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Title:

Variation and mechanisms of life history evolution in insular dwarfism as revealed by a natural experiment

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Abstract

Islands offer a classic topic for evolutionary studies. Few other subjects have historically raised as much fascination as the island large mammals that having evolved into dwarfs. Consensus has been gained that multivariate ecological causes are behind those changes, but what remains largely unexplored are the mechanisms behind them: how life history (age of reproduction, growth rate, longevity) change with insular dwarfism. Previous studies produced contrasting results difficult to compare, as they concerned different species in single islands. The Japanese Archipelago offers worldwide a unique natural experiment, as in its numerous islands of different sizes the same group of organisms, deer, have evolved into different sizes. Using an extensive examination of bone microstructure, statistical modelling of growth rate and demography, we demonstrate different degrees of change in life history in different islands. We discovered how different life parameters amount to a new, *K*-strategy contrasting to that of the parent population.

Introduction

Among the most iconic examples of visible evolutionary change are island mammals that dramatically changed in body size (Foster 1964, Sander et al. 2006, van der Geer et al. 2010). Elephants, hippos and deer in islands across the world became very small, whereas small mammals became larger (van der Geer et al. 2010), what led to the suggestion of an 'island rule' (Foster 1964). A classic example is the pigmy elephant (*Elephas falconeri*) of Sicily, which had a body mass of only 1% of its mainland ancestor (Larramendi and Palombo 2015).

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Several factors have been proposed to be associated with these extreme changes in body size, such as ecological release due to a lack of predators and competitors, resource limitation on islands, founder effects of island immigrants, and the remoteness and duration of isolation, all of which interact in a complex way to establish or obscure the pattern of body size change in focal taxa (Lomolino et al. 2012, Lomolino et al. 2013, Raia and Meiri 2006, van der Geer et al. 2010, van der Geer et al. 2013). Consensus has been gained that what remains largely unexplored are the mechanisms behind those size changes, how life history – age of reproduction, growth rate, longevity – evolves. As these animals are mostly extinct, their bones have been the source of information, via studies of bone microstructure.

The few studies available produced contrasting results, and they were difficult to compare, as they concern different species in single islands. Some studied taxa show a distinctly slower growth rate and delayed somatic maturity compared with continental taxa (Köhler and Moyà-Solà 2009), whereas others do not show extreme modifications in their life histories (Kolb, Scheyer, Lister, et al. 2015, Kolb, Scheyer, Veitschegger, et al. 2015). What is clear is that the reduction of body size in large mammals appears to be associated with modification of the growth trajectory (Long et al. 2019, Palkovacs 2003), indicating that comparisons of trajectories of insular taxa and their mainland relatives would shed light on the process of body size reduction. To achieve this, studies of mainland and insular populations of a single species are particularly useful because they allow confounding factors other than living on islands to be controlled.

The Japanese Archipelago offers worldwide a unique natural experiment, as in its numerous islands of different sizes the same group of organisms, deer, have evolved into different sizes. Using an extensive examination of bone microstructure, CT

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imaging, statistical modelling of growth rate and demography, we demonstrate different degrees of change in life history parameters in different islands. We discovered how different life parameters amount to a new, *K*-strategy contrasting to that of the parent population.

Extant Japanese sika deer (*Cervus nippon*) populations inhabit both the mainland and islands, providing a perfect setting for testing insular effects on the growth rate and life history of a species. Body size of sika deer shows considerable variation among the populations, with deer in the northernmost population showing the largest body size (adult male body mass of ca. 126 kg) whereas those in the southernmost insular population the smallest (adult male body mass of ca. 35 kg) for the species (Kubo and Takatsuki 2015, Ohtaishi 1986). We analyzed and body growth rates and life histories of extant mainland and insular populations of sika deer in Japan using long bone histological and demographic analyses (Figure 1).

To further test the predictions of the model generated by the study of extant forms, we investigated the life history traits of two insular and one gigantic mainland deer that became extinct in the Late Pleistocene (Figure 1). The insular deer (*Cervus astylodon* and Muntiacini gen. et. sp. indet., hereafter referred to as "the Ryukyu muntjac") considered to have migrated to the Okinawa Islands during the Early Pleistocene (Otsuka and Takahashi 2000). *Cervus astylodon* exhibits morphological characteristics that are considered typical of insular dwarfs, e.g., a small body size, shortened limbs, and hypsodont molars (Kubo et al. 2015, van der Geer et al. 2010). By contrast, the gigantic mainland deer (*Sinomegaceros yabei*), from the Late Pleistocene deposits on Honshu mainland, had a comparable body size to the largest extant deer, the moose (*Alces alces*) (Taruno et al. 2017), which has been shown to have the highest growth

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rate among deer (Kolb, Scheyer, Veitschegger, et al. 2015). Therefore, we expected to observe significant variation in the growth rates and life histories among the extant and fossil mainland and insular populations of Japanese cervids, reflecting varying levels of insular effects.

Results

Variation in bone tissue among insular cervids in Japan

There were clear differences in the cortical bone tissues between extant and extinct deer. The cortical bone tissues of *C. astylodon* and the Ryukyu muntjac from Okinawa Island show similar features, having primary parallel-fibered bone tissue with multiple LAGs, which are comparable to the features of an extinct dwarf bovid *Myotragus* (Köhler and Moyà-Solà 2009) and typical extant reptiles [e.g.] (Woodward, Horner, and Farlow 2014). The LAGs are spaced evenly throughout the cortex (Figure 2A–C; SI Appendix, Figures S1A–C and S3A–F) but become closer together as they approach the periosteal surface in older individuals, indicating a decrease in growth rate with age. Only a few areas of the innermost cortex show a prevalence of fibro-lamellar bone tissue with primary osteons, which alternates with nonvascular parallel-fibered bone tissue with flattened osteocytes and LAGs. There is relatively little vascularization showing the longitudinal orientation of the channels compared with other cervids and large mammals (Klevezal 1996, Kolb, Scheyer, Lister, et al. 2015). Haversian bones are seen only at the inner medullary surface and the part of the cortex where the ligaments are strongly attached (e.g., the labium laterale in the femur) in adults.

In contrast, all of the extant deer populations [i.e., the four extant sika deer populations, the extant Reeves's muntjac (*Muntiacus reevesi*)] and the gigantic fossil *S.*

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yabei exhibit a similar bone tissue structure to continental cervids (Calderon et al. 2019, Jordana et al. 2016, Kolb, Scheyer, Lister, et al. 2015), consisting of a fibro-lamellar bone with a high level of laminar to plexiform vascularization and plumped osteocytes in most of the cortex and parallel-fibered bone tissue in the outer cortex (Figure 2G–L; SI Appendix, Figures S2A–I, S3G–I, and S4A–O). Notably, the parallel-fibered bone tissue is more extensively developed and the LAGs at the inner to middle cortex are tighter in the two insular populations (sika deer from Yakushima and Kerama islands) compared with the mainland deer (Figure 2D–F), suggesting a slower growth rate among the insular populations.

Figure 2 summarizes the main histological features of the cervids we examined (also see SI Appendix, Tables S1–S4).

Growth curve models and growth rate comparisons

The LAGs in the long bones of both the extant and fossil deer were well preserved, allowing us to depict a growth curve for each individual (SI Appendix, Figure S7–S14). We then averaged each of three growth curve parameters (the asymptote, growth rate, and inflection point) for each species/population to deduce a representative growth curve for each (Figure 3A, SI Appendix, Figure S15).

The two fossil insular deer (*C. astylodon* and the Ryukyu muntjac) showed remarkably slow and prolonged growth compared to the other cervids, while the two extant insular deer (sika deer from Yakushima and Kerama islands) were intermediate between the fossil insular deer and the extant sika deer from the two mainland areas (Honshu and Hokkaido mainland). The extant Reeves's muntjac showed the fastest growth, corresponding with its early sexual maturity at ca. 6 months (Chiba Prefecture and Deer

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Research Group on Boso 2007). The Pleistocene giant deer *S. yabei* also showed fast growth, suggesting a similar growth pattern to other extant large deer, such as moose (Sand, Cederlund, and Danell 1995). Statistical comparisons of the growth parameters revealed that both the fossil and extant insular deer had significantly slower growth rates than Reeves's muntjac and *S. yabei* (SI Appendix, Tables S9–S11), supporting the results of the bone histological analyses. However, the growth rates of the four extant sika deer populations and the fossil insular deer were not significantly different from each other (SI Appendix, Tables S9–S11), due to the transitional position of the extant insular deer, although the limited numbers of possible samples may also play a role. The inflection point, which marks the transition from the initial phase of exponential growth to the subsequent phase of asymptotic growth, is known to be associated with the timing of sexual maturity (Monteiro and Falconer 1966) and was greatest in *C. astylodon* (SI Appendix, Tables S9 and S11), implying that sexual maturity may be delayed in this species. However, the differences were not always statistically significant, reflecting high levels of variation within species/populations.

Survivorship curve comparisons among extant and fossil cervids.

There was variation in the survivorship curves of *C. astylodon* and the three extant sika deer populations on the Hokkaido and Honshu mainland and Yakushima Island (Figure 3B). The survivorship curve of *C. astylodon* was characterized by very low mortality from the juvenile through to the prime age period, followed by an increase in mortality during senescence, appearing like an inversed L, which is categorized as a type I survivorship curve (Pianka 1978). At the other extreme, extant sika deer from Hokkaido mainland showed high mortality in the younger age class, followed by a more gradual

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decrease in the number of survivors from the prime age to senescence, which is characteristic of a type III survivorship curve. The survivorship curves of sika deer from Honshu mainland and Yakushima Island lay between those of *C. astylodon* and Hokkaido sika deer, being characterized by a steady decrease in survivorship until the prime age (i.e., a type II survivorship curve), though the Honshu population showed an increased mortality rate after the prime age. The maximum longevity of *C. astylodon* was 25 years (Kubo et al. 2011), which was much greater than that of the extant sika deer (17, 19, and 17 years for the Honshu, Hokkaido, and Yakushima Island populations, respectively). Finally, the Ryukyu muntjac had a type I survivorship curve, like *C. astylodon* (Figure 3B), indicating that its mortality rate also did not increase until senescence.

Discussion

The examination of life history and metabolic features in a phylogenetically controlled set of species, based on bone microstructure and other hard tissue markers, reveals the processes associated with dwarfism in the evolution of large herbivorous mammals in islands. There is a common mechanism involved, but the historical background of the populations and the nature of the new environments may result in different evolutionary transformations.

The bone histological analysis showed that the bone tissues of the mainland species/populations are characterized by azonal bone with few LAGs in the outermost cortex, whereas those of the insular populations are characterized by zonal bone with multiple LAGs through the entire cortex, as occurs in the bone tissues of ectotherms (Köhler and Moyà-Solà 2009), indicating that mainland and insular populations may

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have different metabolic rates. Notably, two fossil cervids from Okinawa Island (*C. astylodon* and the Ryukyu muntjac) were found to have zonal bones consisting of parallel-fibered bone, which is unusual for endotherms and indicates a drastic decrease in the bone deposition rate (de Margerie, J. Cubo, and Castanet 2002). We hypothesize this is associated with a lower metabolic rate, comparable to what has been reported for the fossil insular bovid *Myotragus* (Köhler and Moyà-Solà 2009). By contrast, extant sika deer from Yakushima and Kerama islands showed intermediate histological features between the mainland sika deer and the fossil insular cervids, with the retention of fibro-lamellar bone (which is a typical mammalian bone tissue (Kolb, Scheyer, Veitschegger, et al. 2015)), parallel-fibered bone, and multiple LAGs, showing similar histological features to the fossil insular cervid *Candiacervus* (Kolb, Scheyer, Lister, et al. 2015).

Alongside this change in metabolic rate, there was a large difference in relative growth rate among the mainland and insular forms (Figure 3A). Among the extant sika deer populations, we observed a transitional change in growth rate from fast-growing mainland populations (Hokkaido and Honshu) to slow-growing insular populations (Yakushima and Kerama islands). Furthermore, the magnitude of the decrease in growth among the insular deer was related to island size, with a slower rate of growth and a more shrunken body size being observed on the smaller island (Kerama Islands). Sika deer on Kerama Islands are the smallest sika deer in Japan and showed the slowest growth rate among the four studied populations. These deer were introduced to Kerama Islands from the Kyushu mainland ca. 400 years ago by humans (Okinawa Prefectural Board of Education 1996). Although fast change on body size in less than six thousand years have been previously documented in red deer in other islands (Lister 1989), it is

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surprising that this drastic change in growth trajectory could have occurred over such a short period of time, which equates to ca. 80 generations (assuming a generation time of 5 years for the sika deer). This change in growth trajectory was brought about by phenotypic plasticity rather than genetic modification in response to the less abundant understory vegetation in the subtropical evergreen-broadleaved forest and thus the poor nutritional status of Kerama deer (Shiroma and Ohta 1996), which was supported by the observation of a possible malnourished individual (URB-MAM-55; see SI Appendix for details). By contrast, the slower growth rate of Yakushima deer compared with the mainland deer may have a genetic basis because 1) Yakushima deer are not malnourished and have enjoyed a recent population increase (Tsujino, Noma, and Yumoto 2004) and 2) some of their macromorphological features have a genetic basis (Terada and Saitoh 2018, Terada, Tatsuzawa, and Saitoh 2012).

It was not surprising that the fossil gigantic deer *S. yabei* showed fast growth, as this has been reported in a previous study (Kolb, Scheyer, Veitschegger, et al. 2015). However, it is notable that the extant Reeves's muntjac had the highest growth rate among the studied cervids, which fits the expectation given the early sexual maturity of this species (Sand, Cederlund, and Danell 1995), and that this growth rate was statistically equivalent to that of *S. yabei* despite the immense difference in body size between these species. The lowest growth rate was identified for *C. astylodon* followed by the Ryukyu muntjac in the present study (SI Appendix, Table S8). Although the mature body size is similar between Reeves's muntjac and fossil Ryukyu muntjac, their growth rate is different from each other, suggesting the importance of the slow growth rate as an adaptation to the resource-limited island environment. In the study of island adaptation of mammals, it is important to examine not only the body size, proportion,

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and morphology, but also consider growth rate and life history. The histological research is an important method for this purpose.

As with Kerama deer, it is possible that phenotypic plasticity influenced the growth trajectories of the extinct insular deer we studied. We do not currently have any reliable sources of information on the nutritional status of fossil deer, except that they were C_3 plant feeders (Kubo et al. 2015). However, reports of healed bone fractures on a number of leg bones from a museum collection of fossil insular deer of Okinawa Island (Okinawa Prefectural Museum and Art Museum 2009) implies that these deer survived long enough to recover from serious leg injuries, refuting the possibility that fossil Okinawa deer were malnourished and their body growth was suppressed due to a lack of resources.

Comparison of the growth trajectories showed that the extant sika deer populations exhibit a transitional change in somatic maturity from mainland deer (less than 3 years for the Hokkaido and Honshu populations) to insular deer (more than 5 and 7 years for the Yakushima and Kerama Island populations, respectively) (Figure 3A), and these observations have been independently supported by investigations of culled individuals (Ochiai and Asada 1995, Suzuki et al. 2001) and by long-term field observations of wild deer (Agetsuma and Agetsuma-Yanagihara, personal communication). Surprisingly, the two fossil Okinawa deer (*C. astylodon* and the Ryukyu muntjac) were expected to attain somatic maturity at 14 and 7 years old, respectively. Examination of the sexual maturity data for the extant sika deer and Reeves's muntjac populations indicated that the female age at first pregnancy matches or is slightly before the age of somatic maturity (Hokkaido sika deer, 2 years old (Hokkaido Institute of Environmental Sciences 1997); Honshu sika deer, 2 years old (Ochiai and Asada 1995); Yakushima sika deer, 4 years

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old, Agetsuma and Agetsuma-Yanagihara pers. comm.; Reeves's muntjac, 1 year old (Chiba Prefecture and Deer Research Group on Boso 2007)). Thus, it can be predicted that *C. astylodon* and the Ryukyu muntjac would have become sexually mature at 14 and 7 years of age, respectively, indicating that both somatic and sexual maturity were delayed in the insular forms compared with the mainland forms.

Analysis of the survivorship curves also produced intriguing results. The two fossil insular deer showed inversed L-shaped (type I) survivorship curves, indicating lower mortality during the juvenile through prime age periods and increased mortality during senescence. Together with the body growth patterns, this implies that these fossil deer had similar life histories to *K*-strategists (Pianka 1978), such as large anthropoids or elephants. Similar findings have been reported for *Myotragus* (Köhler and Moyà-Solà 2009, Marin-Moratalla, Jordana, and Köhler 2013), implying the commonality of this life history change on predator-free islands. The previous report on dwarfed deer *Candiacervus* having a slower growth from the continental relatives but not as extreme like *Myotragus* (Kolb, Scheyer, Lister, et al. 2015) may result from Crete Island not being such a small island, even in times in which it may have been partitioned (van der Geer et al. 2010). Indeed, all cervids from the mainland of Japan do not show any modifications of life history traits compared with the continental relatives. Furthermore, our study is the first to demonstrate a gradual, transitional shift toward a 'slow life history' from mainland to insular populations, underlying the move toward insular dwarfism. To further test the complete scenario of life history evolution we hypothesize here, it would be relevant to obtain reliable mortality data for Kerama deer, which showed the slowest growth rate among the extant sika deer populations.

We obtained strong evidence that the fossil deer of Okinawa Island had a distinct life

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history based on their growth and mortality patterns. These deer were isolated for a very long time (over 1.5 Ma), and during it they evolved their life history strategy in a small insular environment, including a lower extrinsic mortality due to a lack of predators but limited food resources (Köhler and Moyà-Solà 2009). This process may have started after their initial settlement on Okinawa Island in part through phenotypic plasticity due to a lack of sufficient foods, as possibly occurred in Kerama deer. The life history change would then have become genetically fixed through the natural selection of more *K*-shifted individuals, as appears to have occurred in Yakushima Island deer. The final outcome will have been the development of extreme *K*-strategists, despite their small body size. Unfortunately, this life history trait will have made these species vulnerable to human exploitation, because animals with a *K*-strategy have lower population recruitment (Pianka 1978). Consequently, the two Okinawa deer became extinct at the time of or soon after Paleolithic human arrival to the Okinawa Islands (Fujita et al. 2016, Fujita et al. 2014), possibly due to hunting.

These findings explain the growth variation of insular mammals previously documented (Figure 4). Okinawa deer and *Myotragus*, which dwelt on small islands and underwent exceptionally long time of evolution in predator-free insular environments (over 1.5Ma and 5.2 Ma respectively), showed a dramatic decrease in bone growth rate and an evolution towards a slow life history. On the other hand, other Japanese deer do not show extreme life modifications from the continental relatives, the condition similar to previously reported cases (e.g., a fossil hippo *Hippopotamus minor* from Cyprus, a living cervid *Odocoileus hemionus* from Blakley island and a fossil cervid *Candiacervus* from Crete (Kolb, Scheyer, Lister, et al. 2015, Kolb, Scheyer, Veitschegger, et al. 2015)). These results suggest that the degree of modification in life

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history is strongly affected by both island size and duration of isolation, with smaller islands and longer isolations resulting in life history changes into *K*-strategists. This idea is also supported by an ecological study on a recent insular rabbit *Pentalagus furnessi* in the Ryukyu Archipelago of Japan, having *K*-strategy (Hamada and Mizuta 2020). A report on the Sicilian pigmy elephant *Elephas falconeri*, may represent an exception to this pattern, as the age distribution of the fossil assemblage with an exceptionally high number of calves and immature individuals was interpreted as reflective of a *r*-strategy (Raia, Barbera, and Conte 2003). However, a histological study suggested that this pigmy elephant had a much slower growth rate than its mainland relatives, and sexual maturity was within the range of extant elephants (Köhler et al. 2013). These observations further tempt us to expect an evolutionary optimum of body mass and life history in small predator-free islands, which would be *K*-strategists with body size within small to medium-sized range. Further accumulation of life history information clarified from bone histology should shed light on the generality of life history evolution on islands.

