Intraspecific evolution within an ecologically diverse species can be used to examine evolutionary trends observed in ruminant morphology: a review of case studies of the Japanese sika deer

Mugino Ozaki Kubo

The University Museum, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

E-mail: mugino@um.u-tokyo.ac.jp

Abstract

This review addresses the question "Are interspecific and macroevolutionary trends observed in ruminant morphology found among populations of a single species?" Several case studies of the Japanese sika deer, *Cervus nippon*, are discussed. The Japanese sika deer is a suitable species for this purpose because it inhabits various environments from the northern subarctic forests to the southern subtropical forests. It shows conspicuous variations in feeding habits, body size, and other morphological and behavioral traits. It can be concluded that selective pressures governing interspecific variations among ruminants, e.g., tooth wear due to grazing or mountainous habitat, promote morphological adaptation of local sika deer populations. However, in some cases, genetic differentiation among local populations is relatively small, resulting in a small degree of change compared to that observed between species. Intraspecific comparison of an ecologically diverse species like the Japanese sika deer is useful for elucidating the evolutionary patterns within ruminants and the possible causes for those patterns.

Key words: Sika deer, *Cervus nippon*, Cervidae, evolution, morphology, hypsodonty, teeth, limb, body size, populations.

1. Introduction

1.1 Comparative study of populations: less investigated issues in ruminant evolutionary research

Macroevolutionary trends in ruminant morphology have been examined in numerous studies. These include trends in the body size (e.g., Jarman 1974), skull and teeth, particularly cheek teeth (e.g., Janis 1995), digestive tract (e.g., Hofmann 1989), and cranial ornamentation (e.g., Davis et al. 2011). Based on the interspecific comparison of diverse extant and fossil species, these studies have not only provided a suite of morphological characteristics that are useful for the reconstruction of the phylogeny of ruminants but also indicated the possible selective pressures that drove morphological differentiation. Because ruminants are highly successful herbivores and show continuous feeding variation from grass-dominant diets to browse-dominant diets, morphological characteristics that are relevant to the feeding type are well investigated (Hofmann 1989; Janis 1995; Pérez-Barbería & Gordon 1999; Williams & Kay 2001; Clauss et al. 2003, 2008; Hofmann et al.

2008; Codron & Clauss 2010; Damuth & Janis 2011; Kaiser et al. 2013). Recent statistical advances in phylogenetical comparative methods (PCMs) and increased reliability of the phylogenetic relationships among Ruminantia have greatly contributed to an improved understanding of the relationship between feeding ecology and morphology.

Fewer studies have investigated morphological differentiation among ruminant populations based on the notion that selective pressures shaping macroevolutionary trends among species also effectively drive microevolution within a species (Foster & Endler 1999). This is because 1) ecological variation within a species is normally smaller than that found among the diverse species of ruminants, and 2) recent studies of the population ecology of ruminants pay close attention to what causes differences in specific characteristics of populations, without placing them in the context of macroevolutionary trends. A number of studies of ruminant populations have identified ecological/environmental factors associated with differences in body mass, fecundity, mortality, or recruitment among populations (Clutton-Brock et al. 1984; Gaillard et al. 1993; Clutton-Brock et al. 1997; Mysterud et al. 2001; Festa-Bian-
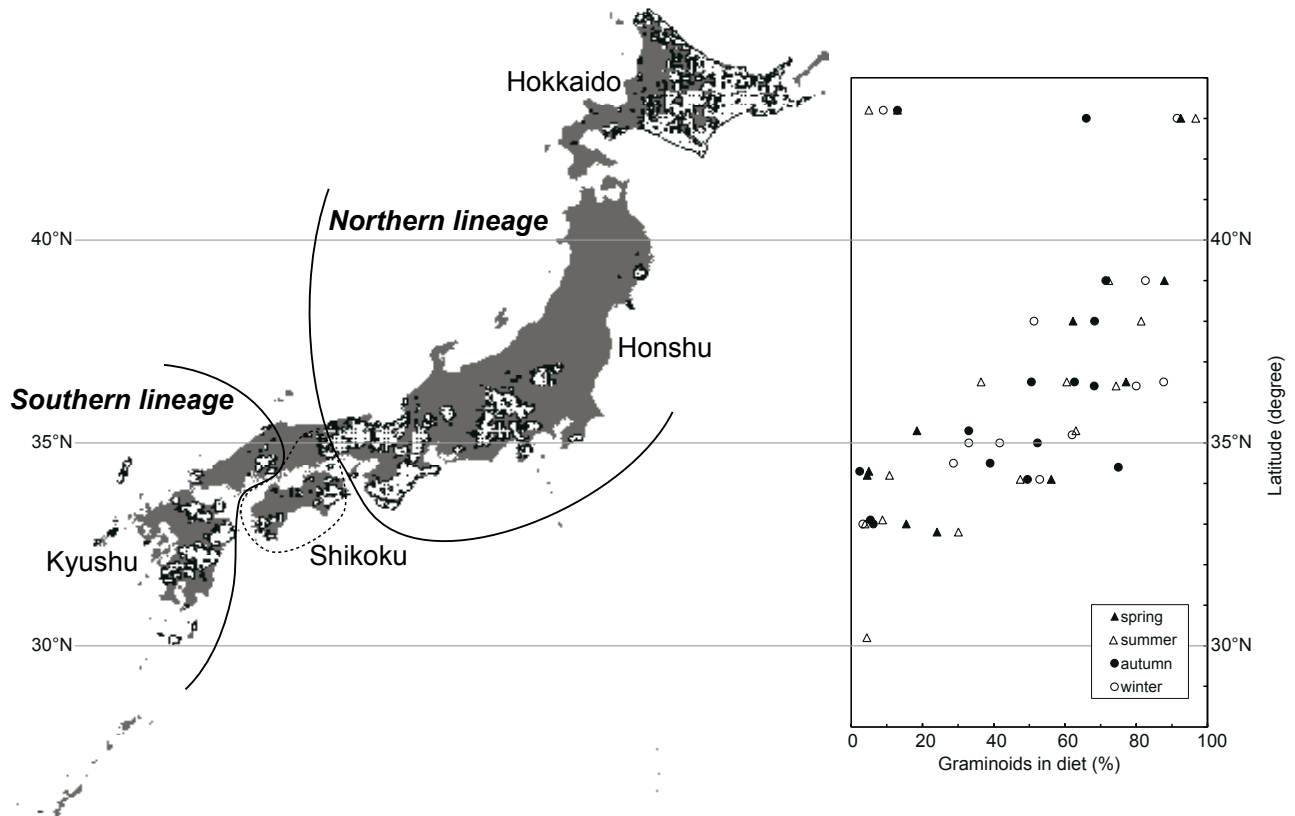
Figure 1: Geographical distribution of the sika deer in Japan, along with its latitudinal variation in diet. The dotted areas of the left map are sika deer habitats. The solid lines represent the boundary between the two phylogenetic lineages: the northern lineage inhabits the area from Hokkaido to the central Honshu, whereas the southern lineage inhabits the southwestern part of Honshu, Kyushu, and the islets surrounding Kyushu. A possible hybridization area is located in central-western parts of Honshu and Shikoku. The scatter plot on the right shows latitudinal variation in the diet (modified from Takatsuki 2009). The x-axis represents the percentage of graminoids in the diet determined using quantitative dietary analyses of rumen contents or feces.

