A mutant gene for albino body color is widespread in natural populations of tanuki (Japanese raccoon dog)

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A mutant gene for albino body color is widespread in natural populations of tanuki (Japanese raccoon dog)

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Albino mutants (white coat and red eyes) of tanuki (*Nyctereutes procyonoides viverrinus*) have been repeatedly found in the Central Alps area of Japan. We recently reported that an albino tanuki from Iida, a city in this area, lacks the third exon of the *TYR* gene encoding tyrosinase, which is essential for melanin synthesis. The absence of this exon was due to the chromosomal deletion of a complex structure. In the present study, we analyzed *TYR* of another albino tanuki that was found in Matsusaka, a city located outside the mountainous area. In this animal, the third exon was also lost, and the loss was due to a deletion in which the structure was identical to that of the Iida mutant. Our results indicate, in consideration of the complex structure of the deletion, that the two albino animals inherited a single deletion that arose in their common ancestor. Iida and Matsusaka are approximately 170 km apart. This is, to our knowledge, the first report of an albino mutant gene that is widely distributed in mammalian natural populations. As the origin of this mutation is not known, the distance covered by the mutant gene remains unclear. If we assume that the mutation occurred halfway between Iida and Matsusaka, we can predict the migration distance to be approximately 85 km; however, if the mutation occurred at any other place, a longer distance would be predicted. Natural selection against albino tanuki may be relaxed because of a recent increase in food resources and refuge in urban areas.

Key words: albinism, wildlife, migration, mutation, deletion

INTRODUCTION

Oculocutaneous albinism is the most extreme mode of albinism. It is characterized, in mammals, by a white coat and red eyes resulting from the absence of the melanin pigment in the hair and retina, respectively (Steingrímsson et al., 2006). This phenotype is considered to result in a survival disadvantage. The main putative factors for this disadvantage include a decrease in UV blocking efficiency, a higher chance of being detected by predators and of being noticed by prey, and a decrease in visual acuity. Mutant alleles, even if they are recessive to the wild-type allele, tend to be eliminated from natural populations on a long time scale when strong natural selection acts against homozygotes. However, the situation in tanuki differs from this population genetics prediction. Animals exhibiting oculocutaneous albinism have been repeatedly found in the Central Alps area of Japan. According to the records of the Iida City Zoo, the zoo received tanuki with oculocutaneous albinism, which were found in vermin control traps, in July 1979, April 1990, November 1999 and January 2017. We used the newest animal for genetic analysis in our previous study (Mae et al., 2020). In addition, one of the authors of this paper is a professional wildlife photographer who has frequently photographed albino tanuki with motion-activated cameras set up at various locations in this area (Miyazaki, 1988). One interesting photograph, taken

in August 2008, shows a family consisting of a mother with wild-type pigmentation and six offspring of which four are wild-type and two are of albino phenotype (Fig. 1). Assuming that this albinism was caused by a recessive allele, the family variation observed indicates that the mother was heterozygous for the albino and wild-type alleles, and that the father was either heterozygous or homozygous for the albino allele.

In our recent study (Mae et al., 2020), we analyzed the *TYR* gene of an albino tanuki that is cared for in Iida City Zoo. This animal, named Ryu, was born in the wild and found in a suburb of Iida City (Nagano Prefecture), which is located in the Central Alps area (Fig. 2). We showed that the albino phenotype of Ryu is caused by a mutation in the *TYR* gene (Mae et al., 2020). This gene, consisting of five exons, encodes the tyrosinase enzyme that catalyzes the first two reactions in melanin biosynthesis with no alternative pathway (Körner and Pawelek, 1982). The mutation was a deletion of approximately 11 kb that resulted in the loss of the third exon, which carries codons for four amino acid residues that bind copper and are essential for tyrosinase function (Olivares et al., 2002; Goldfeder et al., 2014). An important point related to the present study is that the deletion was not a simple removal of a segment: it exhibits a complex structure that is thought to have been formed by multiple mutational changes (see Fig. 4; models are shown in Mae et al., 2020). Ryu was homozygous for this structurally complicated allele. According to the entry 203100 (oculocutaneous albinism, tyrosinase-negative; https://www.omim.org/entry/203100) of OMIM (Online Mendelian Inheritance in Man), the vast majority of *TYR* mutations that cause oculocutaneous albinism syndromes are recessive to the wild-type *TYR* allele. This suggests that the structurally complicated allele carried by Ryu is recessive.