Conclusion

Extant Japanese sika deer are able to modify their life history traits through a combination of phenotypic plasticity and natural selection. This ability likely underlies the process of insular dwarfism also recorded in fossil deer (*C. astylodon* and the Ryukyu muntjac) from Okinawa Island, showing lower mortality during the juvenile through prime age periods and increased mortality during senescence. Together with the body growth patterns, this implies that these fossil deer had a *K*-strategy life history (Pianka 1978). Combining the results of the present and previous studies on life

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histories of insular mammals, we conclude that the degree of modification in life history is strongly affected by both island size and duration of isolation, with longer isolations on smaller islands resulting in more prominent life history changes. We demonstrate the evolution of a gradual, transitional shift toward a 'slow life history' in large tetrapods from mainland to insular populations as the main life history change accompanying insular dwarfism.

Material and methods

Materials

Samples of extant sika deer were obtained from two mainland populations (Hokkaido and Honshu) and two insular populations (Yakushima Island and Kerama Islands) (Figure 1). The four populations had different properties of their habitat environments and showed considerable differences in body size (Table 1). Samples of extant Reeve's muntjac were obtained from the Honshu mainland (Figure 1). This analysis included two mainland populations of sika deer in the Japanese Archipelago (Hokkaido and Honshu mainland) that differ in their maximum body size, with Hokkaido deer being the largest population of Japanese sika deer, and two insular populations (Yakushima and Kerama islands), both of which inhabit subtropical forests but differ in the habitat area (island size = 505 km^2 and 36 km^2, respectively) and isolation period (ca. 100,000–20,000 years and 400 years, respectively). All extant samples were obtained from wild individuals.

Fossils of the following taxa were examined: *Cervus astylodon* and Muntiacini gen. et sp. indet. (the Ryukyu muntjac), collected from the Hananda-Gama Cave site of the Late Pleistocene (>20 ka (Okinawa Prefectural Museum and Art Museum 2009)) on

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Okinawa Island, Japan; and *Sinomegaceros yabei*, collected from the Kumaishi-do Cave of the Late Pleistocene (16–23 ka (Okumura et al. 2016)) on Honshu mainland, Japan.

Detailed information on the extant and fossil specimens is provided in the SI Appendix and is listed in Table 1 and SI Appendix, Tables S1–S4.

Thin sectioning and X-ray computed tomography (CT) scanning

Life history traits, including growth trajectories, longevity, and sexual maturity can be examined by long bone histology counting the lines of arrested growth (LAGs) [e.g.] (Amson et al. 2015, Klevezal 1996, Köhler et al. 2012, Köhler and Moyà-Solà 2009, Kolb, Scheyer, Lister, et al. 2015, Kolb, Scheyer, Veitschegger, et al. 2015, Marin-Moratalla, Jordana, and Köhler 2013) We used in total 62 extant and 21 fossil long bones for histological analyses (Table 1). All of the specimens were photographed and standard measurements were taken, following which thin sections of the midshaft of the long bones were made based on the methodology described in Chinsamy & Raath (Chinsamy and Raath 1992) and Sander (Sander 2000). For the extant species, we sampled both femur and tibia from the same individual, whereas we used either femur or tibia for fossil specimens, as their skeletal remains were not associated. The thin sections were photographed with a digital film scanner (Pixus Mp 800, Canon) and analyzed using an Optiphot2-pol microscope (Nikon). Microscopic photographs were taken with a Nikon Df camera.

Neonatal specimens of sika deer (Honshu mainland: CBM-ZZ-757) and Reeves's muntjac (CBM-ZZ-4974) were scanned using an experimental animal X-ray CT scanner (Latheta LCT-200, Aloka; 24-μm resolution, 80 kV, 0.2 mA) at the Okayama University of Science, Okayama, Japan, to determine the initial diameter of the cortical

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bone and an open medullary cavity in these species. Image segmentation and visualization were performed using VG-Studio Max (Volume Graphics) version 3.1.

The nomenclature and definitions of the bone microstructures included in the following descriptions are based on Francillon-Vieillot et al. (Francillon-Vieillot et al. 1989) and Castanet et al. (Castanet et al. 1993).

Age assessment

For most of the extant deer samples, age at death was assessed by tooth eruption or the number of dental cementum annuli in the tooth root (SI Appendix, Table S3 and S4). In the case of fossil deer samples, the long bones were not associated with skulls and mandibles, therefore it was required to estimate the age at death from the number of LAGs found in the long bones. Several previous studies have reported a good correlation between the number of LAGs and the age at death in a range of vertebrate groups [e.g.] (Castanet et al. 2004). However, only one study has examined this correlation in an extant deer species [red deer (*Cervus elaphus*)], which reported that the number of LAGs in the tibia corresponds to the actual age but the number in the femur corresponds to the age before the deposition of the external fundamental system (EFS) (Calderon et al. 2019). Therefore, the relationship between the actual age at death and the number of LAGs in the bones of the extant cervids sampled was examined first. This showed that the estimated age of the extant deer based on the number of LAGs in both the femur and tibia was highly correlated with the actual age determined by tooth cementum annuli (SI Appendix, Figure S5 and Table S5). While some old adults that were over 8 years of age (HOUMVC-00037, CBM-ZZ-412, and URB-MAM-193) had fewer LAGs in their long bones than their actual age, the diameters of their expanded medullary cavities were

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identical or even larger than the bone diameters observed in neonates and fawns (<1 year old), indicating that the first (and also second in URB-MAM-193) LAG is likely eliminated in old individuals (SI Appendix, Table S5). Thus, since the number of LAGs matched the actual age in extant Japanese cervids, the age of individuals of fossil taxa was also estimated by counting the number of LAGs in their long bones (SI Appendix, Table S6).

Body mass estimation

The body mass during ontogeny was estimated from the bone diameters at the LAGs (Lehman and Woodward 2008). Body mass estimation formulas for both the femora and tibiae were obtained from Scott (Scott 1990) and the formula that uses the mediolateral diameter (MLD) of the femora and tibiae was selected for use in this study (see SI Appendix for details). In addition to the diameters at the LAGs, the external diaphysial measurements [anteroposterior diameter (APD) and MLD] were collected and used to estimate the body mass at death. Raw measurement data of diameters at the LAGs together with the estimated body mass used for growth curve modeling are presented in Supplementary Table S14.

Comparison of growth patterns

Growth curves of body mass were constructed for each individual specimen that were older than yearlings (i.e., those that had more than one LAG). The exception to this was the extant Reeves's muntjac, for which a fawn and yearling were included in the growth curve fitting because this species is known to have rapid growth (Chiba Prefecture and Deer Research Group on Boso 2007). Before fitting the growth curves, neonatal body

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mass data were obtained for both the extant and fossil taxa as a starting point (SI Appendix, Table S7, and Figure S6).

To fit growth curves to the obtained data, the number of LAGs was first transformed into age in years. For each specimen, the body mass (W, in kg) was then formulated with age (x, in years) using the Gompertz curve, which is described as:

$$W = a\mathrm{Exp}(-\mathrm{Exp}(-b(x-c))$$

where a, b, and c represent the asymptote (i.e., the body mass when body growth ceases), growth rate, and inflection point, respectively. The growth curve parameters were then averaged for each population/species to produce a representative growth curve for each group, and the growth rate (b) and inflection point (c) were statistically compared among species/populations using the Tukey–Kramer method. These analyses were conducted separately for the femora and tibiae, and all statistical analyses were conducted in JMP Pro 14.2.0 (SAS Institute Inc.).

Comparison of survivorship curves

Kubo et al. (Kubo et al. 2011) estimated the age at death for *C. astylodon* based on the height of the lower third molar by applying the molar wear rate of extant sika deer. Here, the same age data (n = 45) were used to depict survivorship curves following the method of Caughley (Caughley 1977). In addition, age data were collected from three sika deer populations that were used to investigate the long bone histology (Hokkaido, Honshu, and Yakushima Island populations). These data were collected from the literature (Hokkaido Institute of Environmental Sciences 1997, Kubo et al. 2011) for the Hokkaido and Honshu mainland populations. However, no reliable published age data were available for the Yakushima Island population, so instead histological analysis of

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the cementum layers of the lower first incisors was performed on museum specimens housed in the University Museum of the University of Tokyo, the Tochigi Prefectural Museum, and the Hokkaido University Museum to identify their age at death. Age data were collected from 1060, 594, and 74 individual deer in the Hokkaido, Honshu, and Yakushima Island populations, respectively.

The age at death of the fossil Ryukyu muntjac was also estimated by evaluating the wear score of the lower third molars. Ozaki (Ozaki 2009) previously estimated the age at death of the Ryukyu muntjac by applying the molar wear scoring method developed by Chapman et al. (Chapman, Brown, and Rothery 2005) for extant Reeves's muntjac. While no age data of extant Reeves's muntjac from Honshu mainland have been reported to date, despite their ecology being well investigated for management purposes (Chiba Prefecture and Deer Research Group on Boso 2007), age data have been reported for extant Reeves's muntjac from England (Chapman, Brown, and Rothery 2005). Therefore, these age data were used to depict the survivorship curve of extant Reeves's muntjac. Fossil *S. yabei* and extant Kerama Islands deer were not included in these survivorship curve comparisons due to a lack of reliable age data.

For more details on the materials and methods used in this study, see the SI Appendix.

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Additional information

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Competing interest

We have no competing interests to declare.

Additional files

Supplementary files

- Supplementary file 1. Supplementary information including additional text, figures

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and data set.

- Supplementary file 2. Raw data of measurements (Table S14).

Data accessibility

All data not presented in the manuscript are available as electronic supplementary information and uploaded to Dryad Digital Repository (https://doi.org/10.5061/dryad.wdbrv15n8).

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Figures

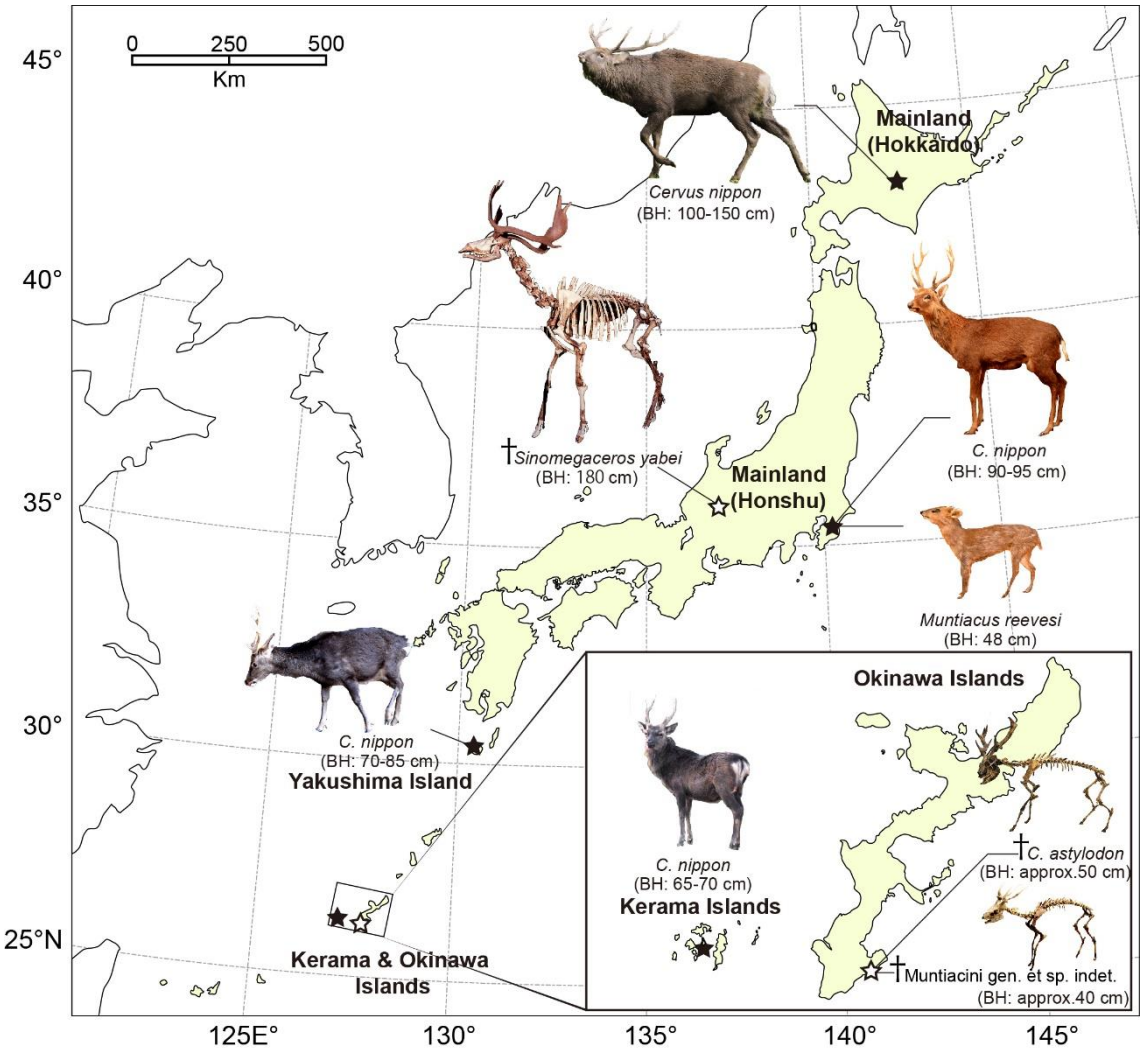

Figure 1. Locations of the sampled cervids in Japan. † indicates fossils excavated from Late Pleistocene deposits. The localities of extant taxa are shown in closed stars and those of extinct taxa are in open stars. The approximate body height (BH) is indicated for each species/population. Photography of fossil cervids courtesy of Gunma Museum of Natural History (*Sinomegaceros yabei*) and Okinawa Prefectural Museum & Art Museum (*Cervus astylodon* and Muntiacini gen. et sp. indet.).

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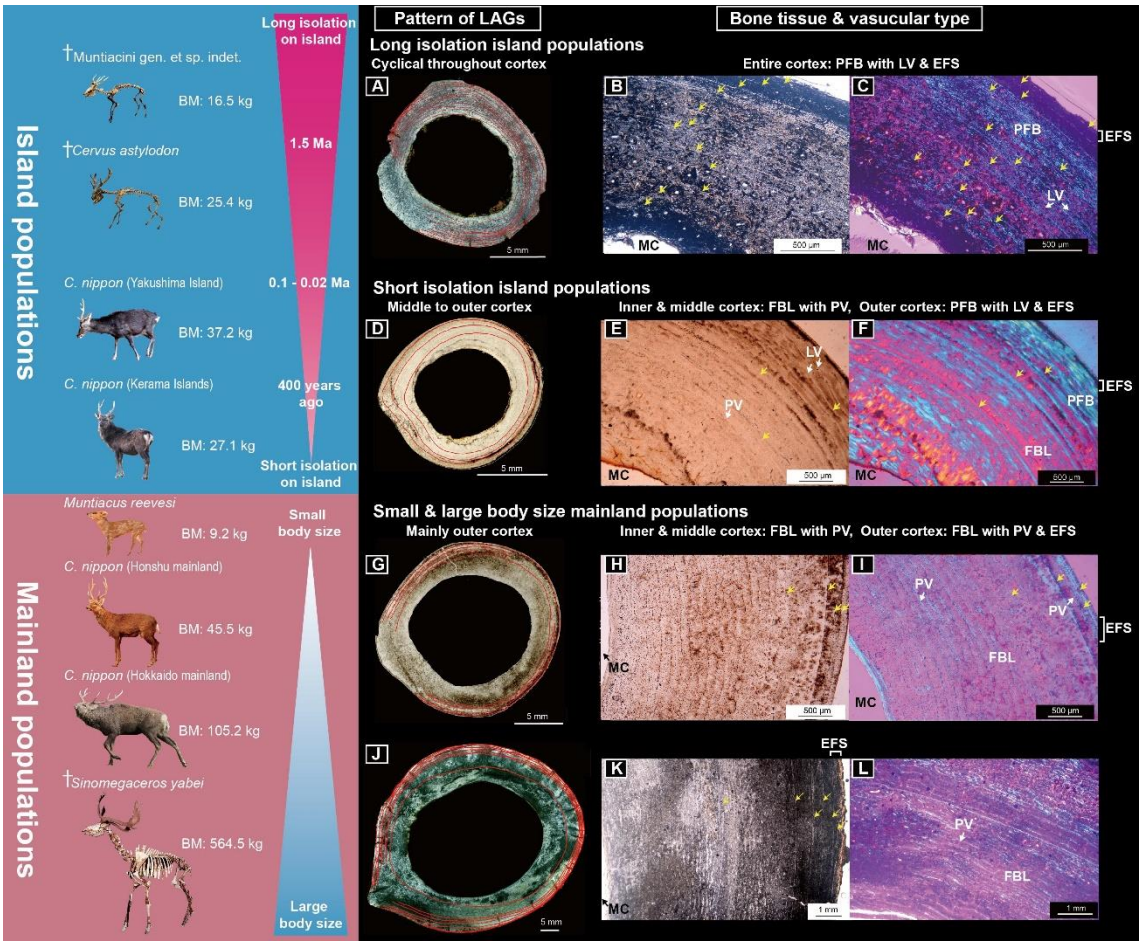

Figure 2. Bone histological features of the Japanese cervids. (A–C) Midshaft cross section (A), cortex in normal light (B), and cortex in polarized light (C) of a femur of fossil *Cervus astylodon* (OPM-HAN07-1603). (D–F) Midshaft cross section (D), cortex in normal light (E), and cortex in polarized light (F) of a femur of extant sika deer (*Cervus nippon*) from Yakushima Island (TPM-M-313). (G–I) Midshaft cross section (G), cortex in normal light (H), and cortex in polarized light (I) of a femur of extant sika deer from Honshu mainland (CBM-ZZ-412). (J–L) Midshaft cross section (J), cortex in normal light (K), and cortex in polarized light (L) of a femur of fossil *Sinomegaceros yabei* (OMNH-QV-4062). Red lines in A, D, G and J and yellow arrows in B, C, E, F, H, I, and K indicate lines of arrested growth. The outer cortex is in the upper right in B, C, E and F and on the right in H, I and K. BM, body mass; EFS, external fundamental system; FBL, fibro-lamellar bone tissue; LAG, line of arrested growth; LV, longitudinal vascular canal; MC, medullary cavity; PFB, parallel-fibered bone tissue; PV, plexiform to laminar vascular canal. † Fossil cervids. Note: only cervids from the Okinawa Islands have a distinctly longer-term geographical isolation history and exhibit bone tissue indicating a slower growth rate (de Margerie, J. Cubo, and Castanet 2002).