chet et al. 2003; Martinez-Jauregui et al. 2009), but these studies can not necessarily determine if these ecological/environmental factors are important for macroevolutionary diversification of ruminants.

To this end, we have been interested in the relationship between microevolution (evolution occurring at the species level) and macroevolution (evolution occurring above the species level or higher taxa). There has been a lack of interaction between those studying the paleontology/macroevolution and others studying the current population ecology of ruminants. If interspecific variation of traits among ruminant species had been the result of natural selection, then the same selective pressure could have produced similar patterns among populations within a species. In this sense, studies of intraspecific variation can provide empirical evidence of recent evolutionary trends.

1.2 The Japanese sika deer as a model for examining macroevolutionary trends

Based on the above mentioned notion, morphological comparisons were made among Japanese sika deer populations (Ozaki et al. 2007, 2010; Kubo et al. 2011, 2013; Kubo & Yamada 2014). These studies

will be introduced in the subsequent section (Section 2), with the primary focus being on the relationship between feeding ecology and dentognathic morphology (Subsections 2.1 and 2.2).

The sika deer (*Cervus nippon*) is widespread throughout eastern and northeastern Asia, from the Ussuri region of Siberia to northern Vietnam, Taiwan, and Japan (Ohtaishi 1986; Geist 1999). Because the distribution of the sika deer in the Japanese archipelago extends from the northern to the southern islands (from 44°N to 26°N, Fig. 1), they inhabit a wide range of environments, from the northern, subarctic coniferous forests to the southern, temperate or even subtropical broad-leaved forests. Such variation in habitat has a strong influence on their ecology, such as feeding habits, seasonal migration, reproduction, and demography (McCullough et al. 2009). Information on the ecology of local populations has been accumulated for management purposes. Skeletal specimens are available for each population from these surveys (collection of animals that have died of natural causes in the field and animals harvested for nuisance culling or for hunting).

Among ecological variations found in sika deer populations, the most notable and informative variation in macroevolutionary research is variation in

Table 1: Sika deer populations used in analyses in Subsections 2.1 and 2.2.

Population	Latitude (°N)	Habitat type	Genetic lineage[*]	Percentage of grass in their diet[**]	Feeding type[***]
1. Eastern Hokkaido	43.4	Deciduous broad-leaved and mixed forest	N	46.3	G
2. Mt. Goyo	39.2	Coniferous or deciduous broad-leaved forest	N	52.5	G
3. Kinkazan Island	38.3	Deciduous broad-leaved forest with some open grasslands	N	65.8	G
4. Nikko	36.8	Deciduous broad-leaved forest	N	73.9	G
5. Ashio	36.7	Deciduous broad-leaved forest with some open grasslands	N	90.0	G
6. Okutama	35.9	Deciduous broad-leaved forest	N	32.1	IM
7. Yamanashi	35.8	Deciduous broad-leaved forest	N	42.2	IM
8. Tanzawa	35.4	Deciduous broad-leaved evergreen broad-leaved forest	N	—	IM
9. Boso Peninsula	35.2	Evergreen broad-leaved forest	N	45.2	IM
10. Izu Peninsula	35.0	Deciduous broad-leaved evergreen broad-leaved forests	N	30.5	IM
11. Nara Park	34.4	Open grassland with planted conifers and deciduous trees	N	81.4	G
12. Shimane	35.4	Evergreen broad-leaved forest	S	38.1	IM
13. Yamaguchi	34.2	Evergreen broad-leaved forest	S	6.5	B
14. Tsushima Island	34.1	Evergreen broad-leaved forest	S	3.4	B
15. Fukuoka	33.5	Evergreen broad-leaved forest	S	7.2	B
16. Mt. Shiraga	32.2	Evergreen broad-leaved forest	S	25.6	IM
17. Yakushima Island	30.3	Evergreen broad-leaved forest	S	4.4	B

[*]The phylogenetic lineage based on mitochondrial DNA haplotype: N, northern lineage; S. southern lineage.

[**]Percentage of graminoids in their diet was obtained from Kubo & Yamada (2014).

