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Inconsistencies Between Morphological and Genetic Subspecies of Grant's Gazelle (*Nanger granti*)

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ABSTRACT

Grant's gazelles exhibit wide variations in coat color and horn shape and nine subspecies have been described using morphological characters. Recently, some authors recognized three subspecies, *N. g. granti*, *N. g. notata* and *N. g. petersi*, based on three distinct genetic clades of Grant's gazelles from Kenya. However, morphological characters for the individuals in the clades and/or genetic characters of the type specimens have not been shown. Thus, there is no evidence to connect the genetic clades to the type specimens and these three subspecies are only tentatively recognized. In this study, the relationships between morphological and genetic subspecies were examined by comparing the morphological and genetic features of four specimens. There were some inconsistencies between morphological and genetic subspecies. Although, NSMT-M 32104 had the genetic type of *N. notata*, the coat color of this specimen was that of *N. g. brighti*. NSMT-M 32284 and 32287 had the genetic type of *N. g. granti*, whereas coat color in these specimens was that of *N. g. serengetae*. NSMT-M 32166 had the genetic type of *N. g. notata*, but the morphological subspecies of this specimen was unclear because much of the body was missing. It is considered that inconsistencies between morphological and genetic subspecies are attributable to insufficient information about morphological variations and insufficient comparison with type specimens.

Key words: Grant's gazelle, *Nanger granti*, taxonomy, morphological subspecies, genetic subspecies

INTRODUCTION

Grant's gazelle (*Nanger granti*) inhabits semi-desert open savannas and treeless plains from southern Sudan and Ethiopia to central Tanzania (Skirka and Swank, 1971; Kingdon, 1982; East, 1999). Grant's gazelle is distinguished from other *Nanger* species by the following characters: extensive area of white hairs around the anus, reddish brown face with a dark nose patch, skull length of 22-27 cm in adults, horns not curving strongly backward, horn tip not facing toward the medial side, a large pygal band in some populations and a lateral band in some populations (Gentry, 1971).

Taxonomy within this species is confused because of the wide variations in horn shape and coat color (Kingdon, 1982). Although nine subspecies were described based on morphological characters, some authors claimed that most of the subspecies were indistinct in the field (Walther, 1972; Leuthold, 1981). Recently, genetic studies revealed three geographic populations in Kenya (Arctander *et al.*, 1996; Lorenzen *et al.*, 2008) recognized the populations as the subspecies

N. g. granti (Brooke 1872), *N. g. petersi* (Gunther 1884) and *N. g. notata* (Thomas, 1987). Groves and Leslie Jr. (2011) regarded these subspecies as distinct species because of the long separations between the clades. However, Lorenzen *et al.* (2008) did not describe the morphological features of the specimens analyzed in their study and did not include any type specimens. Therefore, the subspecies recognized in their study are not evidential. To define subspecies or species, it is necessary to show the connections between specimens and the type specimen.

In this study, four Grant's gazelles were examined using morphological and molecular methods. To verify the relationship among morphotypes, genetic types and type specimens, these four gazelles with the genetic subspecies recognized by Lorenzen *et al.* (2008) and the morphological descriptions of the type specimens were compared.

MATERIALS AND METHODS

Four specimens of Grant's gazelle (NSMT-M 32104, 32166, 32284 and 32287), which were collected in 1958-1988 by the late Watson T. Yoshimoto and donated to the National Museum of Nature and Science, Tokyo and the W.T. Yoshimoto Foundation, Hawaii were examined. These specimens are composed of three mounted skins and a trophy made by the taxidermy company Kleinburger in Seattle (Table 1).

Morphological analysis: Horn shape and coat color were examined (Fig. 1). The distance between the tips of the horns was measured to the nearest 0.5 mm with calipers and the angle of each horn at the root was measured from photographs. The coat color was described using a color chart, RAL D2 (United Color Systems, Inc.), in addition to words because it is difficult to explain the correct color using only words. RAL color is a standard color system and the number is transcribed in order of hue, lightness and chroma based on a hue circle (for example, RAL 030 40 40 for red).

