

Identification of origin of sika deer (*Cervus nippon*) in recently expanded habitat areas in Tokyo Metropolis based on mitochondrial D-loop sequences

東京都で生息域を拡大するニホンジカのミトコンドリア DNA (D-loop 領域) 多型解析

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Abstract: Muscle tissue of 145 sika deer (*Cervus nippon*) collected in Tokyo Metropolis and surrounding regions in southern Kanto were investigated based on mitochondrial D-loop sequences. Four (CN-1-CN-4) of the nine identified haplotypes (CN-1-CN-9) accounted for 94.5% of the total study population. One (CN-1) of these four haplotypes was widely distributed throughout the whole study region, but the other 3 haplotypes (CN-2, CN-3, CN-4) were locally distributed in different areas of the region. Two (CN-2, 3) of these haplotypes were distributed around the Kanto Mountains, whereas the other (CN-4) was around the Tanzawa Mountains. All 6 individuals sampled in Hachioji City, a new habitat area that sika deer in Tokyo Metropolis have recently entered, possessed the haplotype found locally around the Kanto Mountains. Thus, we concluded that the deer captured in Hachioji City have been originated from the Kanto Mountains.

Key-word: habitat expansion, D-loop region, haplotype, Kanto Mountains

要旨: 東京都および周辺で捕獲された計 145 個体のニホンジカの筋肉片サンプルを用いてミトコンドリア DNA の D-loop 領域の多型解析を行った。ハプロタイプは、9 タイプ(CN-1~CN-9)検出され、そのうち 4 タイプが全体の 94.5%を占めていた。これら 4 タイプは、比較的広域に分布する CN-1 タイプと偏域的に分布する CN-2, CN-3, CN-4 タイプに二分された。このうち偏域分布する 3 タイプのうち、CN-2 および CN-3 タイプは関東山地周辺に分布し、CN-4 タイプは丹沢山地周辺に分布していた。都内の生息拡大域にあたる八王子市において捕獲された 6 個体は、すべて関東山地のハプロタイプを有していた。このことから、八王子市に定着したニホンジカは、関東山地個体群がソースとなっていると考えられた。

キーワード: 生息域拡大, D-loop 領域, ハプロタイプ, 関東山地

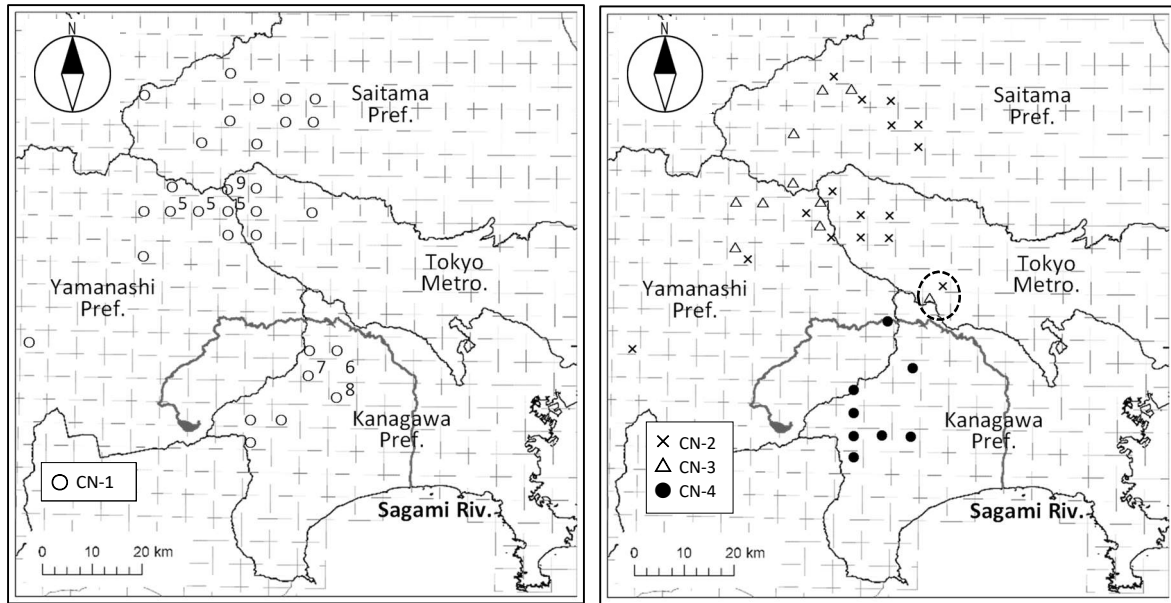


Fig.2. Distribution of D-loop haplotypes in the sampled region.

Wide distributed type (left) and local distributed type (right). “○”, “×”, “△” and “●” signs indicate the presence of the haplotype (CN-1-CN-4) in 5×5km square. Numbers in the left figure denotes haplotype CN-5, 6, 7, 8 or 9. The dashed line indicates the habitat area in Hachioji City into which sika deer is expanding its population.

図-2. D-loop ハプロタイプの地理的分布

左図が広域分布型、右図が偏域分布型のハプロタイプの分布を表す。四角の中の符号は、該当ハプロタイプが5×5kmメッシュの中に存在していたことを示す。左図の数字はハプロタイプCN-5,6,7,8,9のいずれかを表す。破線は生息拡大域(八王子市)。

were used to amplify and sequence the D-loop regions; L15926 and H597 designed by Nagata et al. (4) and a supplemental primer we designed for this study (5'-ATGCTTGACTCA GCAATGG-3'). The PCR products were purified using Exo SAP-IT (Affymetrix), and then these products were used as template for direct sequencing. The sequencing was carried out using an automated sequencer 3130xl Genetic Analyzer (Applied Biosystems), and the sequence alignment was performed using MEGA 6.0 computer software (6). The D-loop region of sika deer contains tandem repeats, each of which consists of approximately 40 bp motifs. The repetition of these units differs greatly between populations (5), so these regions were deleted for the present sequence analysis.