[***]Categorical classification of the feeding type is based on data from Ozaki et al. (2007).

food habits (reviewed in Takatsuki 2009) (Fig. 1). Sika deer populations inhabiting the subarctic coniferous forests or the cool temperate deciduous broad-leaved forests subsist mostly on graminoids, particularly dwarf bamboo (e.g., *Sasa nipponica*), which is dominant within the understories of these forests. In some ways, dwarf bamboo is broadly equivalent to other grass species with respect to the relative amount of neutral detergent fiber (NDF; cellulose, hemicellulose, and lignin) and silica (2.8%–15.9% per dry matter) (Van Soest 1994; Motomura et al. 2000; Yokoyama et al. 2000). Thus, these northern populations can be categorized as "grazers" inhabiting closed habitats (Hofmann 1985). On the other hand, the sika deer that inhabit the temperate evergreen broad-leaved forests feed mainly on the leaves of woody plants, and they are classified as either "intermediate feeders" or "browsers." Most of the browse species have < 1% of silica concentration; for example, Japanese laurel (*Aucuba japonica*), which is a preferred diet for the sika deer in temperate evergreen forests, has 0.58% of silica in the leaves (Ma & Takahashi 2002). Previous studies of the food habits of the sika deer have provided quantitative and comparative data through analyses of rumen contents or feces.

The intraspecific phylogeny of the Japanese sika deer has been described. There are two genetically distinct groups of the sika deer: the northern and southern lineages (Tamate et al. 1998; Nagata et al.
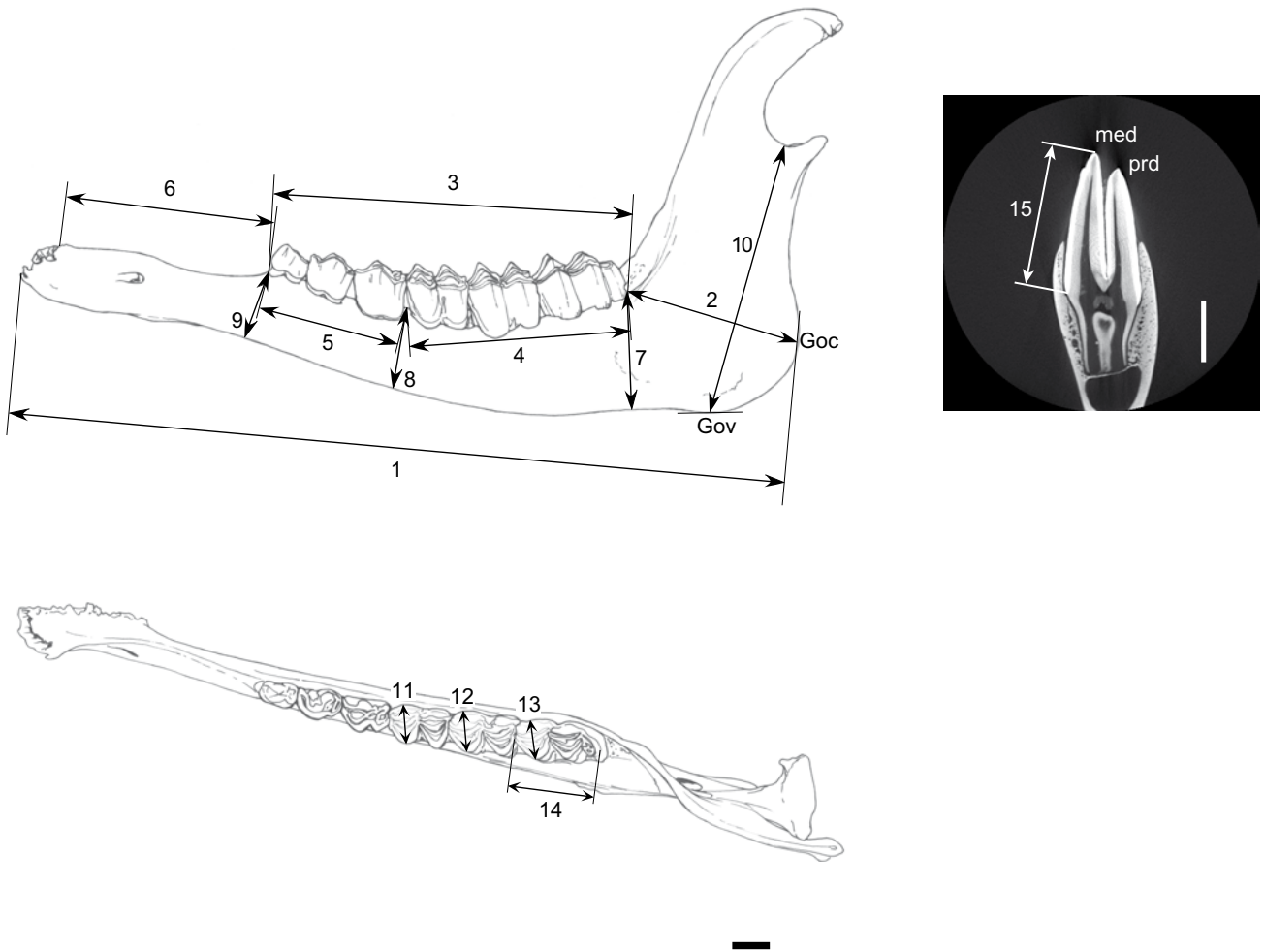
Figure 2: The 15 measurements of mandibles of the sika deer (modified from Ozaki et al. 2007). (1) GL, greatest length of the mandible; (2) MAL, mandibular angle length; (3) CTRL, cheek tooth row length; (4) MRL, molar row length; (5) PMRL, premolar row length; (6) DL, diastema length; (7) MH_M3, height of the mandible behind M_3; (8) MH_M1, height of the mandible in front of M_1; (9) MH_P2, height of the mandible in front of P_2; (10) GOV_NO, gonion ventrale to the deepest point of the mandibular notch; (11) M1B, breadth of M_1; (12) M2B, breadth of M_2; (13) M3B, breadth of M_3; (14) M3L, length of M_3; (15) M3H, height of unworn M_3; and GOC, gonion caudale. The height of unworn M_3 was measured from a cross-sectional image obtained using a microfocus X-ray CT scanning system (see Ozaki et al. 2007 for analytical details). Both scale bars are 1 cm.