The specimens were identified as morphological subspecies by comparison with the original descriptions of nine subspecies (Thomas, 1987, 1901, 1903; Neumann, 1906; Heller, 1913) and other morphological studies (Lydekker, 1914; Grubb, 2000).

Molecular analysis: Using an Ultra Clean™ Tissue DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA), total DNA from each specimen was extracted from bone powder obtained from the cornual process in the horn. The samples were added to 1.5-1.8 mL of 0.5 mM EDTA, pH 8.0 and incubated for 3 days at room temperature. Further procedures followed the manufacturer's protocol. Two primers designed by Kocher *et al.* (1989), HL15926 and HH16397 and four primers designed in this study, DLF220 (5'-TGTCCRCATGCATATAAGCA-3'), DLR125 (5'-TCCACATTTATGAAGCTATATTGATAGTCT-3'), DLF155G (5'-CCTGAAAGACTATCAATATAGCT-3'), DLR330G (5'-CATGTGGACAATCATTTAATGTAC-3') and HL15926modif.2 (5'-TTACACCAGTCTTGTAACCGAAGGA-3'), were used to amplify and sequence the mitochondrial DNA D-loop (Table 2). Polymerase Chain Reaction (PCR) amplification was performed in a 10 µL

Table 1: Core data of our specimens

Museum No.	Sex	Locality	Date	Specimen type
NSMT-M 32104	Male	Dire Dawa, Ethiopia	Oct. 1988	Mounted skin
NSMT-M 32166	Unknown	Narok, Masai Reserve, Nairobi, Kenya	Jan. 1958	Trophu
NSMT-M 32284	Male	Ruvu, Burigi, Tanzania	Jul. 1986	Mounted skin
NSMT-M 32287	Male	Ruvu, Burigi, Tanzania	Jul. 1986	Mounted skin

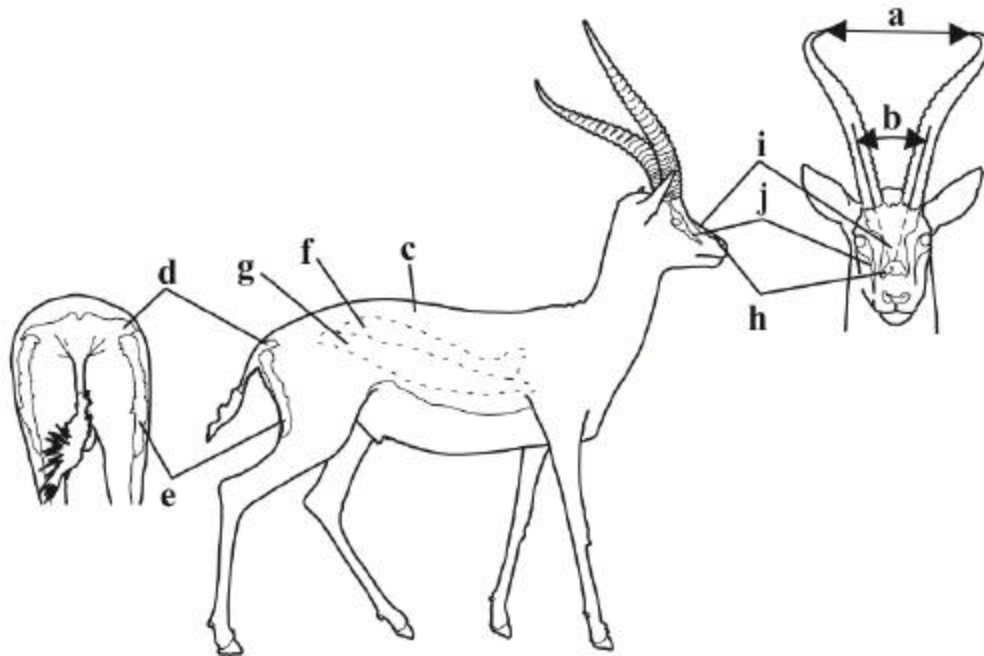


Fig. 1(a-j): Morphological characters examined in this study, a: Horn-spread (distance between the tips of the horns), b: Angle of each horn at the root, c: Back color, d: Color of the white rump patch, e: Color of the pygal band, f: Color of the light lateral band, g: Color of the dark lateral band, h: Color of the nose patch, i: Color of the bridge line at the nose and j: Color of the cheek line

