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

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Abstract

Background

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 processes involved. Recent studies focused on associated change of body size and life history (age of reproduction, growth rate, longevity) – a key to understand the process of insular dwarfism. 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. We investigated eight deer populations exhibiting body size variation, both extant and fossil, to clarify the effect of insularity on life history traits.

Results

We applied several methods to both extant and extinct populations for resolving life history change among deer populations. Skeletochronology using lines of arrested growth successfully reconstructed body growth curves and revealed a gradual change in the growth trajectories reflecting degree of insularity: slower growth with prolonged growth periods in more isolated deer populations. An extensive examination of bone microstructure further corroborated it, and clearly indicated much slower growth and later somatic maturity in fossil insular deer, which had been isolated for more than 1.5 Myr. Finally, mortality patterns revealed by demographic analysis varied among deer populations and life history of insular populations shifted toward “slow-life” of *K*-strategists.

Conclusion

We clarified the evolutionary and ecological process behind insular dwarfism, which occurred in conjunction with life history shifts. The process initiated with phenotypic plasticity responding to the resource-limitation on predator-free islands after the settlements of islanders. Under the insular environments, natural selection favored *K*-selected animals and the life history traits were genetically fixed. Extreme *K*-strategy found in fossil dwarfs on islands would make them vulnerable to anthropogenic changes, that would explain termination of insular dwarfs after human arrival on islands.

Background

Among the most iconic examples of visible evolutionary change are island mammals that dramatically changed in body size [1-3]. Elephants, hippos and deer in islands across the world became very small, whereas small mammals became larger [2], what led to the suggestion of an 'island rule' [1]. A classic example is the pygmy elephant (*Elephas falconeri*) of Sicily, which had a body mass of only 1% of its mainland ancestor [4].

Body size has a close relationship with various aspects of organism's life. Recent studies are focusing on the relationship between body size and life history – growth rate, age of reproduction, longevity – on islands [5-7]. The reduction of body size in large mammals appears to be associated with modification of the growth trajectory [8, 9], and this in turn affects the life schedule, e.g. timing of sexual maturity or longevity. Life history theory, which has been extended from *r*/*K* selection theory in ecology [10], predicts that species adapted to unstable environments, under which high intrinsic rates of natural increase (*r*) are adaptive, suit a set of life history traits such as rapid body growth, early sexual maturity, large number of offspring for a clutch and a short life span, being referred as "*r*-selected". On the other hand, in stable environments the populations are maintained near their carrying capacity (*K*) and resultant intraspecific competitions favor those which have slow body growth, late sexual maturity, small number of offspring for a clutch and a long life span, referred as "*K*-selected". Based on this theoretical background, life history traits were estimated for fossil insular species. Some dwarfed species show a distinctly slower growth rate and delayed somatic maturity compared with continental taxa [6], whereas others do not show extreme modifications in their life histories [11, 12]. Since these studies were based on different

species from single islands, it is hard to generalize body size and life history evolution on islands.

Further important issue awaiting to be explored is the process of body size and life history evolution, and how ecological factors affect the process, e.g. a lack of predators and competitors, resource limitation on islands, founder effects of island immigrants, and the remoteness and duration of isolation [2, 13-16]. Drastic body size changes have been reported for fossil species or populations [2]. Nevertheless, as far as we focused only on the fossil evidence, the ecological process of the changes remains unclear. To achieve this, studies of mainland and insular populations of a single extant species are particularly useful because they allow confounding factors other than living on islands to be controlled. Variation in isolation durations among the populations can mimic the process of insular settlement.