In the present study, we conducted cloning and sequencing analyses of *TYR* from another albino tanuki. In November 2014, an albino adult hit by a car was found on a road in Matsusaka City (Mie Prefecture). This animal was sent to the Oouchiyama Zoo, treated medically, retained there and named Pong. Matsusaka is located outside the Central Alps area (Fig. 2). As described below, our analyses revealed a deletion of the same structure in Pong as that in Ryu. Based on the results obtained, we discuss possible factors for the wide distribution of the albino mutant allele in tanuki natural populations.

MATERIALS AND METHODS

Ethics This study involved a recombinant DNA experiment that was approved in advance by the Recombinant DNA Experiment Safety Committee of Kyoto University (approval number 190058). The study did not include any animal experiments.

Fig. 1. Photographs of tanuki. (A) The three tanuki individuals used in this study. TanW has black eyes, and body color of the regular tanuki pigmentation pattern. TanA1 and TanA2 exhibit oculocutaneous albinism, having red eyes and white hair. (B) A tanuki family living in the wild. The family consists of a mother and six offspring.
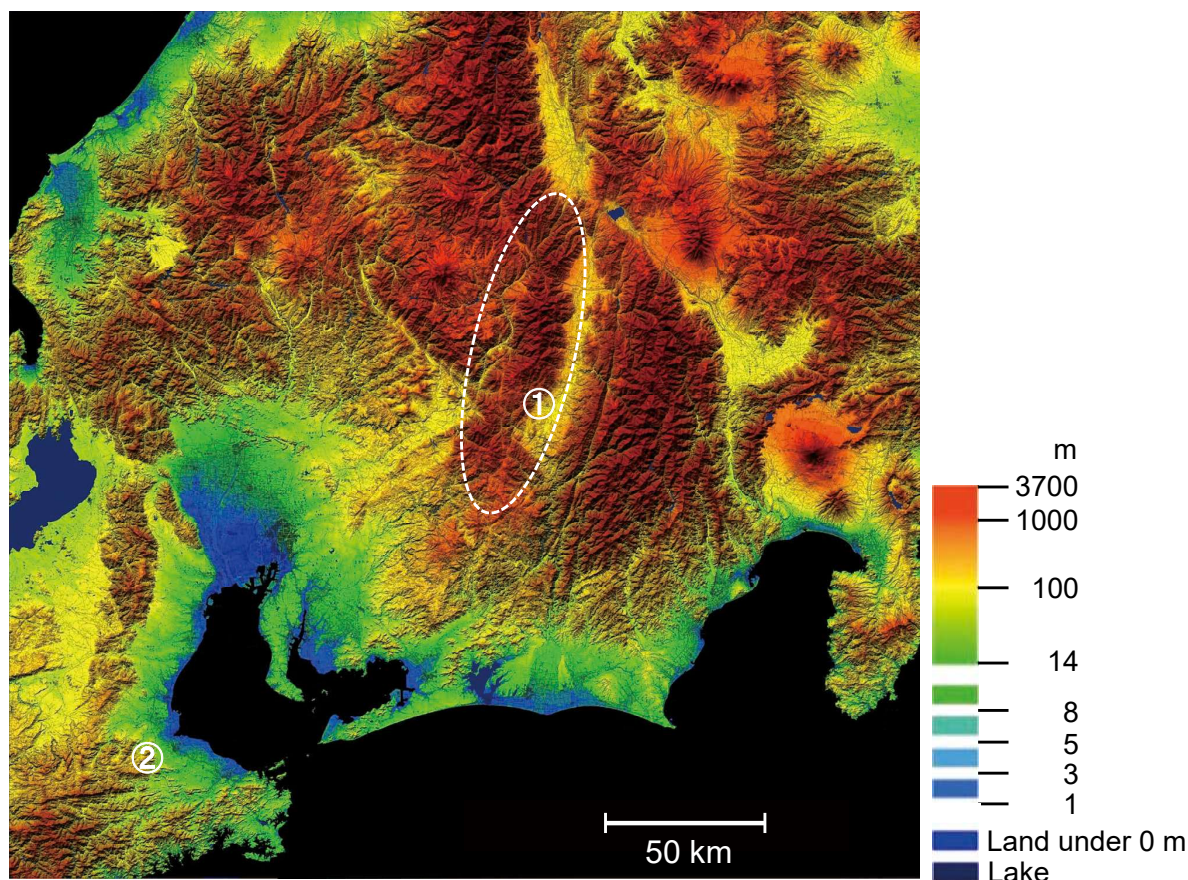
Fig. 2.   Locations at which albino tanuki were found.   TanA1 and TanA2 were found at the points indicated by circled numbers 1 and 2, respectively.   The dashed line indicates the Central Alps area.   The map is part of a topographical chart issued by the Geospatial Information Authority of Japan.