volume containing 10×Ex *Taq* Buffer (Takara Bio Inc., Japan), 2.5 mM MgCl₂, 0.2 mM dNTPs (Takara Bio Inc., Japan), 2 μM forward and reverse primers, 0.5 U of Ex *Taq* (Takara Bio Inc., Japan) and 1.0 μL of template DNA, for a total of 46 cycles: 10 cycles of 94°C for 20 sec, 40°C for 20 sec and 72°C for 30 sec, followed by 36 cycles of 94°C for 20 sec, 49°C for 20 sec and 72°C for 30 sec. Direct sequencing was carried out for 27 cycles of 96°C for 10 sec, 48°C for 5 sec and 60°C for 4 min.

To identify genetic subspecies, our specimens were compared with the genetic subspecies identified by Lorenzen *et al.* (2008). Phylogenetic relationships among the D-loop sequences of our specimens (GenBank accession numbers JN801151-JN801154) and the 27 specimens in Lorenzen *et al.* (2008) (15 *N. g. granti* (EU029808, EU029815, EU029818, EU029819, EU029826, EU029845, EU029852, EU029860, EU029863, EU029866, EU029868, EU029869, EU029878, EU029884 and EU029885), five *N. g. notata* (EU029893, EU029898, EU029899, EU029911 and EU029914) and five *N. g. petersi* (EU029917, EU029925, EU029926, EU029929 and EU029930), were estimated by the neighbor-joining (NJ) method using Kimura's two-parameter model (Kimura, 1980). A blackbuck (*Antilope cervicapra*: AP003422) was used as the outgroup.

RESULTS

Morphological subspecies: Four specimens had variations in horn-spread and the angle between the horns at the roots (Table 2). NSMT-M 32284 had horns like those of *N. g. robertsi*, characterized by a remarkably wide spread (Table 2). Other specimens were not identified to subspecies by horn shape alone.

Table 2: Summary of horn shape and coat color for our specimens and the type specimens of Grant's gazelle

Museum No. or type of subspecies	Horn shape		Body color				Face color					
	Horn- spread	Angle of both side horns at root	Back	Yellow ochre	White-rump patch	Division of white- rump patch	Pygal band	Light lateral band	Dark lateral band	Nose patch	Bridge line of nose	Cheek line
NSMT-M 32104	276.1 mm	21°		Yellow ochre RAL 070 60 30 (<i>brighti</i> -type)	Light ivory RAL 085 90 10	Undivided	Absence (<i>brighti</i> - type)	Absence	Absence	Black brown RAL 050 20 10	Dark black brown RAL 080 20 10	Greenish leghorn RAL 075 80 20
NSMT-M 32166	240.1 mm	21°								Broze RAL 060 40 20	Greenish cream yellow RAL 075 80 30	Absence (<i>granti</i> - type?)
NSMT-M 32284	*496.5 mm (<i>robertsi</i> -type)	27°		Light buff RAL 060 60 30	Pale ivory RAL 090 90 05	Divided partially by insertion within 2.54 cm of back color (serengetae- type)	Burnt umber RAL 060 30 10	Ivory RAL 080 90 10	Absence	Dark brown RAL 060 30 20	Raw sienna RAL 060 50 40	Raw sienna RAL 060 50 40
NSMT-M 32287	287.0 mm	27°		Buff RAL 060 60 20	Light ivory RAL 085 90 10	Divided partially by insertion (within 2.54 cm) of back color (serengetae-type)	Burnt umber RAL 060 30 10	Yellowish leghorn RAL 075 80 20	Absence	Black brown RAL 050 20 10	Yellowish raw sienna RAL 060 50 30	Raw umber RAL 075 40 30
Type of <i>N.</i> <i>g. granti</i>	-	-		Fawn	White		Black	Absence	Absence	Dark color	-	dark above and below eyes (not lines)
Type of <i>N.</i> <i>g. petersi</i>	Scarcely	-										
Type of <i>N.</i> <i>g. notata</i>	-	-					Deep black	Light buff	Black			
Type of <i>N.</i> <i>g. brighti</i>	-	-					Obsolete		Absence			