In the present study, we focused on deer in the Japanese Archipelago, both extant and extinct populations from both islands and mainland (Fig. 1). Our targets cover an extant sika deer (*Cervus nippon*) population recently introduced (c.a. 400 years ago) on a small island, four extant insular and mainland deer populations [sika deer, Reeves's muntjac (*Muntiacus reevesi*)] with variable body sizes, and extinct insular deer (*Cervus astylodon* and Muntiacini gen. et. sp. indet., hereafter referred to as "the Ryukyu muntjac") isolated for more than 1.5 Myr on the Okinawa Island [17], representing different degrees of insular effect. We also included an extinct gigantic mainland deer (*Sinomegaceros yabei*) from the Late Pleistocene deposits on Honshu mainland, having a comparable body size to the largest extant deer, the moose (*Alces alces*) [18]. The immense variation of the island size and duration of isolation found in the eight deer populations enables us to infer the process of body size and life history changes on

islands.

We applied long bone histological analyses established for reconstruction of palaeobiology of fossil species [6, 7, 11, 12, 19-21], which could be applicable to both extant and extinct taxa to clarify variation of body growth rates and life histories of the deer in Japan. Skeletochronology using lines of arrested growth (LAGs) was applied to depict body growth rates, which was further corroborated by detailed qualitative descriptions of bone histology aiming to infer growth pattern and somatic maturity. We also conducted demographic analyses of age data, which were collected either ecological surveys or analysis of tooth wear of fossil deer assemblages, to quantitatively compare mortality patterns among the deer populations. By making the most of these interdisciplinary analytical methods, we expected a gradual change in life history traits in the deer populations according to the intensity of insular effects, with longer isolation on smaller islands resulting in the shift toward *K*-strategy.

Results

Growth curve models and growth rate comparisons using skeletochronology

The lines of arrested growth (LAGs) in the long bones of both the extant and fossil deer were well preserved (SI Appendix, Figure S1 – 4), allowing us to measure the bone diameters at the LAGs. We estimated ontogenetic change in body mass and depicted a growth curve for each individual (SI Appendix, Figure S8, 9, Table S8). We then averaged each of three growth curve parameters (the asymptote, growth rate, and inflection point) generated by the Gompertz curve fitting for each species/population to deduce a representative growth curve for each (Fig. 2A, SI Appendix, Figure S10).

Among the sika deer populations, deer from the two mainland areas (Honshu and

Hokkaido mainland) showed faster growth than those from the two insular populations (Yakushima and Kerama Islands). The growth trajectories of body mass corresponded to those obtained by investigations of culled individuals [22, 23] and by long-term field observations of wild deer (Agetsuma and Agetsuma-Yanagihara, personal communication), validating our estimation method of body growth from skeletochronology. The extant Reeves's muntjac showed the fastest growth, which was also reported in analysis of culled individuals, corresponded with its early sexual maturity at ca. 6 months [24].

Compared to the extant references, the two fossil insular deer (*C. astylodon* and the Ryukyu muntjac) showed remarkably slow and prolonged growth, whereas the Pleistocene giant deer *S. yabei* had fast growth, suggesting a similar growth pattern to other extant large deer, such as moose [25]. 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 S10, S11). 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 S10, 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 [26] and was greatest in *C. astylodon* (SI Appendix, Tables S10, S12), 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.

Variation in bone tissue among deer populations in Japan

The nomenclature and definitions of the bone microstructures in the following descriptions are based on Francillon-Vieillot et al. [27] and Castanet et al. [28].

Histological characteristics of long bones corroborated the above statistical comparisons of growth curves. We focused on the following bone tissue characters: 1) type of primary periosteal bone tissue and vascularization pattern as an indicator of relative bone growth rate [e.g. 29] and 2) the presence of the external fundamental system (EFS), which is constituted by parallel-fibred bone tissue without vascularization and forming an edge of slow-growing bone tissue, as a sign of somatic maturity [e.g. 30, 31] . Fig. 3 summarizes the main histological features of the cervids we examined (also see SI Appendix, Tables S1–S3).