1999; Goodman et al. 2001; Nagata 2009) (Fig. 1). The northern lineage includes populations in Hokkaido and most of Honshu, except for the southwestern region. The southern lineage includes populations in southwestern Honshu, Kyushu, and southwestern islands (e.g., Tsushima Island, Yakushima Island). It is believed that the separation of the two lineages occurred on the Asian continent ca. 0.3–0.5 mya, prior to their respective migration to the Japanese archipelago (Tamate et al. 1998; Nagata et al. 1999; Nagata 2009), although the timing and routes of the respective migration events have not been fully clarified.

Based on this information, there are two different levels at which microevolutionary hypotheses can be tested: between the two phylogenetic lineages and among populations within each lineage, the latter reflecting a more recent divergence than the former.

2. Case studies

2.1 Mandibular morphology

The morphology of sika deer mandibles has been examined in specimens collected from all over the Japanese archipelago (Ozaki et al. 2007). Based on prior interspecific comparative studies in ruminants, a number of morphological characteristics have been shown to correlate with grazing: e.g., broader snouts, larger areas for the insertion of the masseter muscle, longer diastemas, shorter premolar rows, and higher mandibular corpora (reviewed in Janis 1995). Some of these morphological characteristics were not statistically different after adjusting for phylogeny; however, molar row volume, molar hypsodonty, and masseter muscle mass were still significantly larger in the grazing species after adjustment (Pérez-Barbería & Gordon 1999; Williams &

Table 2: Results of regression analyses testing differences in mandibles of the sika deer. Significant differences between the northern and southern lineages or between the grazer and intermediate feeder populations of the northern lineage are in bold. *In the case of the M_3 hypsodonty index being the response variable, log-transformed SIZE was not included in the model because the M_3 hypsodonty index was independent of the body size in the sika deer. Abbreviations of the mandibular measurements are the same as the legend of Fig. 2.