Table 2: Continue

Museum No. or type of subspecies	Reference	Horn shape		Body color				Face color				
		Horn- spread	Angle of both side horns at root	Black	White-rump patch	Division of white- rump patch	Pygal band	Light lateral band	Dark lateral band	Nose patch	Bridge line of nose	Cheek line
Type of <i>N.</i> <i>g. robertsi</i>	(Thomas, 1903)	Tips outward and were very far apart	-	-	-	Undivided	-	Obsolete ness	-	-	-	-
Type of <i>N.</i> <i>g. lacuum</i>	(Neumann, 1906)	Neally pararel	-	-	White small	-	-	-	Absence	-	-	-
Type of <i>N.</i> <i>roosevelti</i>	(Heller, 1913)	463 55 mm	-	Vinaceous -cinnamon	Pure white	-	Clove-brown	Light buff	-	Clove- brown g.	Cinnamon -rufous	Dusky cinnamon
Type of <i>N.</i> <i>g. serengetae</i>	(Heller, 1913)	241.3 mm	-	Mikado- brown	Pure white	Divided completely by insersion to tail	Black	-	Absence	Clove- brown	Cinnamon -rufous	Dusky cinnamon
Type of <i>N.</i> <i>g. raineyi</i>	(Heller, 1913)	171.45 mm	-	Light vinaceous-	Pure white	Undivided	Bister	Light buff	-	Dark sepia	Cinnamon -rufous	Dusky cinnamon

The diagnostic character of each subspecies described in Heller (1913) and/or Lydekker (1914)

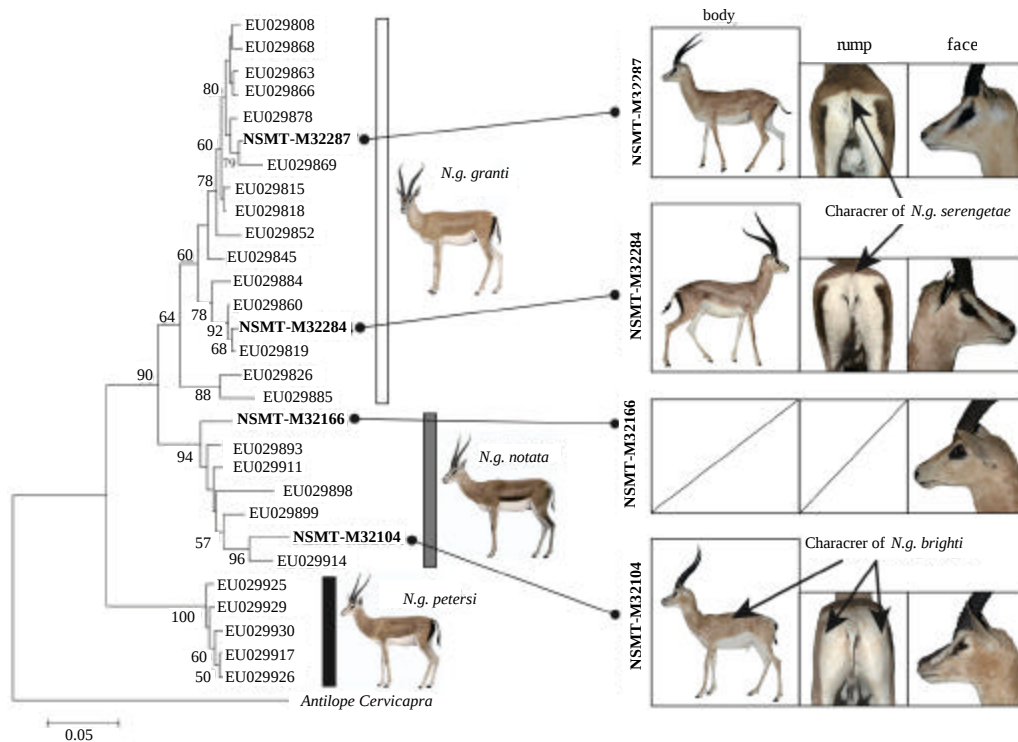


Fig. 2: Relationships among the genetic and morphological subspecies for our specimens, Phylogenetic relationships were established using our specimens and the specimens in Lorenzen *et al.* (2008), The figures of *N. g. granti*, *N. g. notata* and *N. g. petersi* were cited from Groves and Leslie Jr. (2011)