Tanuki animals   Pong was the animal whose genetic material was used in the sequencing analysis of *TYR* in the present study.   For simplicity, Ryu and Pong will be denoted hereafter by TanA1 (TanA in our previous study) and TanA2, respectively.   TanW is an animal with regular tanuki coloring that was used for comparison in our previous study.   TanA1 and TanA2 exhibit oculocutaneous albinism, which is easily distinguishable from the wild-type pigmentation of TanW (Fig. 1).

PCR, cloning and sequencing   To achieve an accurate comparison with our previous study, we did not make any change in the PCR primers or condition settings for PCR, cloning or sequencing (see Mae et al. (2020) for experimental details, including primer names).

RESULTS

Structure of *TYR* exon regions   We conducted PCR amplification of all five exon regions, using genomic DNA from TanW, TanA1 and TanA2 (Fig. 3).   The results reproduced our previous results: amplification of a single fragment of the expected length in all five exon regions

from TanW DNA, and lack of amplification for the exon 3 region in TanA1.   TanA2 exhibited an amplification pattern that was identical to that of TanA1.

Structure of the deletion   We cloned and sequenced the PCR fragments originating from TanA2.   The nucleotide sequences obtained were identical to those of TanA1 in all clones.   Wave patterns at and around the deletion breakpoints are shown in the lower part of Fig. 4.