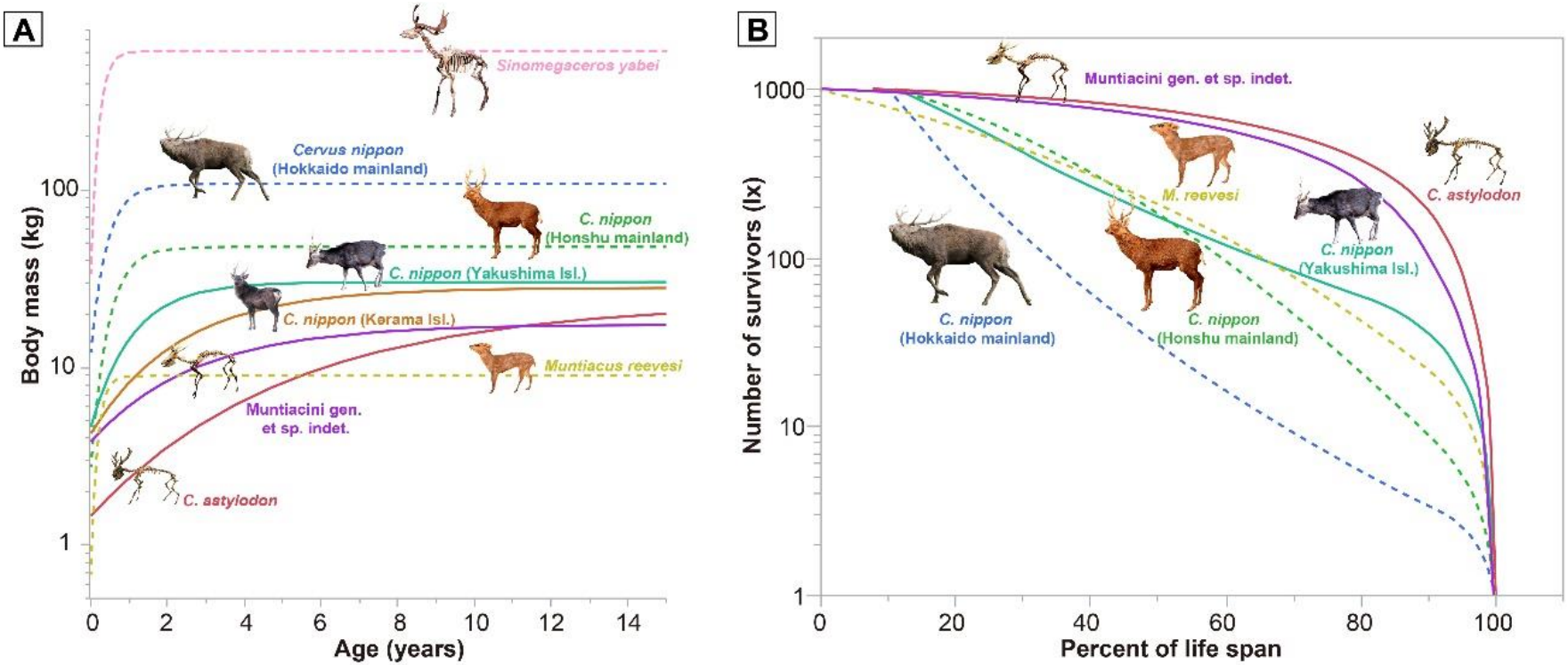

Figure 3. (A) Growth and (B) survivorship curves for Japanese cervids. Insular deer have a significantly slower growth rate and a delayed growth plateau (somatic maturity) compared with mainland deer (A), and there is an ecological shift from the *r*- to *K*-strategy between the insular and mainland deer (B). Growth and survivorship curves of insular cervids are solid lines and those of mainland cevids are dashed-lines.

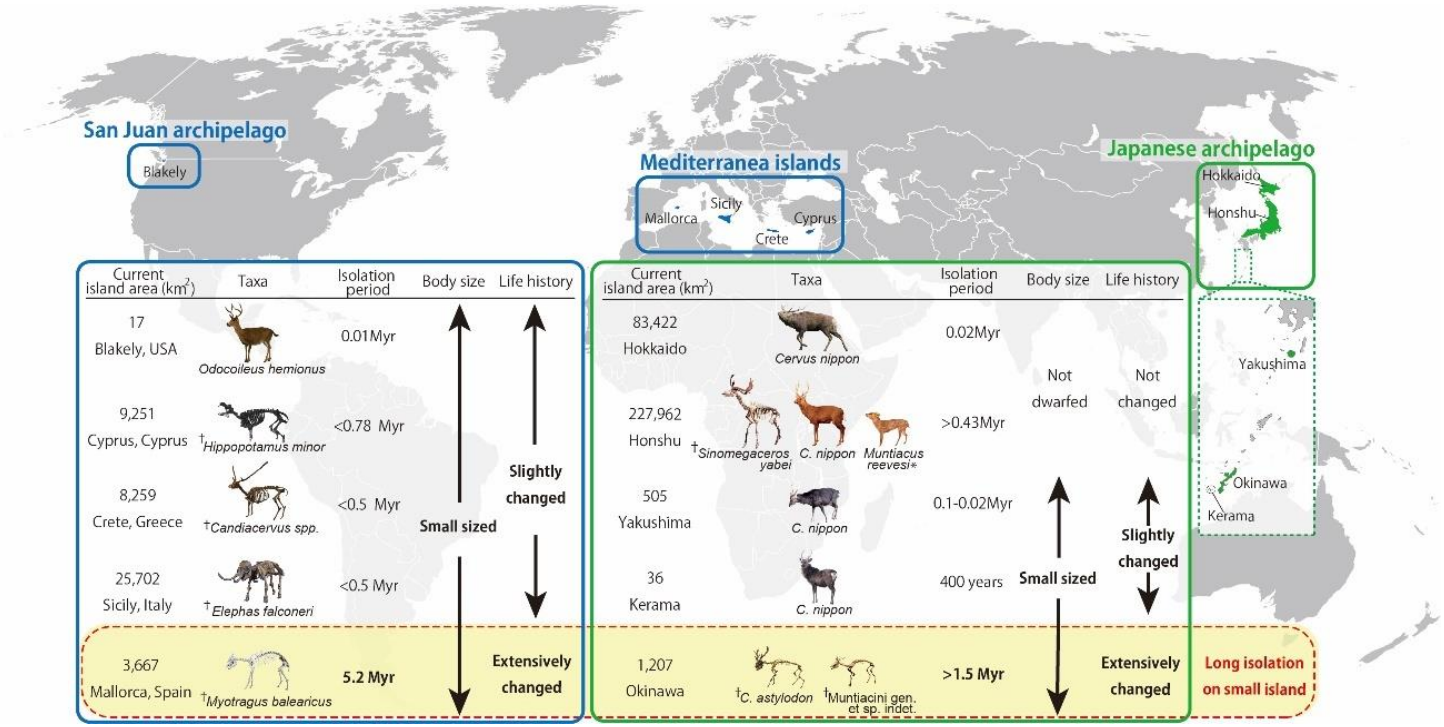

Figure 4. Summary of the relationship between insularity, body size and life history found in extant and fossil large mammals. The cervid species/populations of Japanese Archipelago were investigated in the present study and information of animals in other islands were obtained from references: *Odocoileus hemionus* (Long et al. 2019), *Hippopotamus minor* (van der Geer et al. 2010), *Candiacervus* spp. (van der Geer et al. 2010), *Elephas falconeri* (Palombo 2001, van der Geer et al. 2010), *Myotragus balearicus* (Köhler and Moyà-Solà 2009). Photographs of fossil mammals of Mediterranea islands courtesy of Gunma Museum of Natural History (*Elephas falconeri*), Alexandra van der Geer (*Hippopotamus minor* and *Candiacervus* spp.), and Meike Köhler (*Myotragus baleanicus*). * *Muntiacus reevesi* is an artificially introduced animal in 1980's, therefore the isolation period of > 0.43 Myr is not applicable to it (Chiba Prefecture and Deer Research Group on Boso 2007).

Table 1. Summary of the general information of sampled Japanese insular cervids.

Species/Population	Locality (island size)	Timing of immigration/isolation (reference)	Habitat type	EBM (kg) ‡	N of histological samples (Femur, Tibia)	N of demographic analysis samples (reference of data)
Extant species/population						
Cervus nippon (Hokkaido mainland)	Hokkaido (83,424 km^2)	Separation from Honshu mainland population: Late Pleistocene (ca. 0.02 Ma) (Kawamura 2009)	Subarctic coniferous forests or mixed forests	105.2	F: 7, T: 7	1060 (Hokkaido Institute of Environmental Sciences 1997)
Cervus nippon (Honshu mainland)	Honshu (227,962 km^2)	Middle Pleistocene (0.43 Ma) (Kawamura 2009)	Temperate evergreen broadleaved forests	45.5	F: 8, T: 8	594 (Kubo et al. 2011)
Muntiacus reevesi		Recent introduction (1960–1980) (Chiba Prefecture and Deer Research Group on Boso 2007)		9.2	F: 5, T: 5	84 (Chapman, Brown, and Rothery 2005)
Cervus nippon (Yakushima Island)	Yakushima Island (505 km^2)	Isolation from Kyushu mainland: Late Pleistocene (ca. 0.1–0.02 Ma; see SI Appendix for details and references there in)	Subtropical evergreen broadleaved forests	37.2	F: 4, T: 4	74 (this study)
Cervus nippon (Kerama Islands)	Kerama Islands (36 km^2)	Historic introduction (ca. 400 years ago) (Shiroma and Ohta 1996)	Subtropical evergreen broadleaved forests	27.1	F: 7, T: 7	NA
Fossil species						
†*Sinomegaceros yabei*	Honshu (227,962 km^2)	Middle Pleistocene (> 0.43 Ma) (Taruno et al. 2017)	NA	564.5	F: 2, T: 2	NA
†*Cervus astylodon*	Okinawa Island (1,207 km^2)	Early Pleistocene (ca. 1.5 Ma) (Otsuka and Takahashi 2000)	NA	25.4	F: 6, T: 5	45 (Kubo et al. 2011)
†Muntiacini gen. et sp. indet.				16.5	F: 4, T: 2	65 (Ozaki 2009)

† Fossil cervids.

‡ The estimated body mass (EBM) was obtained from fitted growth curves (SI Appendix, Fig. S7–S14) by averaging asymptotes for each species/population.

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Supplementary information

Variation and mechanisms of life history evolution in insular dwarfism as revealed by a natural experiment

by Hayashi and Kubo et al.

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Supplementary text

1. Locality and specimen information for the sampled cervids used in the histological analyses

A summary of the sampled taxa is provided in Tables S1–S4.

1-1. Extant taxa

Sika deer (*Cervus nippon*) from Hokkaido mainland. All individuals were wild and were hunted in Hokkaido Prefecture (43°N, 143°E), northern Japan (Figure 1). Hokkaido is one of the four main islands constituting the Japanese archipelago and was considered "mainland" for the purposes of this study. Hokkaido sika deer inhabit subarctic coniferous forests mixed with cool temperate deciduous trees and are known to be the largest subspecies [*C. nippon yesoensis* (Groves 2006, Ohtaishi 1986)]. The complete skeleton of each individual is now housed in the Hokkaido University Museum (HOUMVC), Sapporo, Japan. The age at death was assessed based on tooth eruption for fawns and yearlings and the tooth cementum annuli formed at the roots of the incisors for adults.

Sika deer and Reeves's muntjac (*Muntiacus reevesi*) from Honshu mainland. All individuals were wild and were hunted or collected in Chiba Prefecture (35°N, 140°E), which is in the central region of Honshu (Figure 1). Honshu is on(Terada, Tatsuzawa, and Saitoh 2012)e of the four main islands constituting the Japanese archipelago and was considered "mainland" for the purposes of this study. Both of these species inhabit evergreen broadleaved forests in the temperate zone. Reeves's muntjac was introduced to Chiba Prefecture ca. 1960–1980 following an escape from a private park (Chiba Prefecture and Deer Research Group on Boso 2007). The source population is unknown, but the body size of these deer is closer to that of the Taiwanese population. The complete skeletons of these individuals are stored at the Natural History Museum and Institute, Chiba (CBM), Japan. The age at death was assessed based on tooth eruption or the cementum annuli for the Honshu sika deer and from tooth eruption and the molar wear condition for Reeves's muntjac.

Sika deer from Yakushima island. All individuals were wild and were hunted or collected on Yakushima Island (30°18′N, 130°30′E) in Kagoshima Prefecture, Japan, where they inhabit subtropical evergreen broadleaved forests. The sika deer on Yakushima Island are considered to be the subspecies *C. nippon yakushimae* (Ohtaishi 1986) and exhibit considerable phenotypic differences from the mainland populations, such as a small body size, simplified antlers, and shortened limbs (Ohtaishi 1986,

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Terada, Tatsuzawa, and Saitoh 2012). The sika deer of Yakushima Island diverged from the population on Kyushu Island (one of the four main islands constituting the Japanese archipelago) ca. 0.1 Mya when the Ohsumi Strait was formed between the two islands (Ohshima 1990). Yakushima Island was possibly connected to Kyushu Island by a land bridge during the Last Glacial Maximum (ca. 25,000 ya), but the genetic differentiation between the two populations implies a much longer period of isolation for Yakushima deer (Nagata et al. 1999, Terada and Saitoh 2018). The complete skeleton of each individual has been stored at Tochigi Prefectural Museum (TPM), Utsunomiya, Japan. The age at death was assessed based on tooth eruption for fawns and yearlings and the tooth cementum annuli of the incisors and molars for adults.

Sika deer from Kerama islands. All individuals were wild and were collected as carcasses resulting from natural deaths on Kerama Islands (26°12′N, 127°24′E) in Okinawa Prefecture, Japan, by two of the authors (MI and TS) during their fieldwork. Sika deer were introduced to Kerama Islands from Kyushu ca. 400 years ago (Okinawa Prefectural Board of Education 1996) and inhabitat subtropical evergreen broadleaved forests. These deer exhibit the phenotypic characteristics of insular dwarf deer (similar to Yakushima Island deer) and so are also regarded as a subspecies of sika deer (*C. nippon keramae*) (Ohtaishi 1986). Most individuals were complete skeletons, but some bone elements were lost at the time of collection in the field for some individuals. All of the specimens have been stored at the Faculty of Science, University of the Ryukyus (URB), Nishihara, Japan. The age at death was assessed based on tooth eruption for fawns and yearlings and the tooth cementum annuli for adults. However, no reliable age at death was obtained for URB-MAM-34.

1-2. Fossil taxa

***Cervus astylodon* and Muntiacini gen. et sp. indet. (the Ryukyu Muntjac).** Both of these fossil deer species were excavated from the Hananda-Gama Cave site (26°8′7″N, 127°45′32″E) in the southern part of Okinawa Island (Okinawa Prefectural Museum and Art Museum 2009). These animal remains are mainly isolated materials consisting of limb bones and teeth and are considered to have fallen into the cave through fissures or sinkholes following decomposition of the carcasses (Okinawa Prefectural Museum and Art Museum 2009). The detailed geological age of these fossils is not clear due to a lack of suitable specimens for radiocarbon dating. However, based on radiocarbon dating at other sites that have yielded fossils of these species, the geological age of both species has been estimated as the Latest Pleistocene (Okinawa Prefectural Museum and Art

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Museum 2009). *Cervus astylodon* has been recognized as a typical insular dwarf due to its very small body size and shortened limbs (van der Geer et al. 2010). The Ryukyu muntjac used to be referred to as *Dicrocerus* sp. (van der Geer et al. 2010), which is a genus that is known mostly from Miocene Europe. However, the finding that the antlers have a clear burr and grow from the posterior parts of the frontals oriented backward clearly indicates that it does not belong to this genus (Gertrud Rößner, personal communication to MOK). Rather, it appears to be closely related to *Muntiacus* based on the presence of tusk-like canines and a lack of antlers in the females, neither of which is found in *Dicrocerus* (Azanza et al. 2011). Full revision of this species is required, including a detailed description and comparisons with other fossil and extant taxa. Therefore, in this paper, we present it as Muntiacini gen. et sp. indet. and refer to it as "the Ryukyu muntjac." All of the specimens that were examined are stored at Okinawa Prefectural Museum and Art Museum (OPM), Naha, Japan.

***Sinomegaceros yabei*.** This fossil deer had a gigantic body size that was comparable to that of Irish elk (*Megaloceros giganteus*) (Okumura et al. 2016, Taruno et al. 2017). The skeletal remains were excavated from the Kumaishi-do Cave site (35°45″N, 137°′00″E) in Hachiman-cho, Gujo city, Gifu Prefecture, central Japan. Most of the animal remains obtained from this site are isolated materials, but an incomplete skeleton that includes the skull has also been discovered (Okumura et al. 2016). The geological age of these fossils is the Late Pleistocene based on radiocarbon dating of the bones of cervids and proboscideans (Okumura et al. 1982). All of the specimens we sampled are stored at the Osaka Museum of Natural History (OMNH), Osaka, Japan.

2. Growth curve modeling using the femora and tibiae

2-1. Validity of using the number of lines of arrested growth (LAGs) to estimate Age. We first checked the correlation between the numbers of LAGs observed in the long bones and the actual ages at death assessed by the numbers of dental cementum annuli formed in the tooth roots using 20 extant sika deer from both mainland and island localities (Table S5). Since the date or season of death had been recorded for all of these samples together with the age at death, we counted how many times deer experienced winter (i.e., the season when LAGs are usually formed) and compared this with the number of LAGs. The scatter plot clearly showed that, with the exception of four individuals, the number of LAGs equaled the number of winter seasons the deer experienced in their lifetime (Figure S5). The exceptions to this were one sika deer from

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Hokkaido mainland (HOUMVC00037), one sika deer from Honshu mainland (CBM-ZZ-412), and two sika deer from Kerama Islands (URB-MAM-55 and -193). HOUMVC00037, CBM-ZZ-412, and URB-MAM-193 had fewer LAGs (7, 7, and 8, respectively) than their actual age at death (8, 8, and 10, respectively). Based on the expansion of the medullary cavity in these old individuals, it seemed likely that the first (and also second in URB-MAM-193) LAG had been eliminated, and this was supported by the estimated body mass at the first LAG, which was greater than that of younger deer of the same population and sex (based on a comparison of HOUMVC00037 with HOUMVC00033, CBM-ZZ-412 with CBM-ZZ-218, and URB-MAM-193 with URB-MAM-211). Therefore, we considered that these three individuals had lost their initial growth information. In the case of URB-MAM-55, the number of LAGs (7) was greater than the actual age at death (4). This individual had limb bones without epiphyseal fusion, indicating that the bones were still actively growing in a longitudinal direction. However, observation of the long bone histology showed that appositional growth was decelerated and marked with many LAGs. Therefore, it is possible that this deer was malnourished and its growth was arrested intermittently even during the growing season. Consequently, we excluded this individual from the following growth curve analysis.

For the extant Reeves's muntjac specimens, the age at death was estimated based on tooth eruption and the molar wear condition. Assessment of the correspondence between the number of LAGs and the age assessed by tooth eruption and wear for Reeves's muntjac showed that the two were perfectly matched. Therefore, for this species, we considered the age assessed by tooth eruption and wear to be the actual age at death.