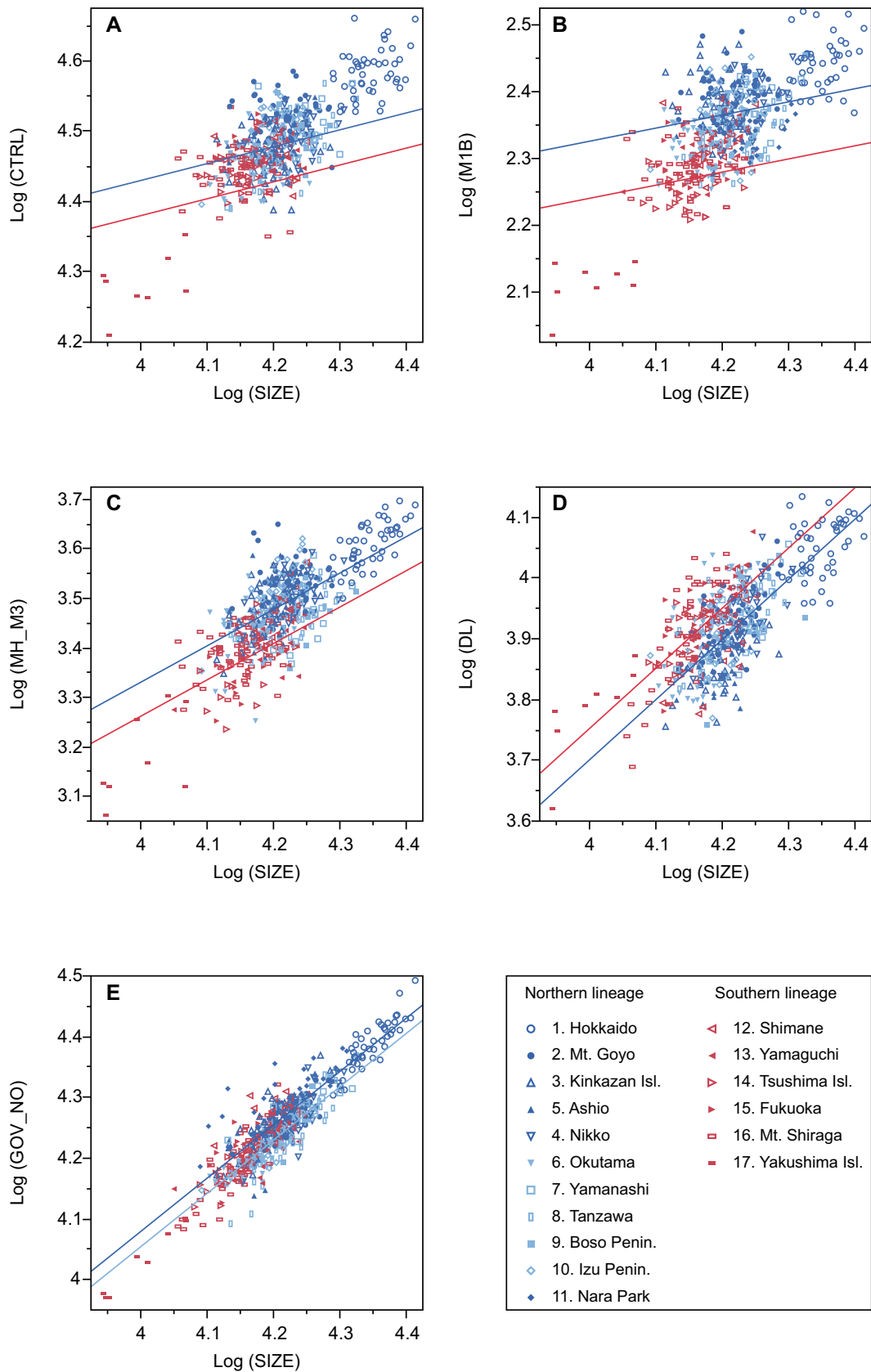| Response variable	Northern vs. southern			Grazer vs. intermediate feeder populations of northern lineage		
	Explanatory variable	Parameter estimate	*P*-value	Explanatory variable	Parameter estimate	*P*-value
Log (MAL)	Intercept	−0.194	0.462	Intercept	0.039	0.908
	Log (SIZE)	0.983	<0.001	Log (SIZE)	0.929	<0.001
	Northern	0.008	0.305	Grazer	0.004	0.698
Log (CTRL)	Intercept	3.445	<0.001	Intercept	3.361	<0.001
	Log (SIZE)	0.243	<0.001	Log (SIZE)	0.269	<0.001
	Northern	**0.025**	**0.026**	Grazer	0.009	0.310
Log (MRL)	Intercept	2.863	<0.001	Intercept	2.942	<0.001
	Log (SIZE)	0.268	<0.001	Log (SIZE)	0.256	<0.001
	Northern	**0.029**	**0.023**	Grazer	0.015	0.211
Log (PMRL)	Intercept	3.102	<0.001	Intercept	2.922	<0.001
	Log (SIZE)	0.096	0.069	Log (SIZE)	0.146	0.018
	Northern	**0.031**	**0.024**	Grazer	0.014	0.170
Log (M1B)	Intercept	1.497	<0.001	Intercept	1.565	<0.001
	Log (SIZE)	0.196	<0.001	Log (SIZE)	0.190	0.001
	Northern	**0.043**	**0.002**	Grazer	0.013	0.144
Log (M2B)	Intercept	0.449	0.025	Intercept	0.816	0.001
	Log (SIZE)	0.474	<0.001	Log (SIZE)	0.392	<0.001
	Northern	**0.019**	**0.039**	Grazer	0.008	0.384
Log (M3B)	Intercept	0.005	0.987	Intercept	0.511	0.116
	Log (SIZE)	0.579	<0.001	Log (SIZE)	0.461	<0.001
	Northern	0.006	0.378	Grazer	0.001	0.903
Log (M3L)	Intercept	0.719	0.001	Intercept	1.023	<0.001
	Log (SIZE)	0.557	<0.001	Log (SIZE)	0.491	<0.001
	Northern	**0.024**	**0.003**	Grazer	−0.002	0.810
Log (DL)	Intercept	−0.237	0.279	Intercept	0.074	0.788
	Log (SIZE)	0.991	<0.001	Log (SIZE)	0.911	<0.001
	Northern	**−0.026**	**0.002**	Grazer	−0.007	0.373
Log (GOV_NO)	Intercept	0.399	<0.001	Intercept	0.555	<0.001
	Log (SIZE)	0.915	<0.001	Log (SIZE)	0.878	<0.001
	Northern	0.000	0.947	**Grazer**	**0.013**	**0.009**
Log (MH_M3)	Intercept	0.356	0.117	Intercept	0.578	0.033
	Log (SIZE)	0.735	<0.001	Log (SIZE)	0.691	<0.001
	Northern	**0.034**	**0.002**	**Grazer**	**0.018**	**0.038**
Log (MH_M1)	Intercept	−3.104	<0.001	Intercept	−3.446	<0.001
	Log (SIZE)	1.476	<0.001	Log (SIZE)	1.554	<0.001
	Northern	−0.011	0.176	Grazer	−0.011	0.181
Log (MH_P2)	Intercept	−1.336	<0.001	Intercept	−1.460	<0.001
	Log (SIZE)	1.016	<0.001	Log (SIZE)	1.046	<0.001
	Northern	0.001	0.771	Grazer	0.006	0.285
M_3 hypsodonty index*	Intercept	1.789	<0.001	Intercept	1.833	<0.001
	Northern	**0.042**	**0.018**	Grazer	−0.026	0.104

Figure 3: Scatter plots of log-transformed mandibular measurements against those of the mandibular size (SIZE) of the sika deer: **(a)** cheek tooth row length, **(b)** M_1 breadth, **(c)** mandibular height behind M_3, **(d)** diastema length, and **(e)** ascending ramus height (GIV_NO). The blue (or black in black and white print) solid lines represent regression lines for the northern lineage, whereas the red (or gray in black and white print) ones represent those for the southern lineage, except for **(e)** in which the blue (or black) line represent those for the grazer population and the pale blue (or gray) one represent those for the intermediate feeder population. Significant differences between the two lineages (differences in the height of the intercepts) are found in the variables related to the cheek tooth size and the diastema length. Significant differences between grazer and intermediate feeder populations are found only in the ascending ramus height.

Kay 2001; Clauss et al. 2008). These morphological characteristics have been interpreted as adaptations to ingesting and masticating a relatively larger amount of less nutritious, more fibrous, and abrasive graminoids compared with browsers or intermediate feeders. Therefore, these morphological characteristics were compared between the two phylogenetic lineages as well as between grazer and intermediate feeder populations within the same lineage.