All of the extant deer populations (i.e., the four extant sika deer populations, the extant Reeves's muntjac) and the gigantic fossil *S. yabei* exhibited a similar bone tissue structure to continental cervids [11, 32, 33], consisting of fast-growing periosteal bone tissue known as a fibro-lamellar bone with a high level of laminar to plexiform vascularization in most of the cortex (Fig. 3G–L; SI Appendix, Figures S2A–I, S3G–I, and S4A–O). Slow-growing parallel-fibered bone tissue was observed in the outer cortex. Notably, the parallel-fibered bone tissue was more extensively developed in the two insular populations (sika deer from Yakushima and Kerama Islands) with the LAGs at the inner to middle cortex being tighter than in the mainland deer (Fig. 3D–F, SI Appendix, Figures S2D–F, and S3G-I, J-L), suggesting a slower growth rate among the insular populations. The cortical bone tissues of two fossil insular deer, *C. astylodon* and the Ryukyu muntjac, were different from those of the extant deer and the gigantic fossil *S. yabei*, in that they were primary parallel-fibered bone tissue with multiple

LAGs, implying much slower growth rate than other deer. Their bone tissues were similar to those of the extinct dwarf bovid *Myotragus* [6] and of typical extant reptiles [e.g. 34]. The LAGs were spaced evenly throughout the cortex (Fig. 3A–C; SI Appendix, Figures S1A–C and S3A–F) but became closer together as they approached the periosteal surface in older individuals, indicating a decrease in growth rate with age. Only a few areas of the innermost cortex showed a prevalence of fibro-lamellar bone tissue with primary osteons, which alternated with nonvascular parallel-fibered bone tissue with LAGs, suggesting bone deposited slowly during most of the lifetime. There was relatively little vascularization and the vascular canals oriented longitudinally compared with other cervids and large mammals [11, 21]. Since the growth rate correlates with intensity and pattern of vascularization [35-37], where lower level of vascularization associated with longitudinal orientation of canals indicated slower bone depositional rate, the bone histological features of the fossil insular deer further supported their slow growth rates.

All extant specimens with EFS had fused epiphyses both in femora and tibiae (SI Appendix, Figure S5, Table S3). The proximal epiphysis of the tibiae and femora fused later than the distal epiphysis, and EFS developed contemporaneously with the proximal fusions. In two mainland sika deer populations, the timing of EFS development and proximal epiphyseal fusion showed good correspondence with the age when the rate of growth decelerated (SI Appendix, Figures S8, S9, Table S3) and the reported age of somatic maturity in the analyses of culled individuals [22, 23] . Notably, the extant sika deer from the Kerama Islands showed delayed EFS development and epiphyseal fusions compared with other investigated deer. Based on the observation of extant specimens, we further investigated the relationship between EFS development and epiphyseal

fusion in fossil specimens (SI Appendix, Figure S5, Tables S1, 2). Though most of the fossil specimens did not preserve both proximal and distal epiphyses, all the specimens with fused proximal epiphysis had EFS and vice versa. Therefore, the correspondence of epiphyseal fusion and EFS was also held in fossil species. Two insular extinct deer from Okinawa Island showed much delayed EFS development (Min. 9 years, Max. 16 years for *C. astylodon*; Min. 5 years, Max. 11 years for the Ryukyu muntjac, see SI Appendix, Table S1, S2) than other investigated deer, suggesting the delay of somatic maturity.

Survivorship curve comparisons among extant and fossil cervids.

To clarify life history differences in mortality patterns, we compared the survivorship curves of fossil *C. astylodon* (from age data of n = 45) and the four extant sika deer populations on the Hokkaido mainland (n = 1060), Honshu mainland (n = 594), Yakushima Island (n = 74), and Kinkazan Island (n = 264) (Fig. 2B). The deer mortality data of the Kinkazan Island population was additionally included as it provided the mortality pattern free from hunting pressure [38], that is also the case for *C. astylodon* and the Ryukyu muntjac inhabiting on Pleistocene Okinawa Island without human and other predators [38, 39]. As the age estimation of *C. astylodon* was based on the lower third molar height, we confined the survivorship curves to juvenile and onwards for *C. astylodon* and the sika deer. 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 [40] and characteristic to *K*-selected species. At the other extreme, extant sika deer from Hokkaido mainland showed relatively high