2-2. Age assessment. We estimated the age at death of the fossil specimens from the number of LAGs. However, several specimens showed explicit expansion of the medullary cavity, which may have erased the record of early growth. Therefore, we also estimated the number of lost LAGs due to bone remodeling. Several estimation methods have been proposed in paleo-histological studies (Cooper et al. 2008, Erickson 2014, Kolb et al. 2015). Here, we applied a simple method that uses a reference specimen with no apparent bone remodeling or expansion of the medullary cavity and compares this with specimens with possible dissipation of the initial LAGs. We chose this method because backward estimation from fitted growth curves (Cooper et al. 2008) resulted in overestimation of the number of lost LAGs, generating a slower growth rate for the fossil taxa. Therefore, to yield a more conservative comparison with extant taxa, we

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estimated the number of lost LAGs through comparisons with younger specimens of the same taxa that were well preserved. Table S6 summarizes the estimation of lost LAGs based on comparisons with the reference specimens. The number of lost LAGs was small in all cases except for the femora of *C. astylodon*, which exhibited a more obvious expansion of the medullary cavity, resulting in up to seven LAGs potentially being lost (in OPM-HAN06-155).

Among the extant specimens we analyzed, only one sika deer (Kerama Islands, URB-MAM-34) did not have a reliable age based on the tooth cementum annuli. This specimen had eight LAGs in both the femur and tibia with completely fused epiphyses. Comparison of this specimen with other Kerama deer specimens indicated that one LAG had been erased by bone remodeling. Therefore, the age at death of this specimen was estimated to be 9 years old.

Raw data of LAG and external diaphysial measurements of specimens used in the histological analyses, together with estimated body mass is presented in Table S14 (Separate Excel file).

2-3. Body mass estimation. In addition to measuring the bone diameters at the LAGs, we measured the external diaphysial measurements [anteroposterior diameter (APD) and mediolateral diameter (MLD)] and used these to estimate the body mass at death. The body mass estimation formulae for both the femur and tibia were obtained from Scott (1990), who presented the following linear regression equations of body mass against limb bone metrics for extant artiodactyls:

$$\mathrm{Log}_{10}(\text{body mass in kg}) = 0.8737 + 2.7585*\log_{10}(\text{femur APD in cm})$$

$$\mathrm{Log}_{10}(\text{body mass in kg}) = 0.8915 + 2.8771*\log_{10}(\text{femur MLD in cm})$$

$$\mathrm{Log}_{10}(\text{body mass in kg}) = 0.9834 + 2.9356*\log_{10}(\text{tibia APD in cm})$$

$$\mathrm{Log}_{10}(\text{body mass in kg}) = 0.8689 + 2.7672*\log_{10}(\text{tibia MLD in cm})$$

We compared the two formulae for each of the femur and tibia and selected those that produced the least discrepancy in estimated body mass with the femur and tibia metrics of extant cervids. This resulted in the formulae using MLD being selected for both bones.

Accurate fitting of a growth curve requires knowledge of the starting point (i.e., the neonatal body mass). For extant sika deer (Honshu and Hokkaido mainland, Yakushima Island) and Reeves's muntjac, we collected neonatal or perinatal body mass data from the literature (Suzuki and Ohtaishi 1993) or zoo reports. However, the neonatal body mass was not known for sika deer from Kerama Islands. Therefore, we used the value of sika deer from Yakushima Island for this population because of their similar body sizes

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as adults. For the fossil taxa, we estimated the neonatal body mass using the following adult–neonate body mass regression equation based on data from 121 extant artiodactyls in the PanTHERIA database [Jones et al. (2009); http://esapubs.org/archive/ecol/E090/184/] (Figure S6):

$$\text{Log}_{10}\,(\text{neonatal body mass in g}) = -0.3009 + 0.806*\log_{10}\,(\text{adult body mass in g})$$

The adult body masses of the fossil cervids, which were obtained from the external diaphysial measurements of the femora and tibiae, were averaged for each species and substituted into the regression equation to predict the neonatal body mass. The estimated neonatal body masses of the fossil cervids are presented in Table S7.

2-4. Growth curve modeling and statistical comparisons of the growth curve parameters. For both the extant and fossil samples, all individuals that were older than yearlings were used for growth curve fitting. In addition, we included a fawn and a yearling in the growth curve fitting for extant Reeves's muntjac. A Gompertz curve was fitted to the longitudinal body mass data for each individual using the formula:

$$W = a\text{Exp}(-\text{Exp}(-b(x-c))$$

where W is the body mass in kg, x is the age in years, and the parameters a, b, and c represent the asymptote, growth rate, and inflection point, respectively. We then obtained growth curves for each species/population and each leg bone (femur or tibia) (Figure S7–S14) and compared two of the parameters (growth rate and inflection point) statistically among the extant and fossil cervids. Two-way analysis of variance (ANOVA) showed that bone type (i.e., femur or tibia) had no significant effect on the growth curve parameters (Table S8). Therefore, we combined the data from the femur and tibia and conducted multiple comparisons using the Tukey-Kramer method to examine differences in the growth parameters among the eight species/populations (Table S9), averaging the growth curve parameters from the femur and tibia for the extant species to avoid repeated sampling from the same individual. In addition, we conducted multiple comparisons using the uncombined femur and tibia datasets (Tables S10 and S11). A comparison of the growth curves is presented in Figure 3A for the femur and Figure S15 for the tibia.

3. Reconstruction of survivorship curves using age data of extant and fossil cervids. We obtained age data for the three extant sika deer populations that were used in the histological investigations either from published papers or through investigation of the cementum annuli of the incisor roots: Hokkaido mainland [n = 1,060 (Hokkaido Institute of Environmental Sciences 1997)], Honshu mainland [n =594 (Ozaki et al.

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2010)], and Yakushima Island (n = 74, cementum annuli of museum specimens). These age data were based on randomly culled individuals and so were considered to be indicative of actual extant populations. To depict the survivorship curves, we constructed life tables following the method described in Caughley (1977), assuming stationary age distributions, and applied probit regression to smooth the age distributions. The survivorship curves were presented with an initial value of 1,000 individuals.

The age data for extant Reeves's muntjac from England and fossil insular cervids from Okinawa Island were obtained from published references [Reeves's muntjac: n = 84 (Chapman, Brown, and Rothery 2005); *C. astylodon*: n = 45 (Kubo et al. 2011); the Ryukyu muntjac: n = 65 (Ozaki 2009)]. Since the age data for these populations were based on natural deaths, the age class frequency was divided by the sample size to obtain a multiple of the frequency of mortality (dx). Life tables were then derived from this mortality frequency (Caughley 1977).

The observed age class frequencies and derived life tables for each deer population are shown in Tables S12 and S13, and the survivorship curves are presented in Figure 3B.

Supplementary figures

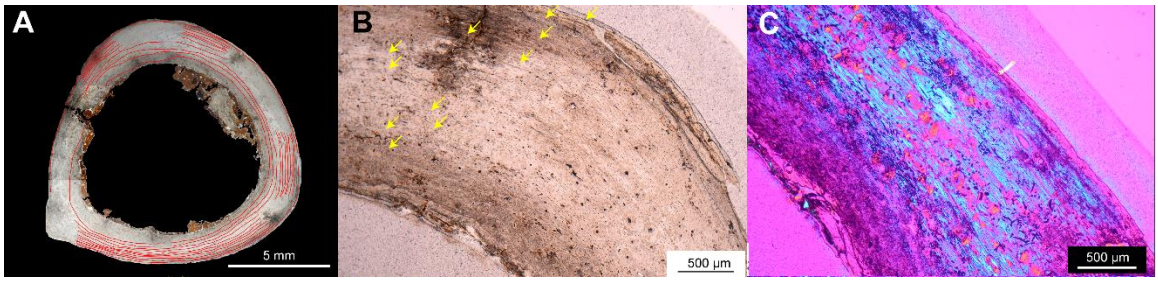


Figure S1. Bone histologies of the femora of fossil Ryukyu muntjac. (A–C) Midshaft cross section in normal light (A), cortex in normal light (B), and cortex in polarized light (C) of femora of the Ryukyu muntjac (Muntiacini gen. et sp. indet.; A and B: OPM-HAN06-163; C: OPM-HAN07-1618). The red lines in A and the yellow arrows in B indicate lines of arrested growth (LAGs). The outer cortex is in the upper right in B and C. Note: LAGs appear at narrow intervals in the inner cortex in the Ryukyu muntjac, which also has an abundance of parallel-fibered bone tissue (blue tissues in C).

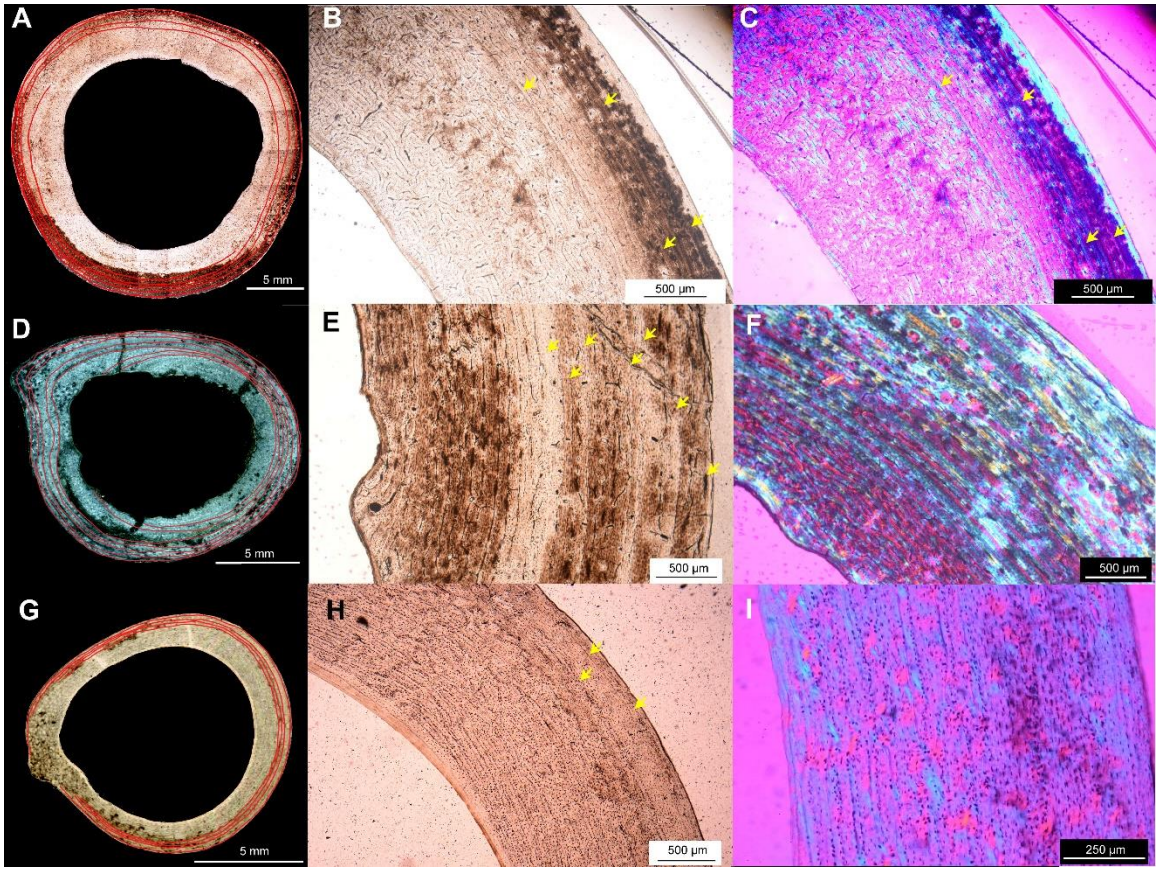


Figure S2. Bone histologies of the femora of extant Japanese cervids. (A–C) Midshaft cross section in normal light (A), cortex in normal light (B), and cortex in polarized light (C) of a femur of sika deer (*Cervus nippon*) from Hokkaido mainland (HOUMVC00035). (D–F) Midshaft cross section in normal light (D), cortex in normal light (E), and cortex in polarized light (F) of a femur of sika deer from Kerama Islands (URB-MAM-183). (G–I) Midshaft cross section in normal light (G), cortex in normal light (H), and cortex in polarized light (I) of a femur of Reeves's muntjac (*Muntiacus reevesi*) from Honshu mainland (CBM-ZZ-2646). The red lines in A, D, and G and the yellow arrows in B, E, and H indicate lines of arrested growth (LAGs). The outer cortex is on the right in E and I and in the upper right in B, C, F, and H.

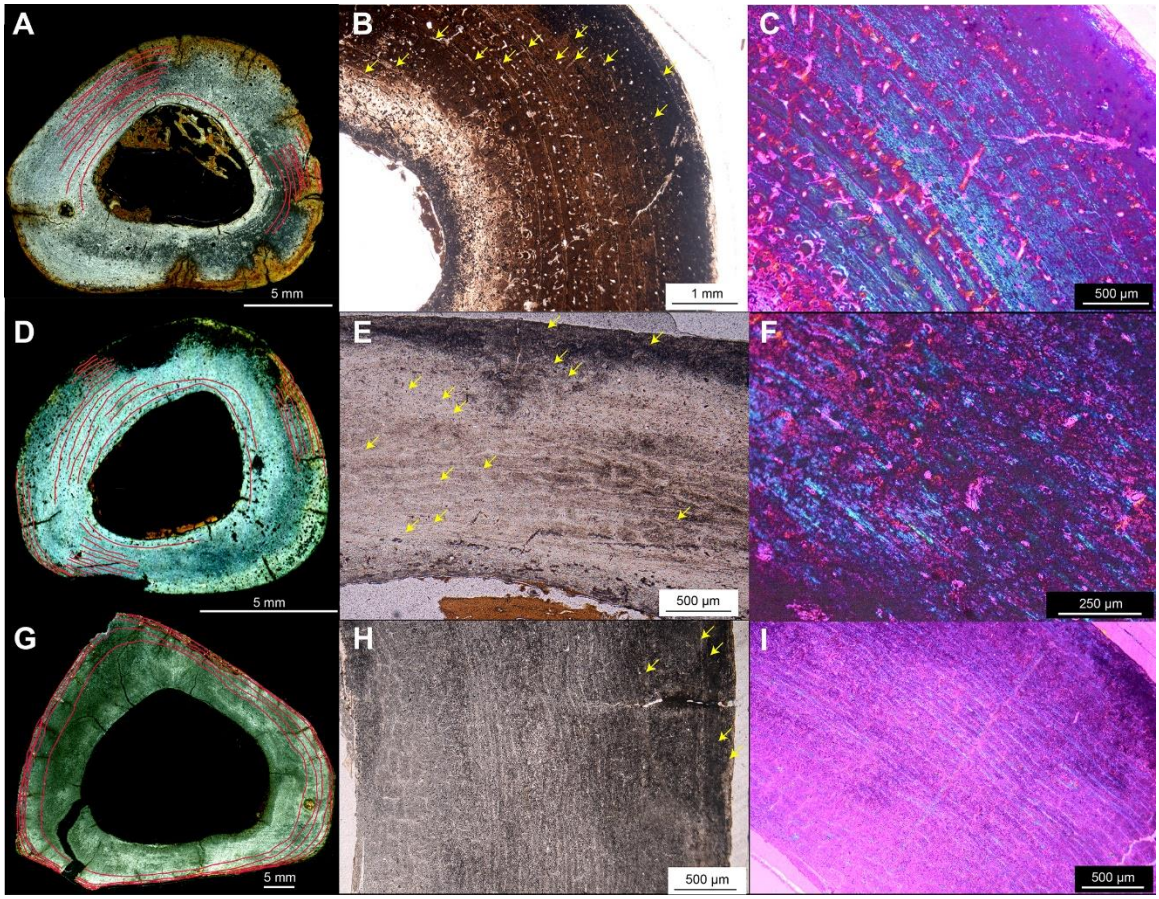


Figure S3. Bone histologies of the tibiae of fossil Japanese cervids. (A–C) Midshaft cross section in normal light (A), cortex in normal light (B), and magnified view of the cortex in polarized light (C) of a tibia of *Cervus astylodon* (OPM-HAN07-1585). (D–F) Midshaft cross section in normal light (D), cortex in normal light (E), and magnified view of the cortex in polarized light (F) of a tibia of the Ryukyu muntjac (Muntiacini gen. et sp. indet.; OPM-HAN07-1599). (G–I) Midshaft cross section in normal light (G), cortex in normal light (H), and cortex in polarized light (I) of a tibia of *Sinomegaceros yabei* (OMNH-QV-4068). The outer cortex is in the upper right in B, C, F, and I, at the top in E, and on the right in H.

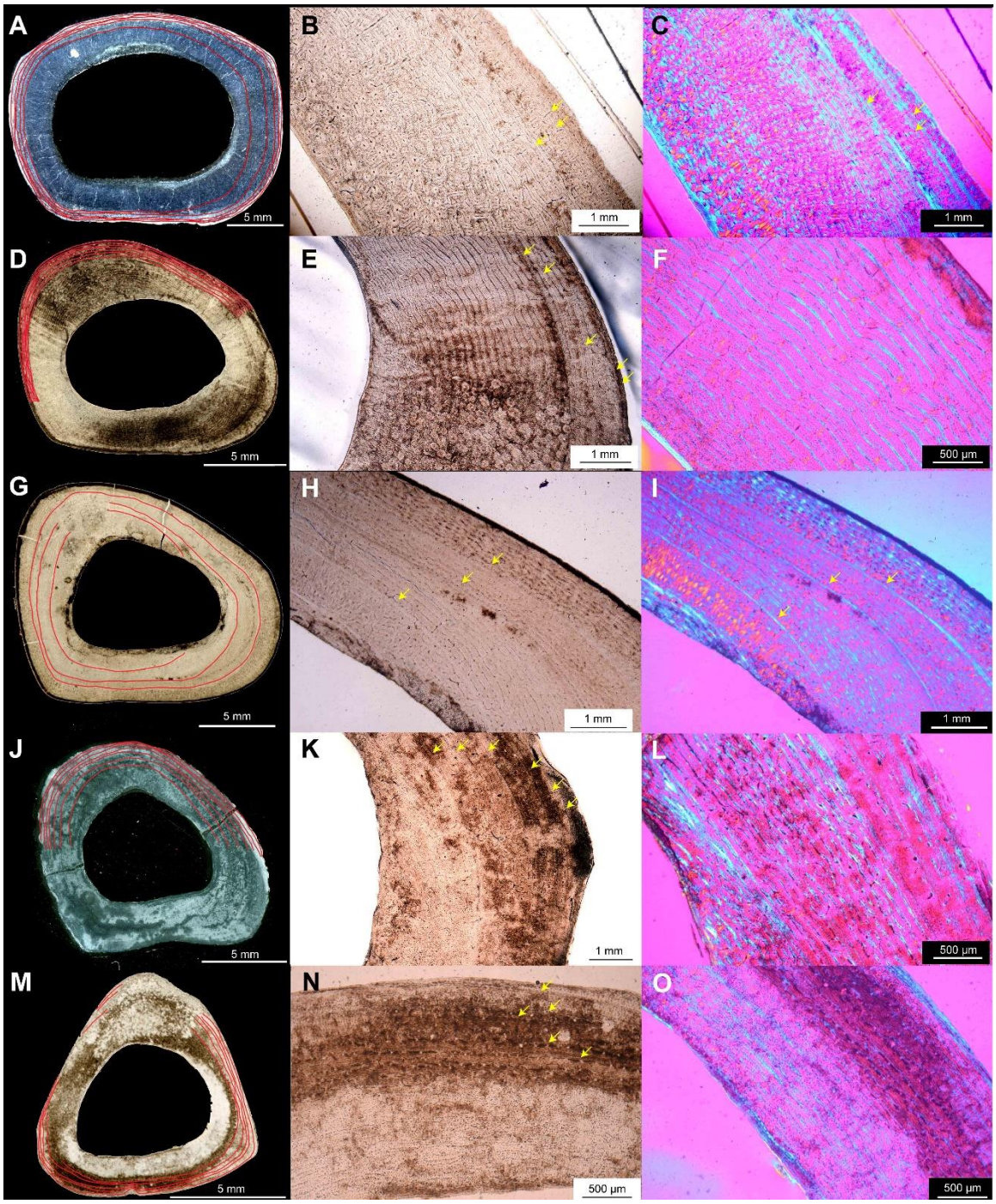


Figure S4. Bone histologies of the tibiae of extant Japanese cervids. (A–C) Midshaft cross section in normal light (A), cortex in normal light (B), and cortex in polarized light (C) of a tibia of sika deer (*Cervus nippon*) from Hokkaido mainland (HOUMVC00035). (D–F) Midshaft cross section in normal light (D), cortex in normal light (E), and cortex in polarized light (F) of a tibia of sika deer from Honshu mainland (CBM-ZZ-412). (G–I) Midshaft cross section in normal light (G), cortex in normal light (H), and cortex in polarized light (I) of a tibia of sika deer from Yakushima Island (TPM-M-313). (J–L) Midshaft cross section in normal light (J), cortex in normal light (K), and cortex in polarized light (L) of a tibia of sika deer from Kerama Islands (URB-MAM-183). (M–O)

Midshaft cross section in normal light (M), cortex in normal light (N), and cortex in polarized light (O) of a tibia of Reeves's muntjac (*Muntiacus reevesi*) from Honshu mainland (CBM-ZZ-2646). The outer cortex is in the upper right in B, C, E, F, H, I, K, L, and O at the top in N.