Follow-up examination of DNA samples   Because the sequence data obtained from TanA2 were identical to those from TanA1, we considered that there might have been an accidental misuse of TanA1 DNA as TanA2 DNA.   To address this possibility, we performed an additional experiment to check whether the mitochondrial DNA also exhibited identical sequences.   Using the primers L15926 and H00651 for the control region (Kocher et al., 1989), we obtained PCR fragments from the same DNA samples of TanA1 and TanA2, as well as TanW, that were used for the main part of our analysis, and then sequenced them.   As depicted in the alignment in Fig. 5, the TanA1 and TanA2 sequences were noniden-
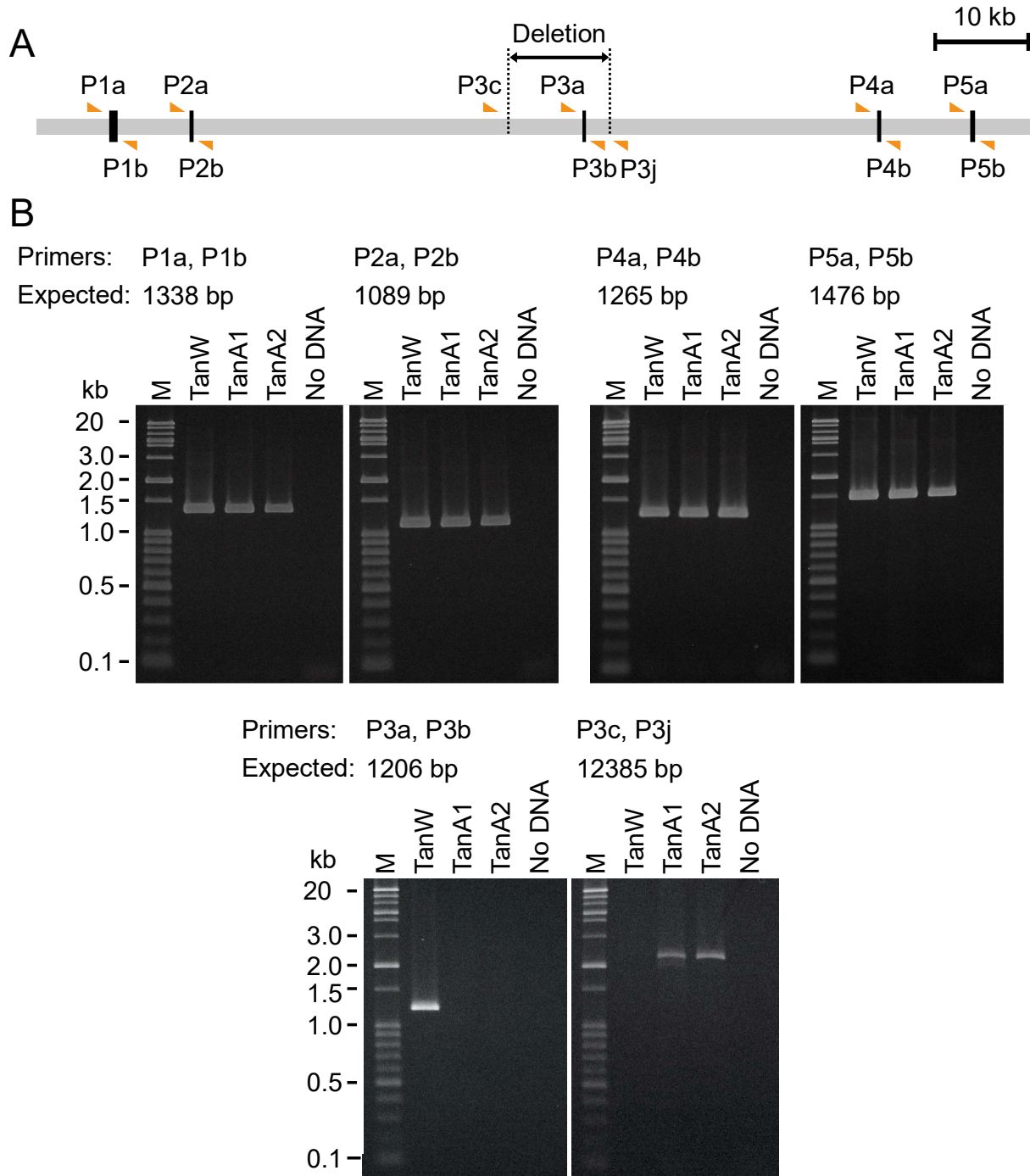
Fig. 3. PCR amplification of the *TYR* exon regions. (A) Overview of the *TYR* gene region. Black rectangles show the five exons of *TYR*. Orange triangles indicate the location and orientation of PCR primers to amplify each exon region (see Mae et al. (2020) for details). The double-headed arrow shows the region that is present in Tyr^+ but absent in Tyr^{del}. (B) Results of PCR amplification. Above each