The body and facial colors of our specimens are summarized in Table 2. All of our specimens in which the body parts were present had a white rump patch and no dark lateral bands (Table 2, Fig. 2). NSMT-M 32104 was distinguishable from the others by lighter back color and no pygal bands (Table 2, Fig. 2) and resembled the type of *N. g. brighti* (Table 2). NSMT-M 32284 and 32287 had similar body colors, although the light lateral band was obscured in NSMT-M 32287 (Table 2, Fig. 2). The white rump patch was partially bifurcated by color from the back. Therefore, these specimens were identified as *N. g. serengetae* (Table 2, Fig. 2). The body color of NSMT-M 32166 was not examined because that part of the body was missing.

Our specimens also varied in facial color (Table 2, Fig. 2). NSMT-M 32284 and 32287 had similar facial colors, while NSMT-M 32104 was distinguished from the others by a darker bridge line of the nose. NSMT-M 32166 did not have a cheek line, which was also the case in the type of *N. g. granti*.

Genetic subspecies: Our specimens exhibited 68 variable sites in a 364 bp fragment of the D-loop. The phylogenetic relationships among the partial D-loop sequences from our specimens and sequences in GenBank showed three clades of subspecies (Fig. 2). NSMT-M 32104, with *N. g. brighti* coat color and NSMT-M 32166, of unknown morphological subspecies, were in the *N. g. notata* clade. NSMT-M 32284 and 32287, with *N. g. serengetae* coat color, were in the *N. g. granti* clade.

DISCUSSION

Coat color and horn shape differed among our specimens, supporting a wide morphological variation in Grant's gazelles (Kingdon, 1982; Gentry, 1971). Our results also showed that morphological subspecies are inconsistent with genetic subspecies.

In the present study, morphological and genetic subspecies were identified by comparison with type descriptions and by following Lorenzen *et al.* (2008), respectively. The inconsistencies between the morphological and genetic subspecies found in our specimens suggest the possibility that Lorenzen *et al.* (2008) misplaced subspecies names to genetic clades. For example, NSMT-M 32104 had *N. g. brighti* coat color but the genetic type of *N. g. notata*, indicating that the genetic clade regarded as *N. g. notata* by Lorenzen *et al.* (2008) was *N. g. brighti*. Lorenzen *et al.* (2008) simply named the genetic clade composed of specimens from northern Kenya as '*N. g. notata*', which was first described in northern Kenya, despite the strong possibility that the population called '*N. g. notata*' is extinct (Grubb, 1994). Groves and Grubb (2011) insisted with no evidence that *N. g. brighti* was a synonym of *N. g. notata*. However, *N. g. brighti* is easily distinguished from *N. g. notata* by coat color (Table 2) and there is no genetic data indicating that these are the same subspecies.

On the other hand, inconsistencies found in NSMT-M 32284 and 32287 may sustain previous taxonomic opinions (Lorenzen *et al.*, 2008; Groves and Grubb, 2011). NSMT-M 32284 had the coat color of *N. g. serengetae* and the horns of *N. g. robertsi* (Table 2), suggesting that these two are the same subspecies. In addition, Walther (1972) reported that *N. g. robertsi* and *N. g. granti* were observed in the same herd and Groves and Leslie Jr., 2011 recognized the horns of *N. g. robertsi* as a variant type of *N. g. granti*. Thus, it is implied that *N. g. robertsi* and *N. g. serengetae* are junior synonyms of *N. g. granti*, making it appropriate for NSMT-M 32284 and 32287, with the coat color of *N. g. serengetae*, to be positioned in the genetic clade of *N. g. granti*.

The present study demonstrated taxonomic inconsistencies in Grant's gazelles. This fact indicates that taxonomy in Grant's gazelles have a problem with unclear diagnostic characters of subspecies. It is considered that these inconsistencies are attributable to insufficient information about morphological variations and insufficient comparison with type specimens.

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