III Results and discussion

We compared the nucleotide sequences of mtDNA D-loop regions from the 145 sika deer and identified nine haplotypes (Table 1.). The most frequent haplotype was designated as CN-1 (46.2%), followed by CN-2 (20.7%), CN-3 (14.5%), CN-4 (13.1%), CN-5 (2.1%), CN-6 (1.4%), CN-7 (0.7%), CN-8 (0.7%), and CN-9 (0.7%). Haplotypes CN-1 to CN-4 accounted

for 94.5% of the total study population, whereas haplotypes CN-5 to CN-9 were each identified in only one to three deer. CN-1 was identical to sequences previously reported in deer in Tochigi Prefecture (AB500007 and AB500008; Registered region include tandem repeat region that is excluded in this study). The CN-6 haplotype, identified in two deer in this study, was identical to a haplotype from Tanegashima Island, Kagoshima Prefecture (AB757723) (7). These two deer with the CN-6 haplotype were considered to be immigrants, based on records of sika deer being imported and released in Kanagawa Prefecture (2).

The geographical distribution of the nine haplotypes is shown in Figure 2. The CN-1 haplotype was widely distributed across the study area, whereas the CN-2, CN-3, and CN-4 haplotypes were locally distributed in different regions. Specifically, the CN-2 and CN-3 haplotypes were distributed around the Kanto Mountains; north of the Sagami River, whereas the CN-4 haplotype was distributed around the Tanzawa Mountains; south of the Sagami River. Genetic divergence between deer from the Tanzawa Mountains and the Kanto Mountains have been identified previously by short sequence repeat (SSR)

analysis (3).

The CN-2 haplotype was found in four of the six sika deer samples collected from Hachioji City, while the other individuals had the CN-3 haplotype. Since the CN-2 and CN-3 haplotypes are characteristic of deer from the Kanto Mountains, it seems that the deer captured in Hachioji City originated from these Mountains. Five of these six sika deer were female. It is known that females tend to be observed later than males in an immigration phase (1). We concluded that sika deer originating from the Kanto Mountains have now settled in Hachioji City.

However, since an ear-tagged male deer from the Tanzawa Mountains was photographed in Hachioji City in 2013 (unpublished), it is virtually assured some sika deer to move from Tanzawa Mountains to Tokyo Metropolis. Further monitoring is needed to estimate the influence of the Tanzawa Mountain population on Tokyo population. We think that the analysis of sika deer migration source presented in this study will help future collaborative efforts by municipal and regional authorities to manage the sika deer population.

IV Conclusions

1. Four of the nine D-loop haplotypes identified in the present study accounted for 94.5% of the analyzed sika deer population; one haplotype was widely distributed (CN-1) and three were locally distributed (CN-2, CN-3, and CN-4).
2. The CN-2 and CN-3 haplotypes were locally distributed in the Kanto Mountains, and the CN-4 haplotype was distributed in the Tanzawa Mountains.
3. The six deer captured in Hachioji City where the population of sika deer has recently expanded into contained haplotypes of Kanto Mountains (CN-2 or CN-3). We consider that the deer captured in Hachioji City have been originated from the Kanto Mountains.

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References

- (1) ASADA, M. (2013) "Lag-phase management" as a population management method in low density areas in sika deer (*Cervus nippon*) and racoon (*Procyon lotor*). Journal of the Mammalogical Society of Japan **53**: 243-255 (in Japanese)
- (2) IIMURA, T. (1965) Tanzawasankai no sika ni kansuru kenkyu. Report of forestry helm in Kanagawa prefecture **13**:1-44 (in Japanese)
- (3) KONISHI, S., HATA, S., MATSUDA, S., ARAI, K., MIZOGUCHI, Y. (2017) Evaluation of the genetic structure of sika deer (*Cervus nippon*) in Japan's Kanto and Tanzawa mountain areas, based on microsatellite markers. Animal Science Journal **88**:1673-1677
- (4) NAGATA, J., MASUDA, R., KAJI, K., KANEKO, M., Yoshida, M. C. (1998). Genetic variation and population structure of Japanese sika deer (*Cervus nippon*) in Hokkaido Island, based on mitochondrial D-loop sequences. Molecular Ecology **7**: 871-877
- (5) NAGATA, J., MASUDA, R., TAMATE, H. B., HAMASAKI, S., OCHAI, K., ASADA, M., TATSUZAWA, S., SUDA, K., TADO, H., YOSHIDA, MC. (1999) Two genetically distinct lineages of the sika deer, *Cervus nippon*, in Japanese islands: Comparison of mitochondrial D-loop region sequences. Molecular Phylogenetics and Evolution **13**: 511-519
- (6) TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., KUMAR, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution **30**, 2725-2729.
- (7) TERADA, C., YAMADA, T UNO, H., SAITOH, T. (2013) New mtDNA haplotypes of the sika deer (*Cervus nippon*) found in Hokkaido, Japan suggest human-mediated immigration. Mammal Study **38**: 123-129