The data of Ozaki et al. (2007) was reanalyzed including additional data obtained after its initial publication. Mandibles of 623 female sika deer from 11 populations of the northern lineage and 6 populations from the southern lineage were used (Tab. 1). Regression analyses (mixed model) was conducted with each log-transformed mandibular measurement being the response variable. Log-transformed mandibular size (SIZE, a geometric mean of three measurements: GL, MH_M1, and GOV_NO; variable names defined in the legend to Fig. 2) and two categorical factors (lineage or feeding type) were explanatory variables. A population nested within a lineage or feeding type was assigned as a random effect in the mixed model. Among 15 measurements (Fig. 2), the measurements related to the cheek tooth size (lengths of the cheek tooth row, molar row, and premolar row; breadths of M_1 and M_2; length of M_3 and M_3 hypsodonty), mandibular height behind M_3, and diastema length were significantly different between the two lineages after adjusting for size ($P < 0.05$). The northern lineage had larger and more hypsodont cheek teeth, higher mandibular corpus, and shorter diastema than those of the southern lineage (Tab. 2, Figs 3a–d). On the other hand, these measurements were not significantly different between grazer and intermediate feeder populations of the same lineage, except for the mandibular corpus height behind M_3, which was larger in the grazers. In addition, a significant difference between grazer and intermediate feeder populations was observed in the ascending ramus height (Tab. 2, Fig. 3e).

Significant differences between the two lineages were observed in the cheek tooth size. The northern lineage had a buccolingually wider and mesiodistally more elongated cheek tooth row than those of the southern lineage. This result implies that the ancestral population of the northern lineage may have acquired a larger occlusal surface area of cheek teeth, which was adaptive for grazing. The shorter diastema of the northern lineage seems to be associated with the elongation of the cheek tooth row. The occlusal area of the cheek tooth row and M_3 hypsodonty (which was represented by both the M_3 hypsodonty index and mandibular corpus height) were significantly increased in the northern linage. This result implies that the ancestral population of the northern lineage may have adapted to abrasive diets, possibly in a continuous or a recurrent harsh environment during the last glacial period, when possible colonization into Japan considered to have taken place (Ozaki et al. 2007). The evolution of hypsodonty will be discussed in depth later (Subsection 2.2).

On the other hand, the cheek tooth size (length, breadth, and height) was not significantly different between grazer and intermediate feeder populations of the northern lineage. This finding indicates that a further increment of the cheek tooth size may not have occurred in grazer populations. The ascending ramus height was significantly greater in grazer populations. The higher ascending ramus may be associated with a larger area for the insertion of the masseter muscle, which was adaptive for masticating fibrous graminoids.

Two hypotheses may explain the difference between the results of between-lineage comparison and between-feeding type comparison. One is that the ancestral population of the northern lineage may have acquired both larger cheek teeth and a higher ascending ramus, but under the current condition of relaxed selective pressure, the ascending ramus of intermediate feeder populations has shrunk, whereas the cheek tooth size has not changed yet (i.e., a
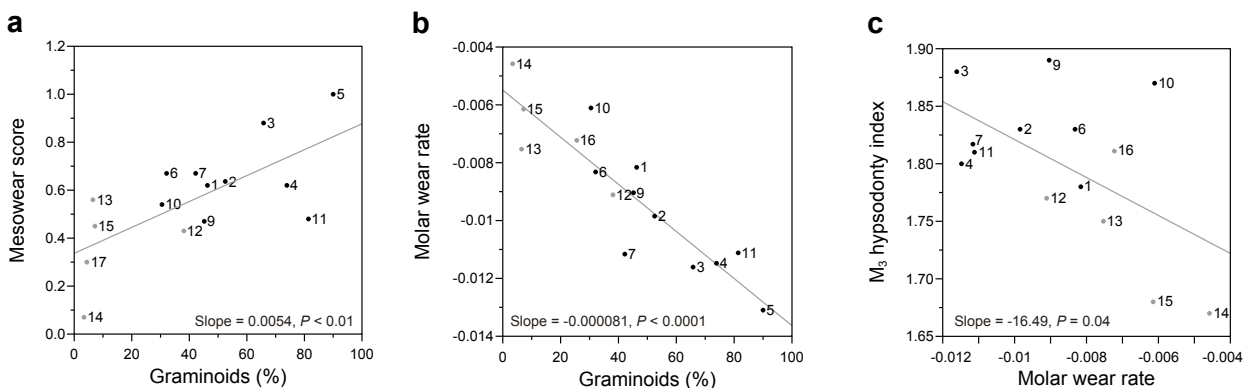

Figure 4: Correlations between the percentage of graminoids in the diet and dental variables (**(a)** mesowear score, **(b)** molar wear rate) and correlations between the molar wear rate and the M_3 hypsodonty index in the sika deer **(c)**. The solid lines represent linear regression lines. Populations of the northern lineage are indicated by black closed circles and populations of the southern lineage are indicated by gray circles. These scatter plots are modified from Kubo & Yamada (2014).

Figure 5: A young female sika deer fed on dwarf bamboo at Mt. Ohdaigahara, central Japan. Note the abundance of dwarf bamboo in the forest floor and the height of the bamboo. Feeding near the ground rarely occurs. (Photograph by Sha Tech).

slower evolutionary response of cheek teeth). Another is that the ancestral population of the northern lineage may have acquired a larger cheek tooth size but not a higher ascending ramus. In populations of the northern lineage, the significant difference in the ascending ramus between grazer and intermediate feeder populations merely reflects a phenotypic (i.e., not evolutionary) response to a diet comprised of a larger amount of fibrous graminoids, which is known based on developmental experiments on laboratory animals reared on hard diets (Ito et al. 1988).