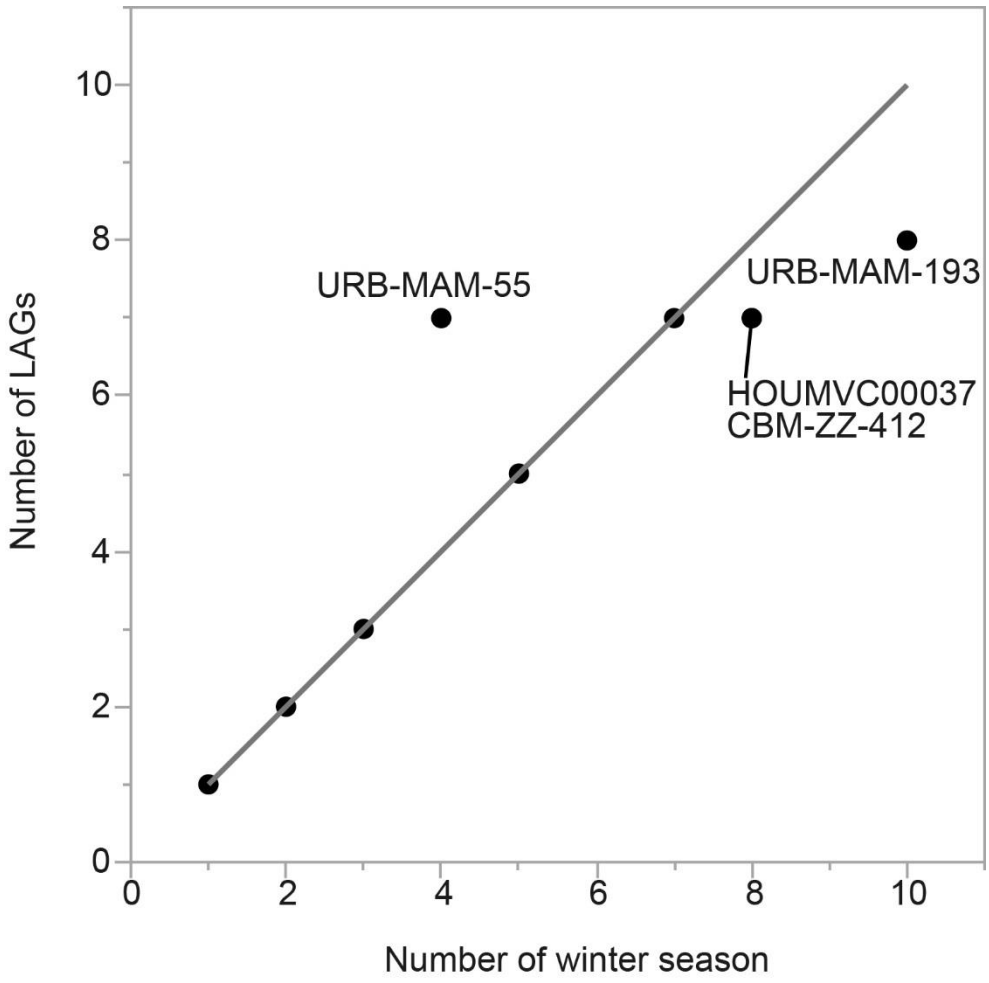


Figure S5. Scatter plot showing the correlation between the number of winter seasons a deer experienced during its lifetime and the number of lines of arrested growth (LAGs). The raw data are presented in Table S5. There was a perfect match between the two variables in extant sika deer (*Cervus nippon*) of known age, with the exception of four individuals (HOUMVC00037, CBM-ZZ-412, URB-MAM-55, and URB-MAM-193). The solid line represents x = y.

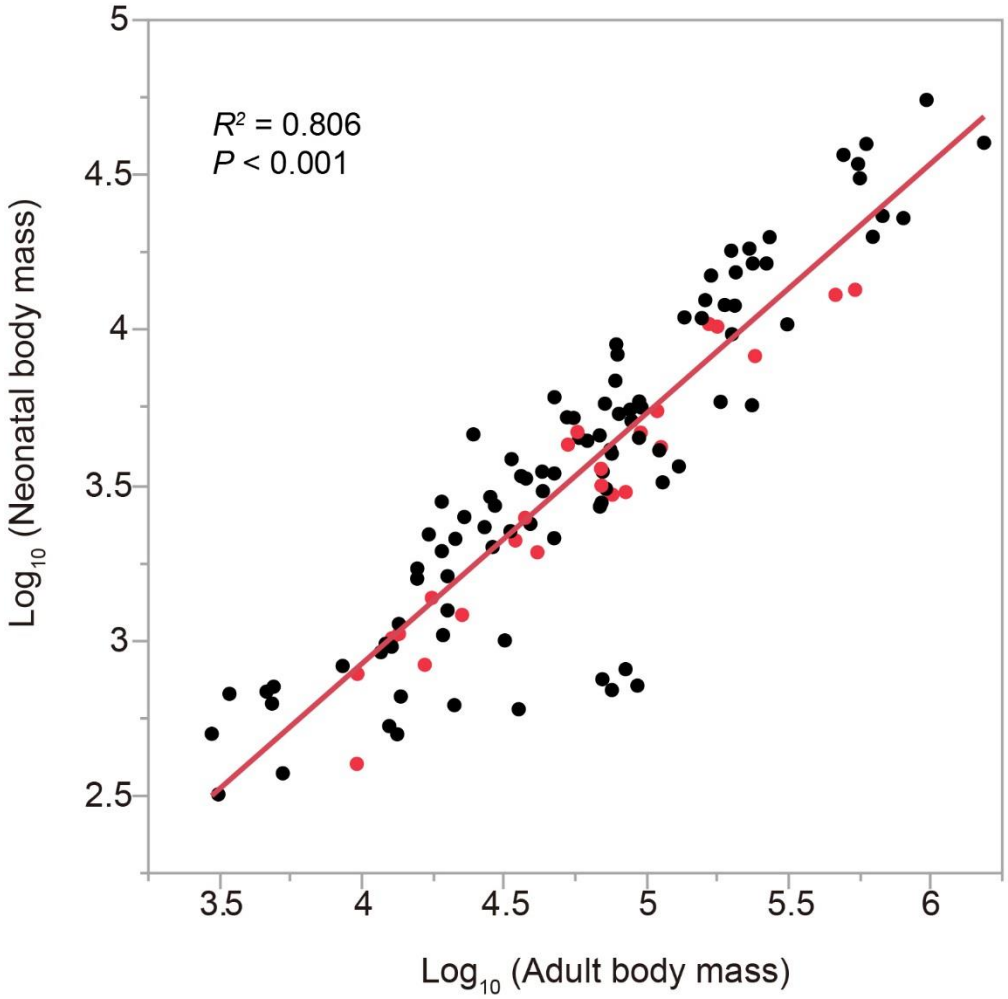


Figure S6. Scatter plot showing the correlation between adult and neonatal body mass in extant artiodactyls (n = 121). The solid line represents the linear regression line. The red dots indicate Cervidae species. Data were obtained from PanTHERIA [(Jones et al. 2009); http://esapubs.org/archive/ecol/E090/184/]. The regression equation was then used to estimate the neonatal body mass of three fossil cervids (see Table S7).

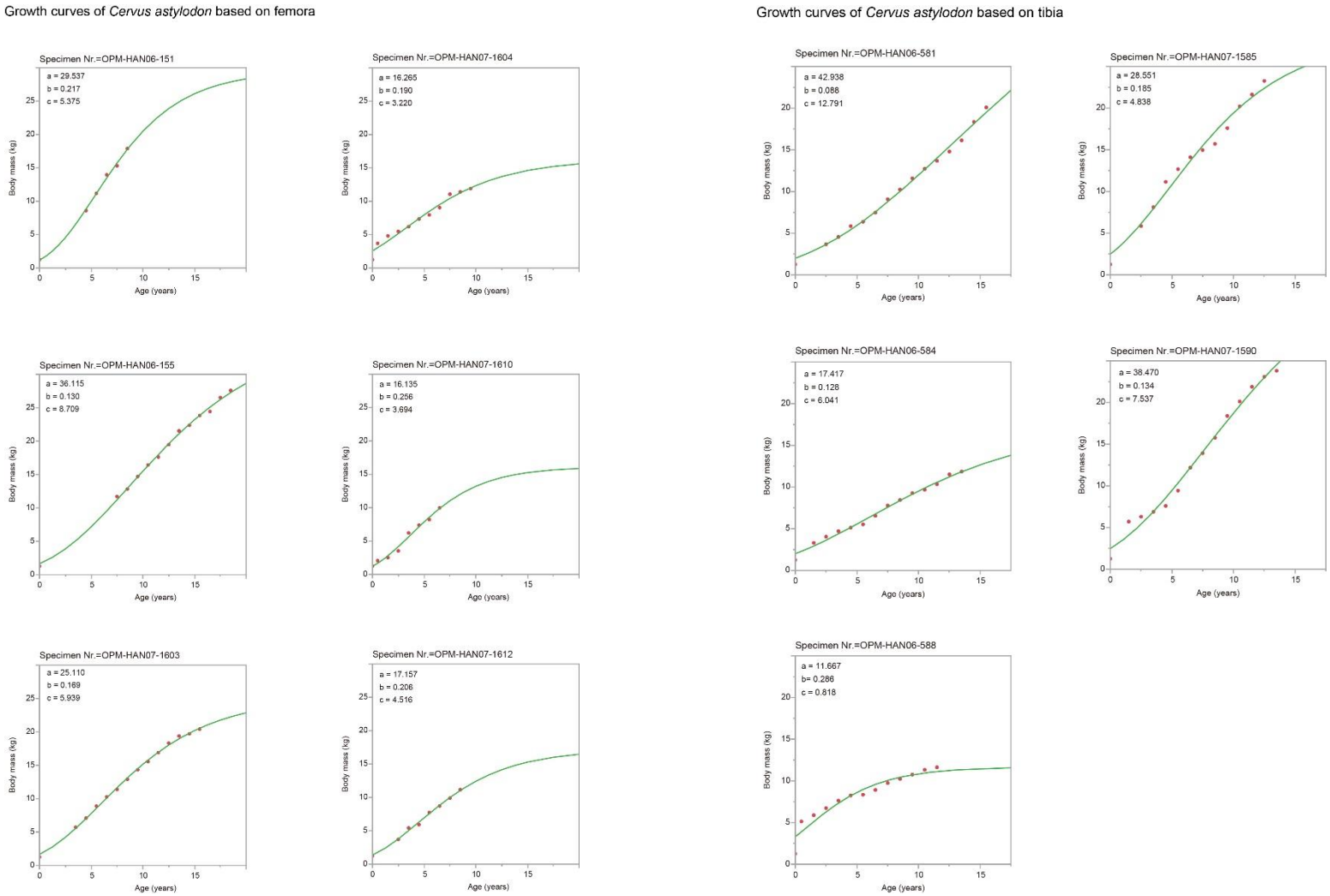
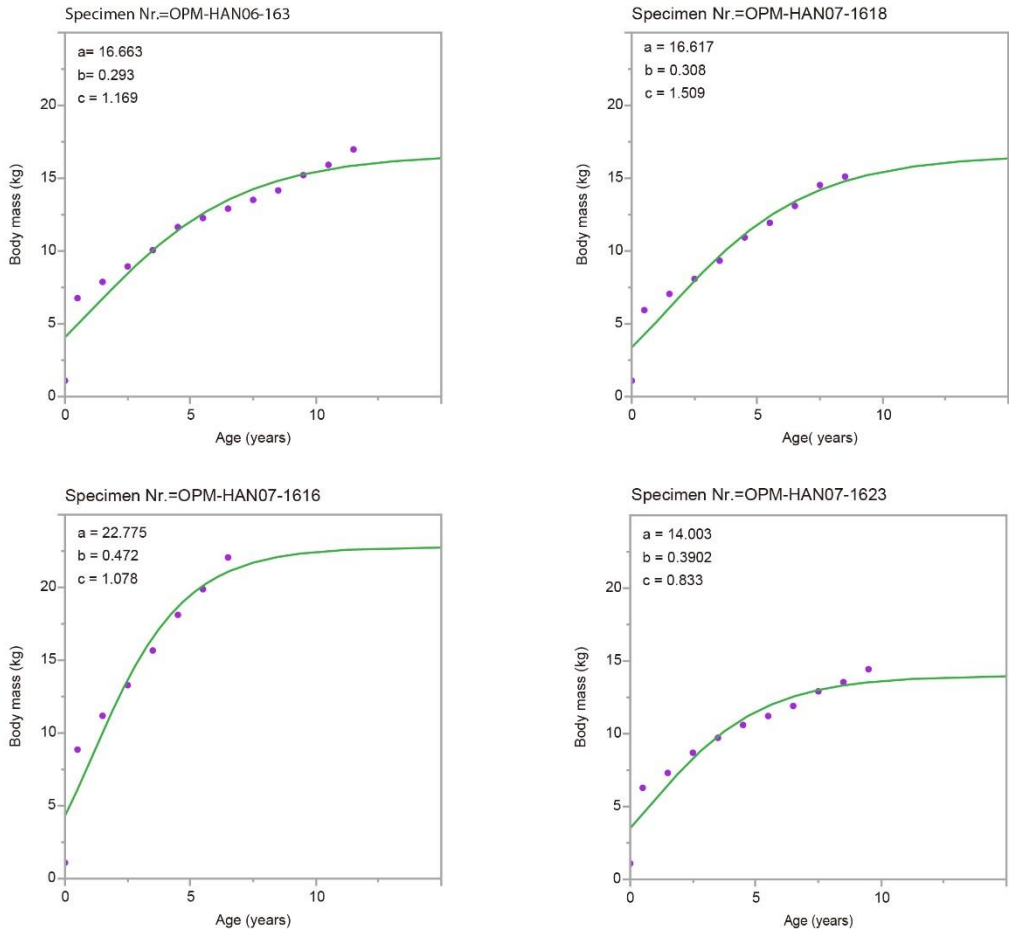


Figure S7. Growth curves of body mass for fossil *Cervus astylodon*. Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote; b: growth rate; and c: inflection point) are shown on the plot for each individual.

Growth curve of Muntiacini genus et sp. indet. (Ryukyu muntjac) based on femora



Growth curve of Muntiacini genus et sp. indet. (Ryukyu muntjac) based on tibia

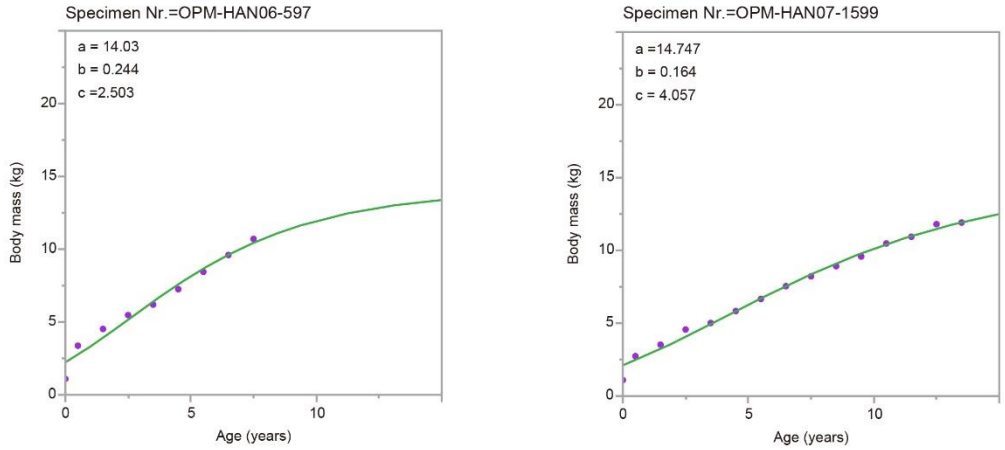


Figure S8. Growth curves of body mass for fossil Ryukyu muntjac (Muntiacini gen. et. sp. indet.). Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote; b: growth rate; and c: inflection point) are shown on the plot for each individual.

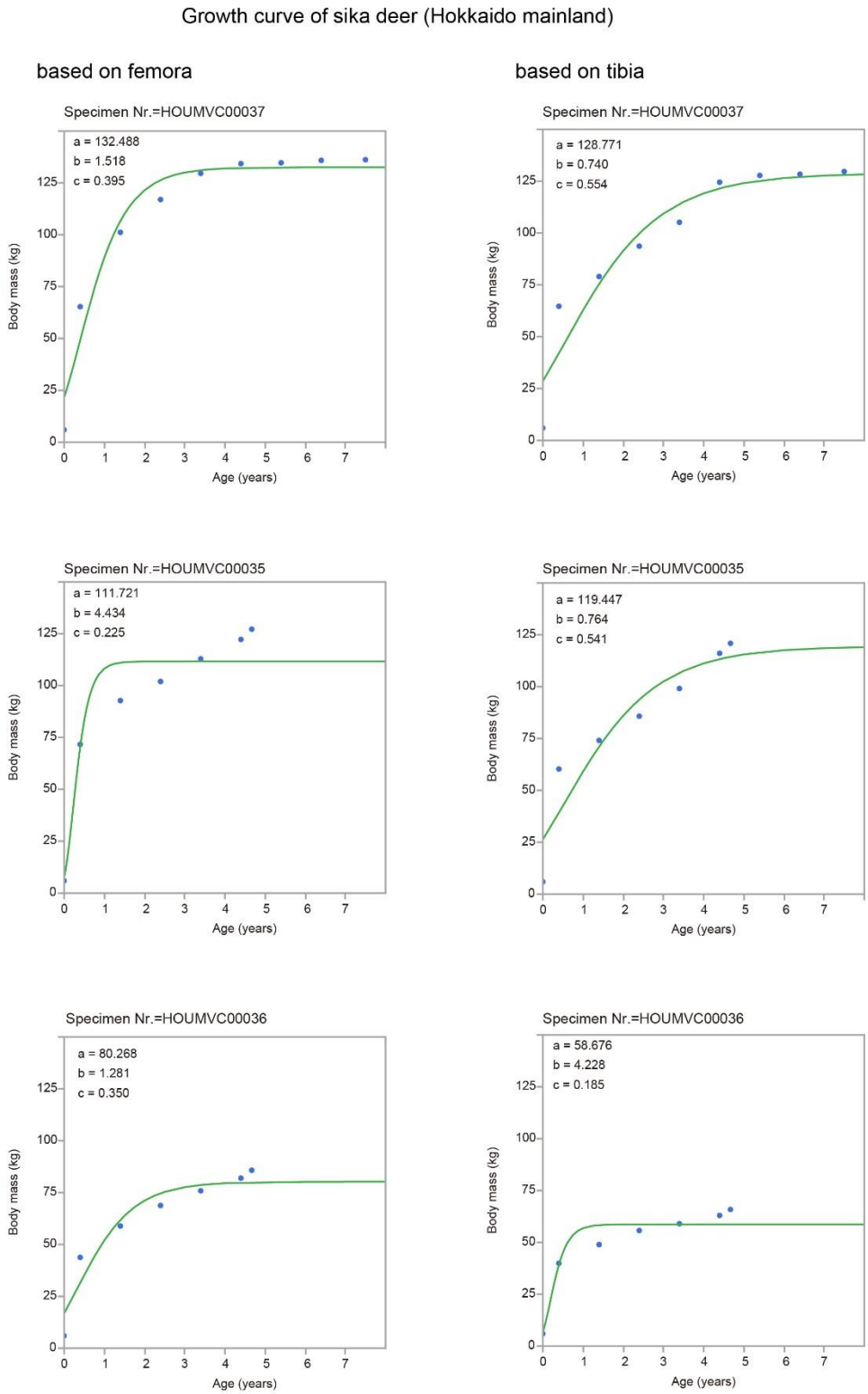


Figure S9. Growth curves of body mass for extant sika deer (*Cervus nippon*) from Hokkaido mainland. Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote; b: growth rate; and c: inflection point) are shown on the plot for each individual.

