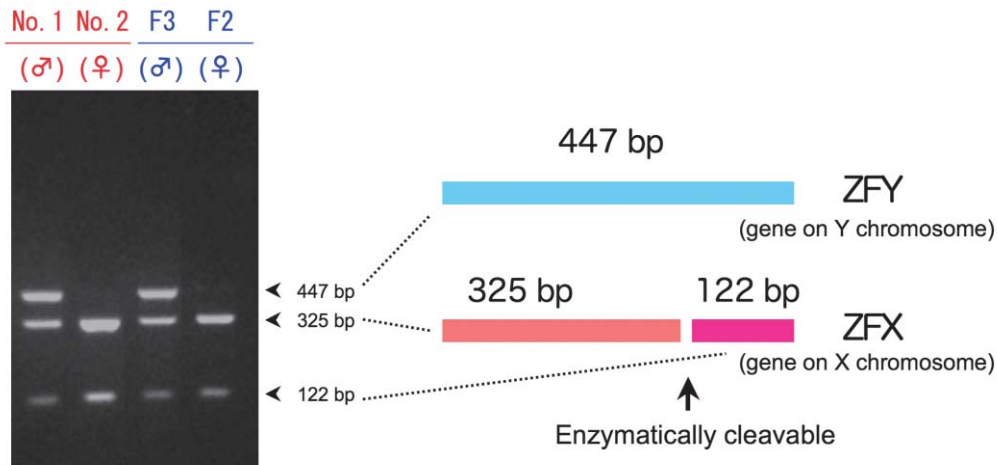


Fig.2 Sex identification of raccoon dogs using ZFX/ZFY genes

ZFX gene is enzymatically cleaved into two DNA fragments, while ZFY gene cannot be cleaved because of the difference in the nucleotide sequence. Therefore, via enzymatic treatment, male (XY) is detected as three DNA fragments with different sizes, and females (XX) as two DNA fragments. No.1 and No.2 show analysis results of DNA extracted from muscle tissues; F2 and F3 of DNA extracted from feces collected outdoors.

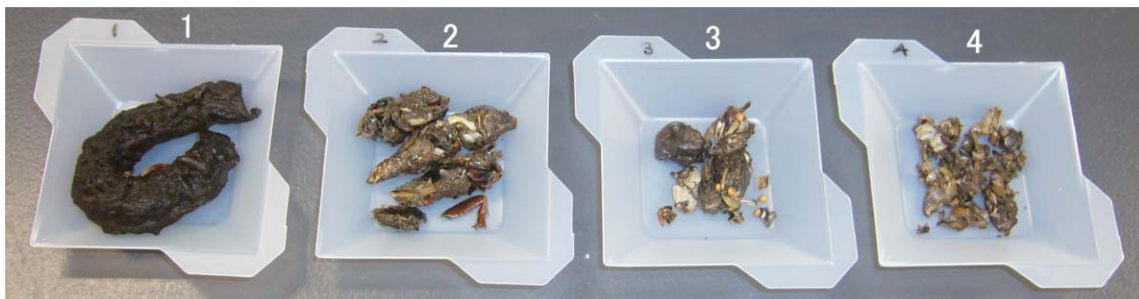


Fig.3 Species identification of feces of Mustelidae by DNA analysis

DNA was extracted from feces of Mustelidae collected outdoors to perform species identification by the cytochrome b gene on mitochondrial DNA. As a result, it was shown that 1, 2 and 3 were of marten, and 4 was of weasel.