2.2 Molar hypsodonty and the molar wear rate

Among the morphological traits characterizing grazing ruminants, increased hypsodonty is the most remarkable and well-studied trait because of its importance for paleodietary and paleoenvironmental inferences. It is generally accepted that increased hypsodonty in herbivorous mammals is an adaptation to an abrasive diet, whereas the source and relative contribution of abrasive materials, i.e., intrinsic grass phytoliths or extrinsic abrasive materials (dust, grit, or sand), are still disputed (Sanson et al. 2007; Damuth & Janis 2011; Jardine et al. 2012; Kaiser et al. 2013; Lucas et al. 2013; Schulz et al. 2013; Stroemberg et al. 2013). The sika deer can provide a rare opportunity to answer this question because it inhabits forest environments; thus, a confounding habitat effect can be excluded. In a recently published paper (Kubo & Yamada 2014), the data of Ozaki et al. (2007, 2010) were reanalyzed with additional populations (16 populations in total, Tab. 1) to quantitatively evaluate the influence of dietary and environmental factors on 3 dental variables: mesowear score (MS), molar wear rate, and M_3 hypsodonty index.

We found that the percentage of graminoids in diet of the sika deer significantly correlated with MS and the molar wear rate (regression slope of molar height against age determined using the cementum

annuli of an incisor root). A larger amount of graminoids in the diet resulted in a rounded molar cusp and a faster molar wear rate (Fig. 4a and 4b). Annual precipitation in the habitat and types of grass species (effect of feeding height) did not influence molar wear. The observed correlation between molar wear rate and proportion of graminoids in diet was not considered to be a by-product of feeding near the ground and ingesting more soil when deer crop grass. This is because the height of dwarf bamboo, on which the northern sika deer predominantly rely, is 40–60 cm; feeding near the ground rarely occurs (Fig. 5). We also found a significant correlation between estimated silica content (%) in deer diet and molar wear rate (see the supplementary analysis of Kubo & Yamada 2014 and discussion therein). Therefore, grass phytoliths can be abrasive and possibly serve as effective selective pressure for increased hypsodonty of cheek teeth, at least for Japanese sika deer. Additional analysis on the amount of soil/grit in deer diet is needed to evaluate the relative importance of grass phytoliths against that of the external abrasives. We also found a positive correlation between the molar wear rate and the M_3 hypsodonty index (Fig. 4c). These data indicated an evolutionary increment in the molar height corresponding to the molar wear rate (selective pressure). However, we have some reservations regarding this conclusion because the degree of hypsodonty differed significantly between the northern and southern lineages (Tab. 2). In Fig. 4c, the hypsodonty index increased with the accelerating molar wear rate in the southern lineage but not in the northern lineage. Genetic evidence indicated that the greatest genetic differentiation was found among populations of the southern lineage, whereas among populations of the northern lineage, this differentiation occurred more recently because of habitat fragmentation. Therefore, according to the findings of comparative analyses of mandibles (Subsection 2.1), we hypothesize that the ancestral population of the northern lineage acquired more hypsodont molars in the past, possibly under strong environmental pressure that promoted molar wear. We also hypothesize that after subdivision into local populations, molar height did not change measurably because of relaxed selection and/or insufficient duration of selection, particularly in populations of the northern lineage. In the paleontological, macroevolutionary context, it has been discussed once evolution of increased hypsodonty occurred, it does not easily reverse because cost of maintaining hypsodonty may be low (see discussion in Fortelius 1985, Hummel et al. 2010). This interpretation can be concordant with our observation that intermediate feeder populations of the northern lineage have the equivalent degree of hypsodonty to those of the grazer populations.

2.3 Limb morphology

Interspecific variation in the limb length in ungulates has been investigated from both aspects of biomechanics (McMahon 1973, 1975; Alexander et al. 1979) and locomotory adaptation to specific habitats (Scott 1985, 1987; Garland & Janis 1993; Janis et al. 2012). The former predicts an allometric relationship between body mass and the limb length; the limb length is proportional to (body mass)$^{0.25}$, whereas the latter focuses on the departure from the predictions with reference to ecological characteristics. It is of great interest if general biomechanical predictions are also applicable even at the species level, and if this body size-limb length relationship is related to some ecological or behavioral characteristics.

In the sika deer, postcranial morphological traits have been less investigated because most specimens of the sika deer housed in museums are limited to skulls and mandibles. Based on measurements of the culled sika deer of a northern population, Takatsuki (1992) showed that the sika deer cannot live in areas with heavy snowfall, where snow accumulates at a level higher than the limb length of the sika deer. Ochiai & Asada (1995) reported a growth pattern of the body size of the sika deer from a population in a temperate region (Boso Peninsula) and found that the hind limb length was relatively (compared with shoulder height) shorter than that of other northern populations. This finding implies that the amount of snowfall or habitat closedness may influence variation in the hind limb length. In the subsequent paper, Ochiai & Asada (1997) compared the size of the skull and limb bones of the Boso population with those of a northern population (Nikko). It was shown that the limb length was proportional to (body mass)$^{0.24}$, thus confirming the biomechanical prediction of McMahon (1973). These data also implied that the positive deviation of the Nikko deer from the allometric prediction line can be explained by snow depth of the habitat. Recently, Terada et al. (2012) showed morphological differentiation in limbs among southern island populations of the sika deer. They used 8 populations belonging to the southern lineage and measured both the skull size [condylobasal length (CBL)] and the length of limb bones (scapula, humerus, radius, metacarpus, femur, tibia, and metatarsus). They showed that there was significant variation in the measurements among the populations, particularly in CBL and the metacarpal length. In analyses, they regressed the log-transformed metacarpal length against log-transformed CBL (a proxy of the body size) and obtained the regression slope and intercept for each population. The slope did not differ significantly among the populations and had a value of 0.74, which was significantly smaller than 1 (i.e., negative allometry). This value is close to the biomechanical prediction as well as to the empirical regression slope obtained for other sika deer populations (Ochiai & Asada 1997), provided that CBL is isometric to body mass (CBL^3 = body mass). On the other hand, the regression intercept (relative carpal length adjusted for the body size) negatively correlated with habitat topology. This was quantitatively evaluated using a geographical information system (GIS); the steeper the habitat area, the shorter the metacarpal length. As Terada et al. (2012) discussed in their paper, this result is in agreement with the interspecific pattern of bovid species; species in mountainous habitats have shorter limbs than those in open habitats (Scott 1985, 1987). Considering the fact that annual snowfall in less than 5cm for the studied habitats of sika deer, Terada et al. (2012) discussed that shorter metacarpal may be favored because it can increase stability in rugged terrain. The relationship between habitat topology and limb morphology has not been studied in other populations, particularly in those of the northern lineage. Together with the observations of Takatsuki (1992) and Ochiai & Asada (1997), it is expected that limb morphology of the northern sika deer is affected by both habitat topology and the amount of snowfall.