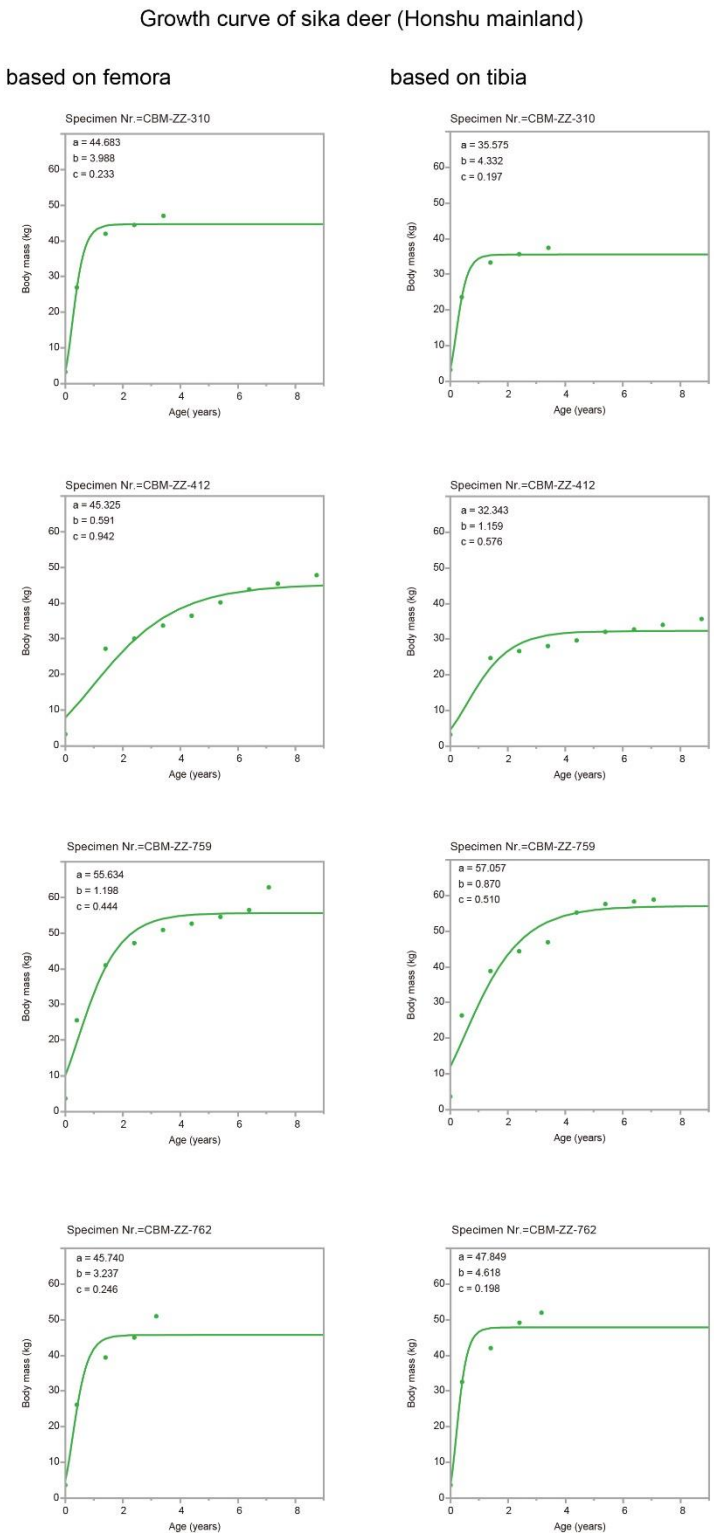


Figure S10. Growth curves of body mass for extant sika deer (*Cervus nippon*) from Honshu mainland. Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote; b: growth rate; and c: inflection point) are shown on the plot for each individual.

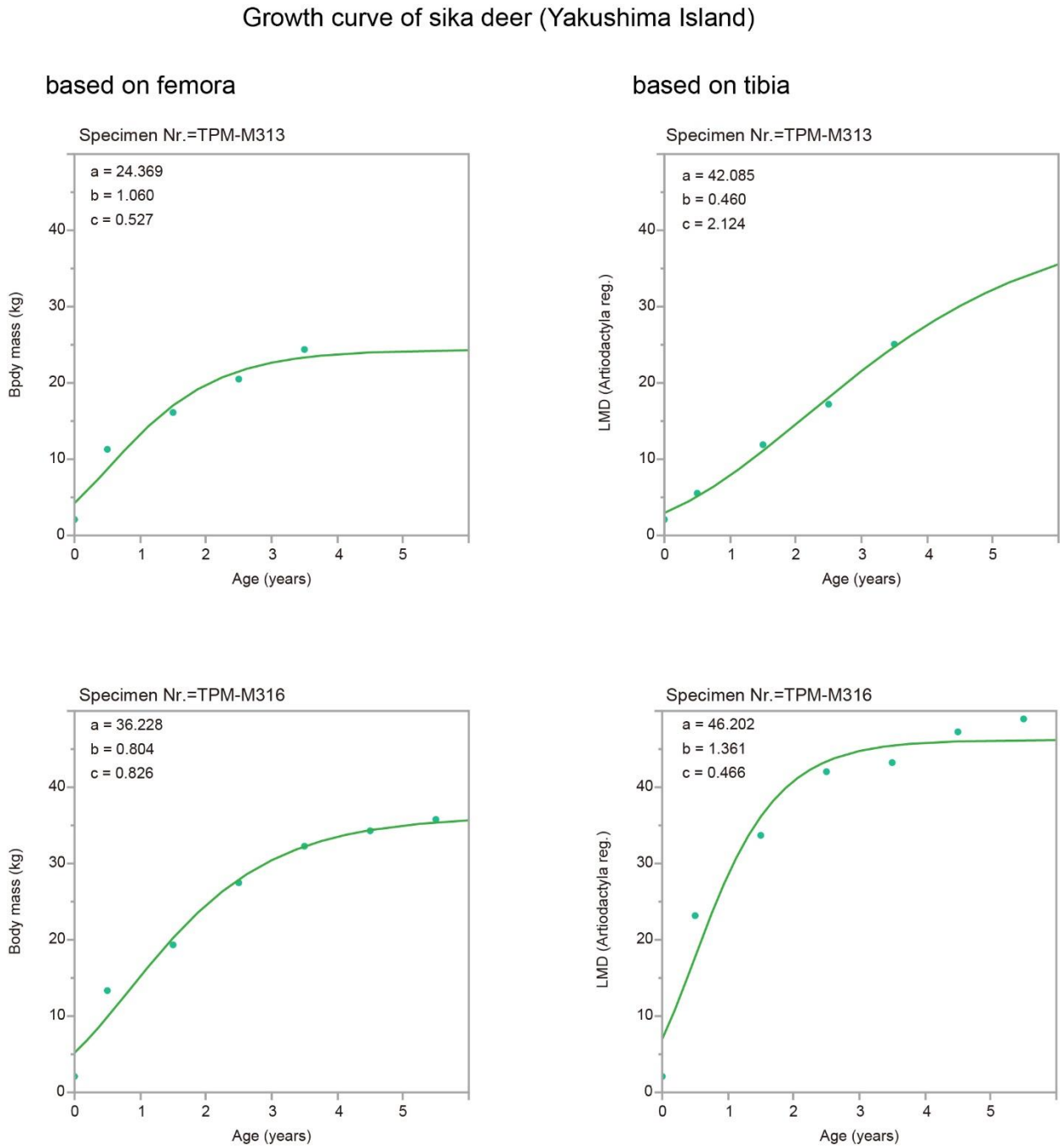


Figure S11. Growth curves of body mass for extant sika deer (*Cervus nippon*) from Yakushima Island. Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote; b: growth rate; and c: inflection point) are shown on the plot for each individual.

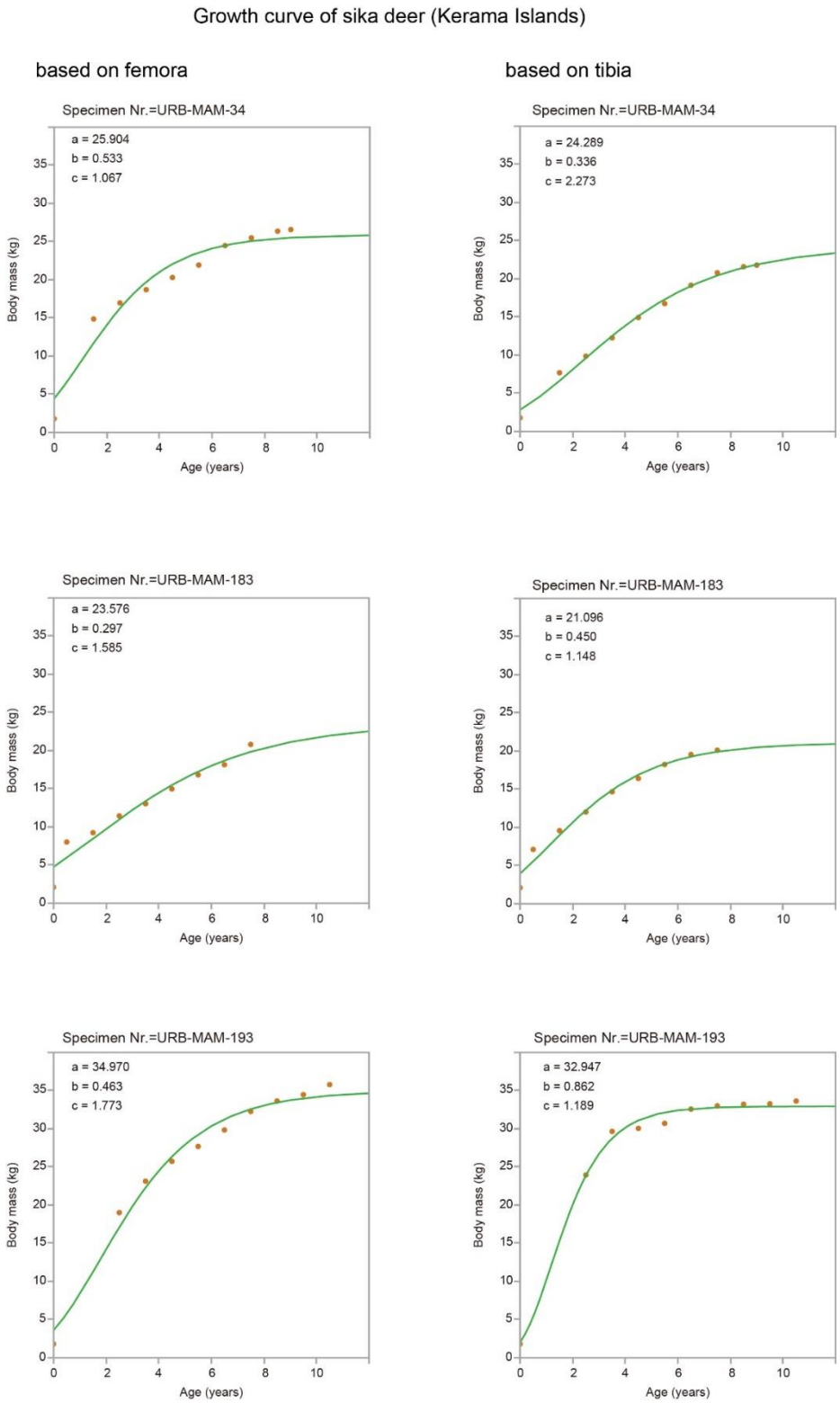


Figure S12. Growth curves of body mass for extant sika deer (*Cervus nippon*) from the Kerama Islands. Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote; b: growth rate; and c: inflection point) are shown on the plot for each individual.

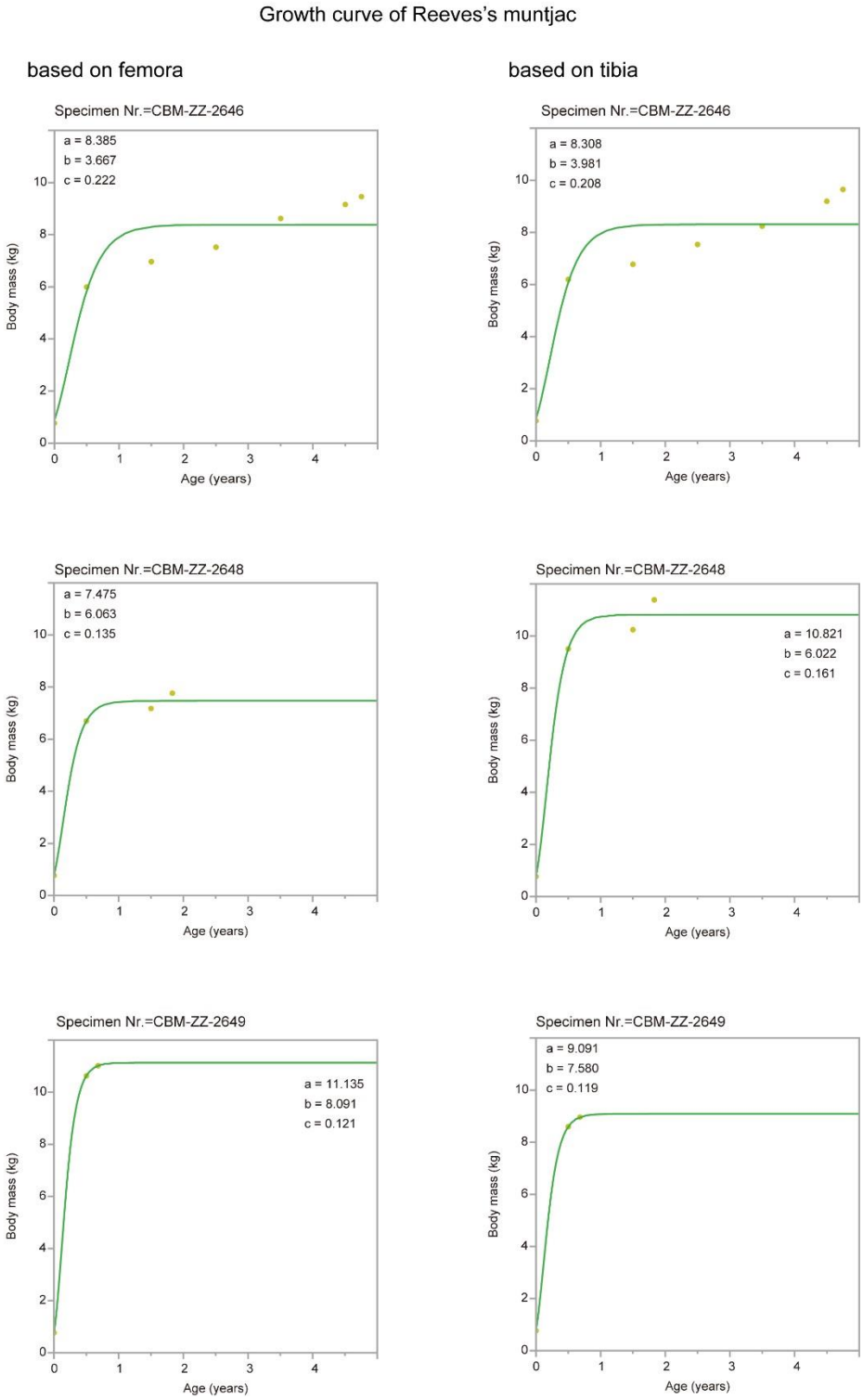


Figure S13. Growth curves of body mass for extant Reeves's muntjac (*Muntiacus reevesi*). Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote, b: growth rate and c: inflection point) are shown on the plot for each individual.

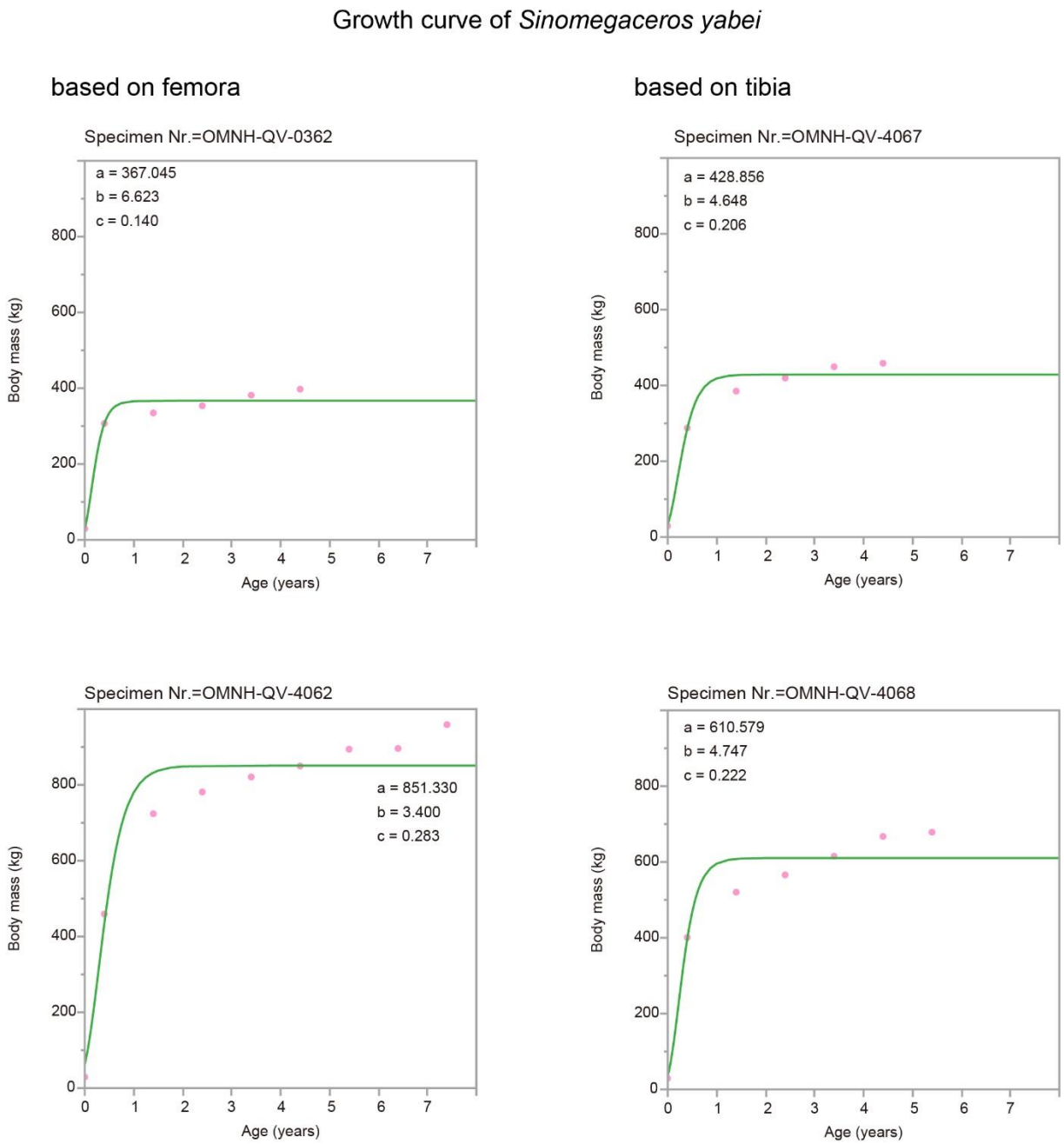


Figure S14. Growth curves of body mass for fossil *Sinomegaceros yabei*. Body mass was estimated from the femur or tibia mediolateral diameter (MLD). Green solid lines represent the fitted Gompertz curves, the parameters for which (a: asymptote; b: growth rate; and c: inflection point) are shown on the plot for each individual.