mortality in the younger age class, followed by a more gradual decrease in the number of survivors from the prime age to senescence, which is characteristic of a type II 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, though the Honshu population showed an increased mortality rate after the prime age. The survivorship curve of Kinkazan Island was also clustered with Honshu and Yakushima Island populations, but showed lower mortality rate in the initial phase and higher rate in the later phase. This slight difference in pattern might be associated with the lack of hunting pressure in the Kinkazan population, however, overall, the survivorship curves were not strongly affected by the intensity of hunting in the sika deer (see the details in SI Appendix). Though the Kinkazan deer inhabited in the predator-free island, still their mortality pattern was different from that of *C. astylodon*, implying further advanced insular effect in the latter. The maximum observed age of *C. astylodon* was 25 years [38], which was greater than that of the extant sika deer (17, 19, 17, and 21 years for the Honshu, Hokkaido, Yakushima Island, and Kinkazan Island populations, respectively; SI Appendix, Table S13, 14).

We additionally compared the survivorship curves between the Ryukyu muntjac (n = 65) and the extant Reeves's muntjac (n = 84) (Fig. 2C). The extant muntjac had a type II survivorship curve as in the extant sika deer from Honshu mainland. Similarly, to *C. astylodon*, the Ryukyu muntjac had a type I survivorship curve, indicating that its mortality rate also did not increase until senescence, again implying strong insular effect on life history traits in Okinawa Island.

Discussion

All of the interdisciplinary analyses of growth rate comparison, histological investigation, and analysis of survivorship curves of both extant and extinct deer species supported our expectation that the evolution of a new combination of life history traits amounts to repeated establishment of a *K*-strategy in insular environments. Our findings are amongst the first to clarify the gradual shift to *K*-selected life history for insular forms, placing the two fossil insular deer from Okinawa Island on the extreme *K*-strategists.

It is possible to infer the ecological process of the life history change from the current dataset of the deer populations. As we have seen in the deer populations in mainlands, any modifications of life history traits happened in the mainland populations (Hokkaido and Honshu mainland sika deer, Reeves's muntjac, and *S. yabei*), similar to the condition of the continental relatives [11]. The Japanese Archipelago is considered as "an island" in the literature [e.g. 2], nevertheless from the current dataset, the Japanese mainland deer may not be under the strong insular effect in the aspect of life history traits. Further investigation of sika deer populations from Asian Continent will shed light on the degree of insularity for Japanese mainland sika deer.

The first step of the life history change on islands could be exemplified in sika deer on Kerama Islands, which showed the smallest body size and the slowest growth rate among the studied extant deer populations accompanied by the bone histological features showing the signs of slow-growth. 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 changes in body size in less than 6,000 years have been previously documented in red deer in other islands [41], it is 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 might have been 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 the Kerama deer [42], which was supported by the observation of a possible malnourished individual (URB-MAM-55; see SI Appendix for details). It would be relevant to obtain reliable mortality data for the Kerama deer in order to clarify the life history shift being observed in its demographic aspect.

The next step of life history evolution would be illustrated by the Yakushima Island deer, which also showed slower growth rate and intermediate histological features, coupled by a moderate shift of survivorship curve toward those of *K*-selected species. Contrary to the situation of the Kerama deer, the slower growth rate of Yakushima deer is considered to have a genetic basis because 1) Yakushima deer are not malnourished and have enjoyed a recent population increase [43] and 2) some of their macromorphological features have a genetic basis [44, 45]. A long-term field observations of Yakushima deer also supported our finding that the life history traits of this population is shifting to *K*-strategist (Agetsuma and Agetsuma-Yanagihara, personal communication).