electrophoresis photograph, the primers used and the sizes expected based on the Tyr^+ sequence are shown. No PCR product was observed in the TanW lane of the P3c/P3j panel. This result is in accordance with that obtained in a previous study (Mae et al., 2020). The reason is that there is an upper size limit in the PCR using fecal DNA as a template, and the expected size (12,385 bp) exceeded this limit. In the previous study, we conducted additional PCR assays using four pairs of primers that divided this long region into four overlapping segments. All of these primer pairs produced fragments of the expected sizes, and sequence assembly resulted in a single sequence of 12,385 bp.
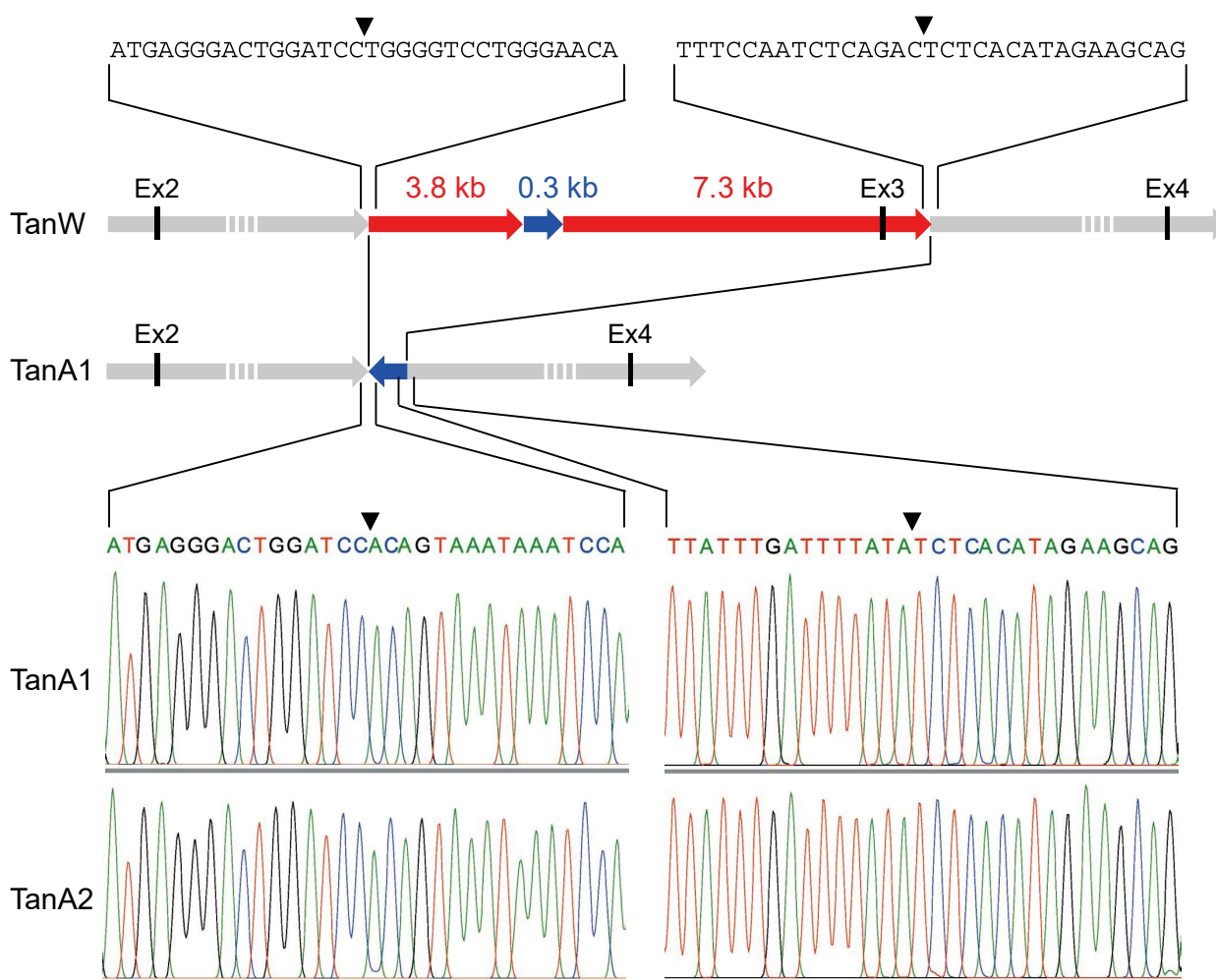
Fig. 4.   Sequence patterns around breakpoints.   The top two lines indicate the structure of the complex deletion that was revealed in our previous study.   The red and blue arrows show the segments that were deleted and retained, respectively, in TanA1 *TYR*.   Under the TanA1 line, wave patterns around breakpoints of TanA1 and TanA2 are shown.   The black triangles indicate the positions of the breakpoints.