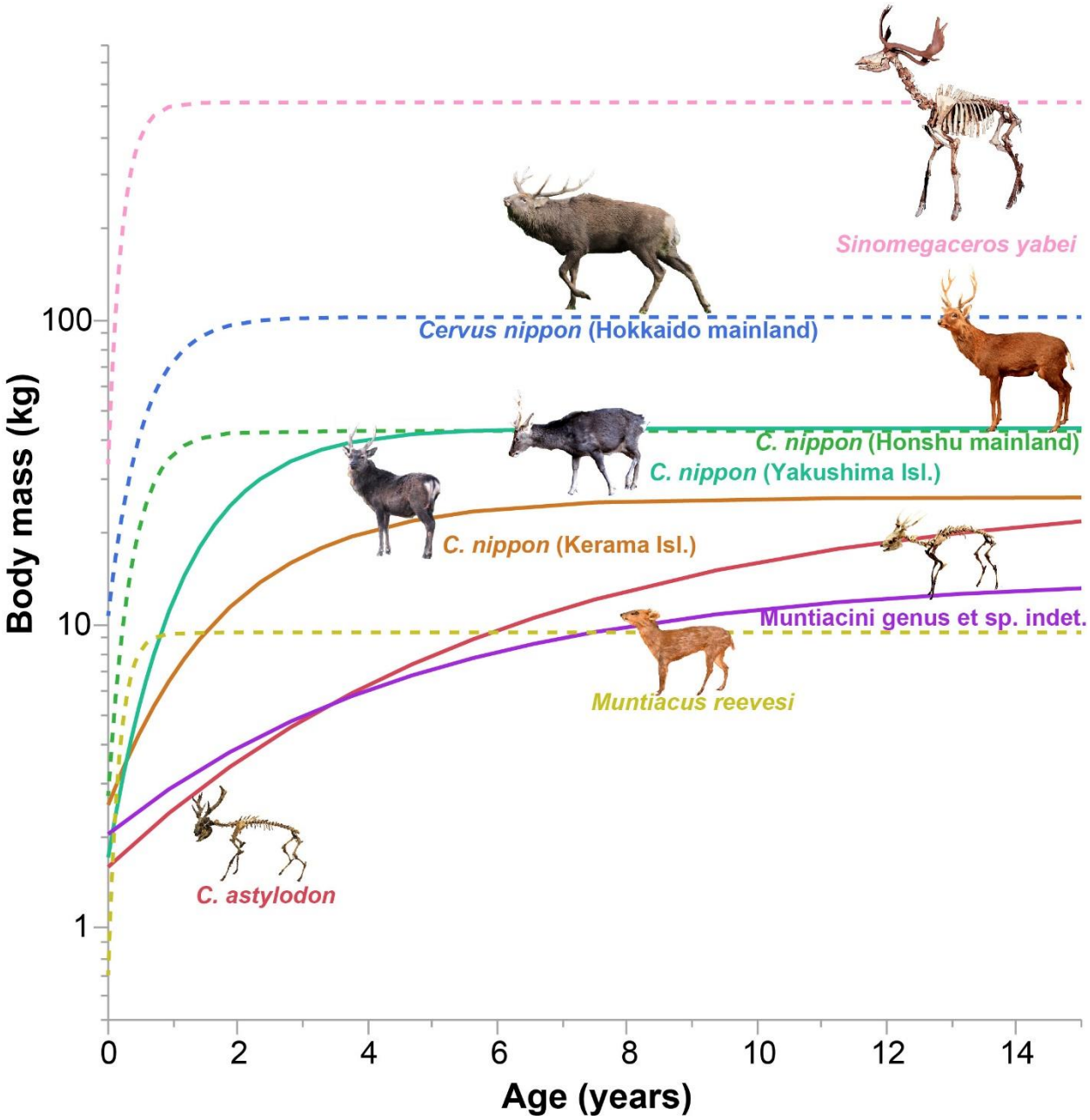


Figure S15. Growth curves of insular cervids in Japan based on lines of arrested growth (LAGs) in the tibiae. Mainland cervids show rapid growth, attaining their maximum body size within 2 years, whereas two fossil Okinawa cervids exhibited an extremely slow growth rate, reaching their maximum body size at ca. 14 years for *Cervus astylodon* and 10 years for the Ryukyu muntjac (Muntiacini gen. et. sp. indet.). Sika deer (*Cervus nippon*) from Yakushima and Kerama islands have an intermediate growth rate, attaining their maximum body size at around 5 years of age. Growth curves of insular cervids are solid lines and those of mainland cevids are dashed-lines.

Supplementary tables

Table S1. Measurements and histological features of the femora of the fossil cervids that were sampled. Note that the presence of EFS is correlated with epiphyseal fusions, suggesting the somatic maturity of the animals [see also Erickson (2007)].

Taxon	Collection no.	GL (mm)	EBM (kg) [†]	Epiphyseal fusion	Number of LAGs	Bone tissue type	Degree of remodeling
Cervus astylodon	OPM-HAN07-1610	101.3 [a]	10.0	Prox: broken Dis: broken	6	PFB	Poor (only inner cortex)
	OPM-HAN07-1612	104.3 [a]	11.2	Prox: broken Dis: unfused	6	PFB	Poor (only inner cortex)
	OPM-HAN07-1604	112.1 [a]	11.9	Prox: fused Dis: broken	9	PFB	Poor (only inner cortex)
	OPM-HAN06-151	124.1 [a]	17.9	Prox: broken Dis: fused	4	PFB, EFS	Extensive (PBTs are present only in the outer cortex)
	OPM-HAN06-155	129.5 [a]	27.6	Prox: broken Dis: broken	11	PFB	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN07-1603	130.4 [a]	20.4	Prox: broken Dis: broken	12	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
Muntiacini gen. et sp. indet. (Ryukyu muntjac)	OPM-HAN06-163	112.9 [a]	17.0	Prox: fused Dis: broken	11	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN07-1618	121.4 [a]	15.1	Prox: broken Dis: broken	8	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN07-1623	134.3 [a]	14.4	Prox: broken Dis: broken	9	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN07-1616	144.4 [a]	22.1	Prox: fused Dis: broken	6	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
Sinomegaceros yabei	OMNH-QV-0362	391.7 [a]	959.2	Prox: unfused Dis: broken	5	FBL	Moderate (only posterior side of the cortex)
	OMNH-QV-4062	465.0	397.6	Prox: fused Dis: broken	7	FBL, EFS	Moderate (only posterior side of the cortex)

Dis, distal epiphysis; EBM, estimated body mass; EFS, external fundamental system; FBL, fibro-lamellar bone tissue; GL, greatest length of femur; LAG, line of arrested growth; PBT, primary bone tissue; PFB, parallel-fibered bone tissue; Prox, proximal epiphysis; SO, secondary osteon.
Collection acronyms: OPM, Okinawa Prefectural Museum and Art Museum, Okinawa, Japan; OMNH, Osaka Museum of Natural History, Osaka, Japan.
[†] The estimated body mass (EBM) was obtained from the estimation equation based on femur mediolateral diameter. [a] The estimated greatest length of the bone based on measurements of the complete femora of other individuals.

Table S2. Measurements and histological features of the tibiae of the fossil cervids from Late Pleistocene deposits that were sampled. See Table S1 for an explanation of the abbreviations. Note that the presence of EFS is correlated with epiphyseal fusions.

Taxon	Collection no.	GL (mm)	EBM (kg) [†]	Epiphyseal fusion	Number of LAGs	Bone tissue type	Degree of remodeling
Cervus astylodon	OPM-HAN06-588	118.9 [a]	11.6	Prox: broken Dis: fused	11	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN06-584	122.5 [a]	11.9	Prox: broken Dis: fused	12	PFB,EFS	Moderate (scattered SO s are present throughout the entire cortex)
	OPM-HAN06-581	146.0 [a]	20.1	Prox: broken Dis: fused	13	FBL (from perimedullary region to 3rd LAGs), PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN07-1585	147.0 [a]	23.3	Prox: broken Dis: broken	10	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN07-1590	148.1 [a]	23.8	Prox: broken Dis: broken	12	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
Muntiacini gen. et sp. indet. (Ryukyu muntjac)	OPM-HAN06-597	112.2 [a]	10.7	Prox: broken Dis: fused	7	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
	OPM-HAN07-1599	117.2 [a]	11.9	Prox: broken Dis: broken	13	PFB, EFS	Moderate (scattered SOs are present throughout the entire cortex)
Sinomegaceros yabei	OMNH-QV-4067	406.7 [a]	458.6	Prox: broken Dis: broken	4	FBL, EFS	Moderate (only anterior side of the cortex)
	OMNH-QV-4068	444.9 [a]	678.6	Prox: fused Dis: broken	5	FBL, EFS	Moderate (only anterior side of the cortex)

[†] The estimated body mass (EBM) was obtained from the estimation equation based on tibia mediolateral diameter.

[a] The estimated greatest length of the bone based on measurements of the complete femora of other individuals.

Table S3. Measurements and histological features of the femora of the extant cervids that were sampled.

Taxon	Collection no.	Age[†]	Sex	GL (mm)	EBM (kg) [‡]	Epiphyseal fusion	Number of LAGs	Bone tissue type	Degree of remodeling
Sika deer (*Cervus nippon*) (Hokkaido, mainland)	HOUMVC 00039	8M	♂	235	56.2	Prox: unfused Dis: unfused	1	FBL	Poor (only posterior side of the cortex)
	HOUMVC 00038	8M	♀	218	38.0	Prox: unfused Dis: unfused	1	FBL	Poor (only posterior side of the cortex)
	HOUMVC 00033	1Y8M	♂	284	94.5	Prox: unfused Dis: unfused	2	FBL	Poor (only posterior side of the cortex)
	HOUMVC 00034	1Y9M	♀	271	96.7	Prox: unfused Dis: unfused	2	FBL	Moderate (only posterior side of the cortex)
	HOUMVC 00035	4Y8M	♂	299	127.2	Prox: fused Dis: unfused	5	FBL	Extensive (only posterior side of the cortex)
	HOUMVC 00036	4Y8M	♀	243	85.8	Prox: fused Dis: fused	5	FBL, EFS	Extensive (only posterior side of the cortex)
	HOUMVC 00037	7Y6M	♂	300	175.5[b]	Prox: fused Dis: fused	7	FBL, EFS	Extensive (only posterior side of the cortex)
Sika deer (Honshu, mainland)	CBM-ZZ-757	0 D	NA	104.3	3.5	Prox: unfused Dis: unfused	0	WB	None
	CBM-ZZ-671	6M	♀	170.5[a]	22.2	Prox: unfused Dis: broken	1	FBL	Poor (only a few erosion cavities)
	CBM-ZZ-218	1Y2M	♀	191.4	28.2	Prox: unfused Dis: unfused	1	FBL	Poor (only a few erosion cavities)
	CBM-ZZ-761	1Y2M	♂	203.4	35.6	Prox: unfused Dis: unfused	1	FBL	Poor (only a few erosion cavities)
	CBM-ZZ-310	3Y5M	♀	217.4	47.0	Prox: fused Dis: fused	2	FBL, EFS	Extensive (only posterior side of the cortex)
	CBM-ZZ-762	3Y2M	♂	252	51.0	Prox: partially fused Dis: unfused	3	FBL	Extensive (only posterior side of the cortex)
	CBM-ZZ-759	7Y1M	♂	237.6	62.8	Prox: fused Dis: fused	7	FBL, EFS	Extensive (only posterior side of the cortex)
	CBM-ZZ-412	8Y9M	♀	218.6	47.8	Prox: fused Dis: fused	7	FBL, EFS	Extensive (only posterior side of the cortex)
Sika deer (Yakushima Island)	TPM-M-312	0Y	NA	142[a]	13.5	Prox: unfused Dis: unfused	0	FBL	None
	TPM-M-314	1Y6M	♂	174.9[a]	30.8	Prox: unfused Dis: unfused	1	FBL	Poor (only a few erosion cavities)
	TPM-M-313	3Y6M	♂	179	24.4	Prox: unfused Dis: unfused	3	FBL	Extensive (only posterior side of the cortex)
	TPM-M-316	5Y6M	♂	216.6	35.8	Prox: fused Dis: fused	5	FBL, EFS	Extensive (only posterior side of the cortex)

Sika deer (Kerama Islands)	URB-MAM-80	4M	NA	120.1	7.2	Prox: unfused Dis: unfused	0	FBL	None
	URB-MAM-211	1Y	♀	167.8	13.8	Prox: unfused Dis: unfused	1	FBL	None
	URB-MAM-194	1Y	♂	168.3	18.4	Prox: unfused Dis: unfused	1	FBL	None
	URB-MAM-34	NA	♀	182.7	26.6	Prox: fused Dis: fused	8	FBL, PFB, EFS	Extensive (only inner cortex)
	URB-MAM-183	7Y6M	♂	184.3	20.8	Prox: fused Dis: fused	7	FBL, PFB, EFS	Moderate (only inner cortex)
	URB-MAM-55	4Y6M	♂	185.5	21.6	Prox: unfused Dis: unfused	7	FBL, PFB, EFS	Extensive (only posterior side of the cortex)
	URB-MAM-193	10Y6M	♀	198.4	35.8	Prox: fused Dis: fused	8	FBL, PFB, EFS	Extensive (only posterior side of the cortex)
Reeves's muntjac (*Muntiacus reevesi*)	CBM-ZZ-4974	0D	NA	34.3	0.8	Prox: unfused Dis: unfused	0	WB	None
	CBM-ZZ-2687	5M	♂	110.7	4.9[b]	Prox: unfused Dis: unfused	0	FBL	Poor (scattered erosion cavities on posterior side of the cortex)
	CBM-ZZ-2649	10M	♀	124.6	6.8[b]	Prox: unfused Dis: unfused	1	FBL	Poor (scattered erosion cavities on posterior side of the cortex)
	CBM-ZZ-2648	1Y10M	♀	127.5	8.5[b]	Prox: fused Dis: fused	2	FBL, EFS	Extensive (only posterior side of the cortex)
	CBM-ZZ-2646	4Y9M	♀	126.4	7.2[b]	Prox: fused Dis: fused	5	FBL, EFS	Extensive (only posterior side of the cortex)

D, days old; M, months old; NA, unknown; WB, woven bone; Y, years old. See Table S1 for explanations of all other abbreviations.
Collection acronyms: CBM-ZZ, Natural History Museum and Institute, Chiba, Japan; TPM, Tochigi Prefectural Museum, Tochigi, Japan; HOUMVC, the Hokkaido University Museum, Sapporo, Japan; URB, University of the Ryukyus, Okinawa, Japan.
[†] Age at death was determined through analyses of tooth eruption and the cementum annuli for sika deer and tooth eruption and the molar wear score for Reeves's muntjac.
[‡] The estimated body mass (EBM) was obtained from the estimation equation based on femur mediolateral diameter.
[a] The estimated greatest length of the bone based on measurements of the complete femora of other individuals.
[b] The actual body mass measurement.

Table S4. Measurements and histological features of the tibiae of the extant cervids that were sampled.