Terada et al. (2012) have also convincingly demonstrated that habitat topology can be quantitatively assessed using GIS; this will of great use to interspecific comparison with quantitative categorization of habitat types.

2.4 Body size

Evolutionary changes in the body size is one of the most studied issues in evolutionary biology because it affects so many aspects of the animal´s biology (Schmidt-Nielsen 1984). Among ruminant species, the evolution of body size has been assessed with reference to ecological diversification (Bell 1970; Jarman 1974; Geist 1999; Pérez-Barbería et al. 2002). On the other hand, changes in body size within a species are confounded by phenotypic plasticity due to environmental factors. Body size (mass) can be affected greatly by nutritional conditions. To separate genetic and environmental effects, "common garden" type experiments or reciprocal transplants of target populations must be used. These can be very difficult to conduct for large, wild mammals (see Pelletier et al. 2007; Wilson et al. 2007 for studies of the genetic basis of the body size of wild sheep populations). Therefore, intraspecific studies of body size variation in mammals have focused on identifying an empirical relationship between the body size and environmental factors (Gay & Best 1996; Wigginton & Dobson 1999; Smith et al. 2002; Wolverton et al. 2009; Gür 2010). In many cases, this relationship was discussed from the perspective of Bergmann's rule, which describes an increase in the body size of endotherms at high latitudes (Bergmann 1847).

Because the Japanese sika deer shows considerable latitudinal variation in the body size among local populations, it has been considered as an ex-

ample that follows Bergmann's rule (Ohtaishi 1986). Body mass of adult males during the winter season varies with latitude; 120 kg in the northernmost population (Hokkaido) but only 30 kg in the southernmost population (Kerama Islands) (Ohtaishi 1986). Most mainland (Honshu) populations show intermediate values (60 kg in the central area to 100 kg in the northern area). However, quantitative measurements of the body size of local populations were limited at the time of publication of the report by Ohtaishi (1986). Therefore, further accumulation of body size data is needed to properly evaluate Bergmann's rule for Japanese sika deer.

There have been several reports on geographic variation in the skull size among sika deer populations. Imaizumi (1970) described a latitudinal cline in CBL for the purpose of species classification in what he termed the subgenus *Sika*. His taxonomic splitting of the sika deer into several species is not generally accepted (Ohtaishi 1986; Endo 1996). Imaizumi (1970) reported that "*C. nippon*" (most Japanese populations, except for Hokkaido, Yakushima Island, Kerama Islands, and Tsushima Islands) showed a cline in CBL that is larger in the north and smaller in the south. This regression slope was based on only 7 individual males. Thus, further data are needed to clarify latitudinal variation in the skull size. Shiroma & Ohta (1996) compared skull morphology among 8 populations (Hokkaido, Nikko, Kagoshima, Nozakijima Island, Tsushima Island, Mageshima Island, Yakushima Island, and Kerama Islands) and showed that the northernmost population (Hokkaido) had the largest skull size followed by Nikko, Kagoshima, and other island populations. Because the sampled populations were biased to island populations, it is still unclear whether the latitudinal cline followed Bergmann's rule. Terada et al. (2012) found that CBL was negatively related to temperature and latitude; these findings are congruent with Bergmann's rule among 8 southern island populations.

In summary, although geographical variation in the body size is conspicuous in the Japanese sika deer, there has been no comprehensive study of quantitative data of populations from all over the Japanese archipelago. Recently, both body mass and skull measurements were collected for more than 30 sika deer populations by us. These data will be reported in the near future.

3. Conclusions

The focus of this review was the question "Are interspecific and macroevolutionary trends in ruminant morphology also found among populations of a single species?" Several case studies of the Japanese sika deer were conducted by our group and other researchers. Several conclusions can be drawn from these studies: 1) selective pressures, such as accelerated tooth wear associated with grass consumpti-

on or topographical features of a habitat that require climbing ability, promote local adaptation of populations within an ecologically diverse species, similar to the situations in which these same selective pressures govern interspecific variation among ruminant species; 2) in some cases, genetic divergence among local populations is insufficient in duration, resulting in a smaller extent of morphological change compared with that in subspecific lineages or closely related species; and 3) a phenotypic change resulting from nutritional conditions affects intraspecific comparison to some extent, depending on traits. This may confound the conclusion that the observed morphological differentiation is caused by adaptation. To confirm the third conclusion, some genetic models of traits are needed that involve pedigree analysis or "common garden" experiments, both of which are difficult to conduct for wild mammals.

There are still several traits, not only morphological but also physiological and behavioral, to be investigated for intraspecific variations or trends in the sika deer. For example, geographical variation in the size and morphology of antlers is an attractive topic with reference to the evolution of antlers in Cervidae (Gould 1974; Geist 1999). Miura (1984) reported geographical variation in mating behavior among local populations. This implies that habitat closedness may have a strong impact on the degree of aggregation and thus the mating system of the sika deer. This is in agreement with the evolutionary trend observed across ruminant species (Jarman 1974; Pérez-Barbería et al. 2002). Whether this variation in the mating system is related to antler variation among local deer populations is another intriguing question.

Acknowledgements

4. References

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