tical.   This confirmed that these DNA samples were from different individuals and excluded the possibility of misuse of the samples.

DISCUSSION

Origin of the mutant *Tyr* allele   As explained in detail in our previous study, the deletion in *TYR* in TanA1 exhibited a complex structure: two separate regions of 3.8 kb and 7.3 kb were removed, and a segment of 0.3 kb between these two regions was retained but in the reverse orientation (Fig. 4). This complex structure was also found in TanA2, indicating that the deletion arose in a common ancestor of TanA1 and TanA2 and was inherited by them.   In addition to the deletion, exon 5 of TanA1 *TYR* had previously been shown to carry two nonsynonymous base substitutions (c.1530G>C and c.1535G>C leading to p.L500F and p.R502P, respectively)

(Mae et al., 2020).   In the present analysis we found that exon 5 of TanA2 *TYR* also harbors these base substitutions (data not shown), providing further evidence that a single mutant *TYR* allele was inherited by TanA1 and TanA2.   The *TYR* allele carrying this deletion will be denoted by Tyr^{del}, and other alleles that are dominant over Tyr^{del} and cause melanin pigmentation will be represented by Tyr^{+}.

Expansion in natural populations   Iida and Matsusaka are approximately 170 km apart (Fig. 2).   The present work is, to our knowledge, the first report of an albino mutant gene that is widespread in mammalian natural populations.   Because the origin of Tyr^{del} is not known, the distance that the Tyr^{del} allele has migrated over in natural populations remains unclear. If we assume that the mutation occurred halfway between Iida and Matsusaka, we can predict the migration distance to

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TanW    CATATCCATGTATTGTCACAATAGACATAAACTACTTAATCATACGGTATTTTACTTCAA    60
TanA1   ...............................G............................    60
TanA2   .........................................................G......  60

        GGACATGCTCAAGTTGCATATCACCTAGTCCAATAAGGGATTAATCACCATGCCTCGAGA   120
        ............................................................   120
        ............................................................   120

        AACCATCAATCCTTGCCTGAAGTGTACCTCTTCTCGCTCCGGGCCCATATTAACGTGGGG   180
        ............................................................   180
        ...............T............................................   180

        GTTTCTATCGTGAAACTATACCTGGCATCTGGTTCTTACCTCAGGGCCATGAACTCTCTC   240
        ............................................................   240
        ............................................................   240

        CATCCAATCCTACTAATCCTCTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCC   300
        ............................................................   300
        ............................................................   300

        CATGATCACACATAACTGTGGTGTCATGCATTTGGTATCTTTTATTTTTGGGGGGGGG-A   359
        .......................................................G.     360
        .......................................................-.........-.   358

        ATCTGCTATCACTCAGCTATGACCGCAACGGCACTAACTCTAACCTATTCCTGCATTCAG   419
        ............................................................   420
        ...........................................................A   418

        GGGATATGCCCGTCGCGGCCCTAACGCAGTCAGATAATATGTAGCTGGACTTGCTTTCAA   479
        .......................................................C.....   480
        ............................................................   478

        TCATTAATCAACTCCGTGCACAATTCAAGGTGCTATTCAGTCAATGGTTACAGGACATAA   539
        ............................................................   540
        ............................................................   538

        GAACTTTTACACACGTACACACGTACACACGTACACACGTACACACGTACACACGTAC     597
        ........................G...............G................     598
        ........................G...............................       596
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Fig. 5. Nucleotide sequences of mitochondrial DNA. The control region was PCR-amplified, sequenced and aligned. The entire sequence of TanW is illustrated. For TanA1 and TanA2, nucleotides identical to those in TanW are indicated by dots, and different nucleotides are presented by the letters for the nucleotides. Dashes indicate the absence of a corresponding nucleotide.

be approximately 85 km. If its source differs, then either Tyr^{del} in TanA1 or Tyr^{del} in TanA2 must have migrated over a longer distance. The greater the migration distance, the longer the allele is considered to have persisted in natural populations. From the viewpoint of population genetics, it needs to be taken into consideration that Tyr^{del} causes the most extreme mode of albinism and is therefore likely to have deleterious effects on its host individuals in natural circumstances. This raises the question of what factors contributed to the survival and expansion of Tyr^{del}.

Possibility of mediation by humans One possibility is that an animal carrying Tyr^{del} was transported by humans. For example, a tanuki kept as a pet may have been released at a different place than where it originally lived, or a tanuki may have wandered into a vegetable container that was shipped from farmland to market. These are quite improbable scenarios but cannot be ruled out at present.

Possibility of adaptation to urban environments Next, we assume that transport by humans is not involved in the Tyr^{del} migration. Adaptation of tanuki to urban environments may be an important factor in this allele migration. Urban development by humans pushes wildlife toward elimination in general, but tanuki may be an exceptional species. Competition among tanuki individuals may be weakened because of a recent relative increase in food resources and refuge in urban areas. Weakened competition may relax natural selection against individuals of the albino phenotype. Relaxed natural selection is

expected to increase the relative contribution of random genetic drift to the frequency change of the Tyr^{del} allele.

Establishment of a survey method   To test this hypothesis, it is desirable to develop an experimental method to easily distinguish the Tyr^{+} / Tyr^{del} genotype from Tyr^{+} / Tyr^{+}.   By applying it to wild tanuki animals, we can estimate the degree of geographical expansion of Tyr^{del}.   Fortunately, this method has already been established in a previous study (Mae et al., 2020) as well as the present study. Tyr^{del} yielded a 2.1-kb fragment upon PCR using the P3c and P3j primers (Fig. 3).   It is also beneficial that fecal samples are a usable source of genomic DNA.   Tanuki have a habit (called "tamefun" in Japanese) of defecating at a fixed place on the ground, which is shared by all family members.   A survey at various sample collection sites may reveal a geographical expansion of the Tyr^{del} allele with a wider range than that shown in the present study.

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