Taxon	Collection No.	Age[†]	Sex	GL (mm)	EBM (kg) [‡]	Epiphyseal fusion	Number of LAGs	Bone tissue Type	Degree of Remodeling
Sika deer (*Cervus nippon*) (Hokkaido, mainland)	HOUMVC 00039	8M	♂	282	41.9	Prox: unfused Dis: unfused	1	FBL	Poor (only innermost cortex)
	HOUMVC 00038	8M	♀	270	39.0	Prox: unfused Dis: unfused	1	FBL	None
	HOUMVC 00033	1Y8M	♂	337	69.3	Prox: unfused Dis: fused	2	FBL	Extensive (only anterior side of the cortex)
	HOUMVC 00034	1Y9M	♀	319	76.1	Prox: unfused Dis: unfused	2	FBL	None
	HOUMVC 00035	4Y8M	♂	356	120.9	Prox: unfused Dis: fused	5	FBL	Extensive (only anterior side of the cortex)
	HOUMVC 00036	4Y8M	♀	295	65.9	Prox: fused Dis: fused	5	FBL, EFS	Poor (only innermost cortex)
	HOUMVC 00037	7Y6M	♂	346	175.5[b]	Prox: fused Dis: fused	7	FBL, EFS	Poor (only innermost cortex)
Sika deer (Honshu, mainland)	CBM-ZZ-757	0 D	NA	128.1	3.5	Prox: unfused Dis: unfused	0	WB	None
	CBM-ZZ-671	6M	♀	202	24.3	Prox: unfused Dis: unfused	1	FBL	None
	CBM-ZZ-218	1Y2M	♀	212.6	36.4	Prox: unfused Dis: unfused	1	FBL	Moderate (only anterior side of the cortex)
	CBM-ZZ-761	1Y2M	♂	239.4	38.2	Prox: unfused Dis: unfused	1	FBL	Poor (only a few erosion cavities)
	CBM-ZZ-310	3Y5M	♀	253	37.4	Prox: fused Dis: fused	2	FBL, EFS	Extensive (only anterior side of the cortex)
	CBM-ZZ-762	3Y2M	♂	292.4	52.0	Prox: partially fused Dis: fused	3	FBL	Moderate (scattered SOs are present throughout the entire cortex)
	CBM-ZZ-759	7Y1M	♂	277.6	58.8	Prox: fused Dis: fused	7	FBL, EFS	Extensive (only anterior side of the cortex)
	CBM-ZZ-412	8Y9M	♀	258.8	35.7	Prox: fused Dis: fused	7	FBL, EFS	Extensive (only anterior side of the cortex)
Sika deer (Yakushima Island)	TPM-M-312	0Y	NA	160.8[a]	12.4	Prox: unfused Dis: unfused	0	FBL	None
	TPM-M-314	1Y6M	♂	213.2[a]	36.4	Prox: unfused Dis: fused	1	FBL	Poor (only a few erosion cavities)
	TPM-M-313	3Y6M	♂	207.4	25.1	Prox: unfused Dis: fused	3	FBL	Extensive (only anterior side of the cortex)

	TPM-M-316	5Y6M	♂	249.6	49.0	Prox: fused Dis: fused	5	FBL, EFS	Extensive (only anterior side of the cortex)
Sika deer (Kerama Islands)	URB-MAM-80	4M	NA	147.7	4.5	Prox: unfused Dis: unfused	0	FBL	None
	URB-MAM-211	1Y	♀	195	20.8	Prox: unfused Dis: unfused	1	FBL	None
	URB-MAM-194	1Y	♂	198	23.2	Prox: unfused Dis: unfused	1	FBL	None
	URB-MAM-34	NA	♀	218	21.8	Prox: fused Dis: fused	8	FBL, PFB, EFS	Moderate (only inner cortex)
	URB-MAM-183	7Y6M	♂	215	20.1	Prox: fused Dis: fused	7	FBL, PFB, EFS	Extensive (only anterior side of the cortex)
	URB-MAM-55	4Y6M	♂	229	19.6	Prox: unfused Dis: unfused	7	FBL, PFB, EFS	Extensive (only anterior side of the cortex)
	URB-MAM-193	10Y6M	♀	224	33.6	Prox: fused Dis: fused	8	FBL, PFB, EFS	Extensive (only anterior side of the cortex)
Reeves's muntjac (*Muntiacus reevesi*)	CBM-ZZ-4974	0D	NA	40.6	0.8	Prox: unfused Dis: unfused	0	WB	None
	CBM-ZZ-2687	5M	♂	116.9	4.9[b]	Prox: unfused Dis: unfused	0	FBL	Poor (scattered erosion cavities on posterior side of the cortex)
	CBM-ZZ-2649	10M	♀	126.4	6.8[b]	Prox: unfused Dis: fused	1	FBL	Poor (scattered erosion cavities on posterior side of the cortex)
	CBM-ZZ-2648	1Y10M	♀	135	8.5[b]	Prox: fused Dis: fused	2	FBL	Extensive (only posterior side of the cortex)
	CBM-ZZ-2646	4Y9M	♀	135	7.2[b]	Prox: fused Dis: fused	5	FBL, EFS	Extensive (only posterior side of the cortex)

See Tables S1 and S3 for explanations of the abbreviations.

[†] Age at death was determined through analyses of tooth eruption and the cementum annuli for sika deer and tooth eruption and the molar wear score for Reeves's muntjac.

[‡] The estimated body mass (EBM) was obtained from the estimation equation based on tibia mediolateral diameter.

[a] The estimated greatest length of the bone based on measurements of the complete tibiae of other individuals.

[b] The actual body mass measurement.

Table S5. List of the extant sika deer (*Cervus nippon*) samples that were used to check the validity of using lines of arrested growth (LAGs) to estimate age. Since LAGs form during winter, we compared the number of LAGs with the number of winter seasons a deer had experienced during its lifetime (See also Figure S5).

Taxon	Specimen no.	Sex	Age assessment method	Age at death	Date or season of death	No. of winter seasons	No. of LAGs
Sika deer (Hokkaido mainland)	HOUMVC 00039	♂	Tooth eruption	8 months	1977/2/20	1	1
	HOUMVC 00038	♀	Tooth eruption	8 months	1977/2/23	1	1
	HOUMVC 00033	♂	Tooth eruption	1 year 8 months	1976/2/29	2	2
	HOUMVC 00034	♀	Tooth eruption	1 year 9 months	1976/2/29	2	2
	HOUMVC 00035	♂	Cementum annuli	4 years 8 months	1976/2/26	5	5
	HOUMVC 00036	♀	Cementum annuli	4 years 8 months	1977/2/20	5	5
	HOUMVC 00037	♂	Cementum annuli	7 years 6 months	1955/12/19	8	7
Sika deer (Honshu mainland)	CMB-ZZ-671	♀	Tooth eruption	6 months	1987/12/19	1	1
	CBM-ZZ-218	♀	Tooth eruption	1 year 2 months	1986/8/9	1	1
	CBM-ZZ-761	♂	Tooth eruption	1 year 2 months	1988/8/8	1	1
	CBM-ZZ-310	♀	Cementum annuli	3 years 5 months	1986/11/19	3	3
	CBM-ZZ-762	♂	Cementum annuli	3 years 2 months	1988/8/9	3	3
	CBM-ZZ-759	♂	Cementum annuli	7 years 1 month	1988/7/4	7	7
	CBM-ZZ-412	♀	Cementum annuli	8 years 9 months	1987/3/12	8	7
Sika deer (Yakushima Island)	TPM-M314	♂	Tooth eruption	1 year 6 months	Autumn–winter	1	1
	TPM-M313	♂	Cementum annuli	3 years 6 months	Autumn–winter	3	3
	TPM-M316	♂	Cementum annuli	5 years 6 months	Autumn–winter	5	5
Sika deer (Kerama Islands)	URB-MAM-55	♂	Cementum annuli	4 years 6 months	Autumn–winter?	4	7
	URB-MAM-183	♂	Cementum annuli	7 years 6 months	Autumn–winter?	7	7
	URB-MAM-193	♀	Cementum annuli	10 years 6 months	Autumn–winter?	11	8

Table S6. Estimation of the number of lines of arrested growth (LAGs) that were lost through bone remodeling in fossil specimens.

Taxon	Bone	Specimen no.	No. observed LAGs	Reference specimen	Expansion of medullary cavity	Estimated LAGs lost	Estimated age at death (years)
Cervus astylodon	Femur	OPM-HAN07-1610	6	NA	NA	NA	6
		OPM-HAN07-1612	6	OPM-HAN07-1610	Yes (2 LAGs)	2	8
		OPM-HAN07-1604	9	OPM-HAN07-1610	No	0	9
		OPM-HAN07-1603	12	OPM-HAN07-1610	Yes (3 LAGs)	3	15
		OPM-HAN06-155	11	OPM-HAN07-1610	Yes (7 LAGs)	7	18
		OPM-HAN06-151	4	OPM-HAN07-1610	Yes (4 LAGs)	4	8
	Tibia	OPM-HAN06-588	11	NA	NA	0	11
		OPM-HAN06-584	12	OPM-HAN06-588	Yes (1 LAG)	1	13
		OPM-HAN07-1585	10	OPM-HAN06-588	Yes (2 LAGs)	2	12
		OPM-HAN06-581	13	OPM-HAN06-588	Yes (2 LAGs)	2	15
		OPM-HAN07-1590	12	OPM-HAN06-588	Yes (1 LAG)	1	13
Muntiacini gen. et. sp. indet. (Ryukyu muntjac)	Femur	OPM-HAN07-1618	8	NA	NA	0	8
		OPM-HAN07-1623	9	OPM-HAN07-1618	No	0	9
		OPM-HAN07-1616	6	OPM-HAN07-1618	No	0	6
		OPM-HAN06-163	11	OPM-HAN07-1618	No	0	11
	Tibia	OPM-HAN06-597	7	NA	NA	0	7
		OPM-HAN07-1599	13	OPM-HAN06-597	No	0	13

Sinomegaceros yabei	Femur	OMNH-QV-0362	4	NA	NA	0	4
		OMNH-QV-4062	7	OMNH-QV-0362	No	0	7
	Tibia	OMNH-QV-4067	4	NA	NA	0	4
		OMNH-QV-4068	5	OMNH-QV-4067	No	0	5

Table S7. Estimation of the neonatal body mass of the fossil cervids. Estimates were made using the regression equation of neonatal body mass against adult body mass for 121 extant artiodactyls (see also Figure S6).

	Estimated adult body mass (kg)			Estimated neonatal body mass (kg)
Species	**Femur MLD**	**Tibia MLD**	**Average**	
Cervus astylodon	15.531	17.424	16.477	1.2588
Muntiacini gen. et. sp. indet. (Ryukyu muntjac)	16.273	11.307	13.790	1.0905
Sinomegaceros yabei	959.175	678.566	818.870	29.378

MLD, mediolateral diameter.

Table S8. Results of a two-way analysis of variance testing the effects of species and bone type (femur or tibia) on the Gompertz growth curve parameters.

Parameter	Factor	d.f.	Sum of square	*F*-value	*P*-value
Growth rate	Species	7	197.234	20.634	<0.0001
	Bone type	1	0.009	0.007	0.934
Inflection point	Species	7	231.866	13.059	<0.0001
	Bone type	1	3.571	1.408	0.242

Table S9. Statistical comparisons of the growth curve parameters (growth rate and inflection point) among eight extant and fossil cervids. Since bone type (femur or tibia) did not have a statistically significant effect on the growth curve parameters (see Table S8), the femur and tibia data were combined.

Taxon	Growth rate							Inflection point						
				95% CI		Statistical tests					95% CI		Statistical tests	
Reeves's muntjac (*Muntiacus reevesi*)	3	5.901	0.543	4.788	7.013	A		3	0.161	1.129	−2.152	2.474	A	
Sinomegaceros yabei	4	4.854	0.470	3.891	5.818	A		4	0.213	0.978	−1.790	2.216	A	
Sika deer (*Cervus nippon*) (Honshu mainland)	4	2.499	0.470	1.536	3.462	B		4	0.418	0.978	−1.585	2.422	A	
Sika deer (Hokkaido mainland)	3	2.161	0.543	1.049	3.273	B	C	3	0.375	1.129	−1.938	2.688	A	
Sika deer (Yakushima Island)	2	0.921	0.665	−0.441	2.283	B	C	2	0.986	1.383	−1.847	3.819	A	B
Sika deer (Kerama Islands)	3	0.490	0.543	−0.622	1.603	B	C	3	1.506	1.129	−0.807	3.819	A	
Ryukyu muntjac (Muntiacini gen. et. sp. indet.)	6	0.312	0.384	−0.474	1.098		C	6	1.858	0.799	0.222	3.494	A	
Cervus astylodon	11	0.181	0.284	−0.400	0.761		C	11	5.771	0.590	4.563	6.979		B

CI, confidence interval; SE, standard error. Taxa with different letters in the "Statistical tests" columns are significantly different (Tukey–Kramer method, $P < 0.05$).

Table S10. Statistical comparisons of the growth rates of extant and fossil cervids based on measurements of the femora and tibiae.

Taxon	Growth rate estimated from femur MLD								Growth rate estimated from tibia MLD							
				95% CI		Statistical tests						95% CI		Statistical tests		
Reeves's muntjac (*Muntiacus reevesi*)	3	5.940	0.715	4.444	7.436	A			3	5.861	0.753	4.264	7.457	A		
Sinomegaceros yabei	2	5.011	0.875	3.179	6.844	A	B		2	4.697	0.922	2.742	6.653	A	B	
Sika deer (*Cervus nippon*) (Honshu mainland)	4	2.254	0.619	0.958	3.549		B	C	4	2.745	0.652	1.362	4.128	A	B	C
Sika deer (Hokkaido mainland)	3	2.411	0.715	0.915	3.907		B	C	3	1.911	0.753	0.314	3.507		B	C
Sika deer (Yakushima Island)	2	0.932	0.875	−0.900	2.764		B	C	2	0.910	0.922	−1.045	2.866		B	C
Sika deer (Kerama Islands)	3	0.431	0.715	−1.065	1.927			C	3	0.550	0.753	−1.047	2.146			C
Ryukyu muntjac (Muntiacini gen. et. sp. indet.)	4	0.366	0.619	−0.930	1.661			C	2	0.204	0.922	−1.751	2.159		B	C
Cervus astylodon	6	0.195	0.505	−0.863	1.252			C	5	0.164	0.583	−1.073	1.401			C

CI, confidence interval; MLD, mediolateral diameter; SE, standard error.
Taxa with different letters in the "Statistical tests" columns are significantly different (Tukey-Kramer method, $P < 0.05$).

Table S11. Statistical comparison of the inflection point for extant and fossil cervids based on measurements of the femora and tibiae.

Taxon	Inflection point estimated from femur MLD							Inflection point estimated from tibia MLD						
				95% CI		Statistical tests					95% CI		Statistical tests	
Cervus astylodon	6	5.242	0.424	4.356	6.129	A		5	6.405	0.997	4.292	8.518	A	
Ryukyu muntjac (Muntiacini gen. et. sp. indet.)	4	1.147	0.519	0.062	2.233		B	2	3.280	1.576	−0.061	6.621	A	B
Sika deer (*Cervus nippon*) (Kerama Islands)	3	1.475	0.599	0.221	2.728		B	3	1.537	1.287	−1.191	4.265	A	B
Sika deer (Yakushima Island)	2	0.677	0.734	−0.859	2.212		B	2	1.295	1.576	−2.046	4.636	A	B
Sika deer (Honshu mainland)	4	0.466	0.519	−0.619	1.552		B	4	0.370	1.114	−1.992	2.733		B
Sika deer (Hokkaido mainland)	3	0.323	0.599	−0.930	1.577			3	0.427	1.287	−2.301	3.155		B
Sinomegaceros yabei	2	0.212	0.734	−1.324	1.747		B	2	0.214	1.576	−3.127	3.555	A	B
Reeves's muntjac (*Muntiacus reevesi*)	3	0.160	0.599	−1.094	1.413		B	3	0.163	1.287	−2.565	2.891		B

CI, confidence interval; MLD, mediolateral diameter; SE, standard error.
Taxa with different letters in the "Statistical tests" columns are significantly different (Tukey-Kramer method, $P < 0.05$).

Table S12. Life tables for three extant sika deer (*Cervus nippon*) populations based on age data from randomly culled individuals.

Population	Age (years)	Age (% of maximum observed age)	Sampled fx	Fx (Probit smoothing)	lx	lx*1000
Sika deer (Hokkaido mainland)	2	10	450	409	1.000	**1000**
	3	15	242	236	0.577	**577**
	4	20	113	141	0.345	**345**
	5	25	63	88	0.214	**214**
	6	30	46	56	0.138	**138**
	7	35	34	37	0.091	**91**
	8	40	37	25	0.062	**62**
	9	45	24	18	0.043	**43**
	10	50	14	13	0.031	**31**
	11	55	15	9	0.022	**22**
	12	60	6	7	0.016	**16**
	13	65	6	5	0.012	**12**
	14	70	3	4	0.009	**9**
	15	75	2	3	0.007	**7**
	16	80	2	2	0.005	**5**
	17	85	2	2	0.004	**4**
	18	90	0	1	0.003	**3**
	19	95	1	1	0.003	**3**
			Total: 1060			
Sika deer (Honshu mainland)	2	11	132	126	1.000	**1000**
	3	17	108	108	0.853	**853**
	4	22	78	89	0.708	**708**
	5	28	70	72	0.571	**571**
	6	33	51	56	0.448	**448**
	7	39	42	43	0.342	**342**
	8	44	34	32	0.254	**254**
	9	50	24	23	0.184	**184**
	10	56	25	16	0.130	**130**
	11	61	14	11	0.089	**89**
	12	67	7	8	0.060	**60**
	13	72	7	5	0.040	**40**
	14	78	1	3	0.026	**26**

	15	83	0	2	0.016	**16**
	16	89	0	1	0.010	**10**
	17	94	1	1	0.006	**6**
			Total: 594			
Sika deer (Yakushima Island)	2	11	18	18	1.000	**1000**
	3	17	16	14	0.776	**776**
	4	22	10	11	0.595	**595**
	5	28	6	8	0.457	**457**
	6	33	8	6	0.354	**354**
	7	39	4	5	0.277	**277**
	8	44	3	4	0.219	**219**
	9	50	0	3	0.175	**175**
	10	56	3	3	0.141	**141**
	11	61	2	2	0.114	**114**
	12	67	2	2	0.093	**93**
	13	72	1	1	0.077	**77**
	14	78	0	1	0.064	**64**
	15	83	0	1	0.053	**53**
	16	89	0	1	0.045	**45**
	17	94	1	1	0.038	**38**
			Total: 74			

Table S13. Life tables for extant Reeves's muntjac (*Muntiacus reevesi*) and two fossil insular deer based on age data from individuals that died naturally.

Population	Age (years)	Age (% of maximum observed age)	Sampled fx	dx	lx	lx (Probit smoothing)	lx*1000
Cervus astylodon	2	8	3	0.067	1.000	0.952	**1000**
	3	12	0	0.000	0.933	0.938	**986**
	4	15	0	0.000	0.933	0.923	**970**
	5	19	2	0.044	0.933	0.907	**953**
	6	23	1	0.022	0.889	0.889	**934**
	7	27	3	0.067	0.867	0.870	**914**
	8	31	0	0.000	0.800	0.849	**893**
	9	35	0	0.000	0.800	0.827	**869**
	10	38	3	0.067	0.800	0.803	**843**
	11	42	2	0.044	0.733	0.776	**815**
	12	46	0	0.000	0.689	0.747	**785**
	13	50	0	0.000	0.689	0.716	**752**
	14	54	1	0.022	0.689	0.682	**717**
	15	58	0	0.000	0.667	0.645	**678**
	16	62	2	0.044	0.667	0.605	**636**
	17	65	2	0.044	0.622	0.562	**591**
	18	69	4	0.089	0.578	0.515	**541**
	19	73	5	0.111	0.489	0.464	**487**
	20	77	3	0.067	0.378	0.408	**429**
	21	81	2	0.044	0.311	0.348	**366**
	22	85	4	0.089	0.267	0.283	**297**
	23	88	3	0.067	0.178	0.212	**223**
	24	92	3	0.067	0.111	0.135	**142**
	25	96	2	0.044	0.044	0.051	**54**
			Total: 45				
Muntiacini gen. et sp. indet. (Ryukyu muntjac)	0	0	5	0.077	1.000	0.996	**1000**
	1	9	2	0.031	0.923	0.958	**961**
	2	18	6	0.092	0.892	0.912	**915**
	3	27	1	0.015	0.800	0.858	**861**
	4	36	0	0.000	0.785	0.794	**797**
	5	45	2	0.031	0.785	0.719	**722**
	6	55	3	0.046	0.754	0.630	**632**
	7	64	27	0.415	0.708	0.525	**527**
	8	73	16	0.246	0.292	0.400	**402**
	9	82	2	0.031	0.046	0.254	**254**

	10	91	1	0.015	0.015	0.080	**80**
			Total: 65				
Extant Reeves's muntjac	0	0	12	0.143	1.000	0.920	**1000**
	1	6	21	0.250	0.857	0.798	**868**
	2	13	9	0.107	0.607	0.684	**744**
	3	19	7	0.083	0.500	0.578	**629**
	4	25	3	0.036	0.417	0.482	**524**
	5	31	8	0.095	0.381	0.395	**429**
	6	38	2	0.024	0.286	0.318	**346**
	7	44	3	0.036	0.262	0.251	**273**
	8	50	4	0.048	0.226	0.194	**211**
	9	56	5	0.060	0.179	0.147	**160**
	10	63	1	0.012	0.119	0.109	**118**
	11	69	0	0.000	0.107	0.078	**85**
	12	75	1	0.012	0.107	0.054	**59**
	13	81	2	0.024	0.095	0.037	**40**
	14	88	2	0.024	0.071	0.024	**26**
	15	94	4	0.048	0.048	0.015	**16**
			Total: 84				

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