The most apparent evolutionary transformation is shown in the two fossil deer isolated on Okinawa Island over 1.5 Ma. Their growth pattern, bone histological characters, and mortality pattern were totally different from other deer populations. As with Kerama deer, it is possible that phenotypic plasticity partially influenced the growth trajectories of them. We do not currently have any reliable sources of information on the nutritional

status of fossil deer, except for their dietary habits [39, 46]. However, reports of healed bone fractures on a number of leg bones from a museum collection of fossil insular deer of Okinawa Island [47] 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. It is also notable that exactly the same trend was observed for the fossil deer and muntjac, further supporting the shift toward *K*-strategy as an adaptation to the insular environment. This process may have started after their initial settlement on Okinawa Island in part through phenotypic plasticity of growth trajectory due to a lack of sufficient foods during growing periods, 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 [40]. Consequently, the two Okinawa deer became extinct at the time of or soon after Paleolithic human arrival to the Okinawa Islands [48, 49], possibly due to hunting. This might be also applicable to other island dwarfs extinct during Pleistocene [50, 51], though the definitive role of Pleistocene *Homo* on their extinction has been recently questioned [52].

Our findings were also in good correspondence with the worldwide observations of life history change in insular mammals previously documented (Fig. 4). A living cervid *Odocoileus hemionus* from Blakely Island showed slower growth rate than a mainland population [8]. As the tooth eruption was also delayed in the Blakely Island deer, this

life history change may have a genetic basis, similar to our case of Yakushima Island deer. With longer isolation but variable island sizes, the life history response varied among the mammals from Mediterranean islands. A fossil hippo *Hippopotamus minor* from Cyprus did not show apparent modification in bone histology and life history [12], whereas a fossil cervid *Candiacervus* from Crete showed slight modifications toward slower life history [11]. Sicilian pygmy elephant *Elephas falconeri* provided a contradictory case: in the demographic aspect it was interpret to have *r*-selected life history as the age distribution of the fossil assemblage was skewed to calves and immature individuals [53], nevertheless a histological study suggested that this pygmy elephant had a much slower growth rate than its mainland relatives, and sexual maturity was within the range of extant elephants [54]. These differences in the life history response, however, are not surprising considering the fact that these Mediterranean islands were much bigger than those we have observed apparent life history changes (i.e. Blakely Island, Kerama Islands, Yakushima Island, Okinawa Island, and Mallorca Island). The most explicit change in life history was observed in *Myotragus balearicus* from Mallorca Island, which underwent an exceptionally long time of isolation (5.2 Ma) in predator-free environments. *Myotragus* showed a dramatic decrease in bone growth rate and an evolution towards a slow life history [6, 7, 55], implying the commonality of this evolutionary change with the Okinawa fossil deer. These results suggest that the degree of modification in life 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.

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, which were estimated to have a *K*-strategy life history. Combining the results of the present and previous studies on life 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 mammals 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) (Fig. 1). The four populations had different properties of their habitat environments and showed considerable differences in body size [56](Table 1). Samples of extant Reeve's muntjac were obtained from the Honshu mainland (Fig. 1). All extant samples were obtained from wild individuals.

Three Pleistocene fossil deer species 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, [47]) on Okinawa Island, Japan; and *Sinomegaceros yabei*, collected from the Kumaishi-do Cave of the Late Pleistocene (16–23 ka [57]) 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–S3.

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

We applied histological analyses of long bones to infer life history traits [6, 7, 11, 12, 19-21]. 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 previous studies [58, 59]. 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 bone and an open medullary cavity in these species. Image segmentation and visualization were performed using VG-Studio Max (Volume Graphics) version 3.1.

Age assessment

To allow comparison of growth patterns, we estimated ages as follows. 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, Tables 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 [60]. 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) [32]. 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 including those appeared in EFS in both the femur and tibia was highly correlated with the actual age determined by tooth cementum annuli (SI Appendix, Figure S6 and Table S4). 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 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 S4). 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 S5). Haversian bones were 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, indicating the good preservations of the primary bone tissues in most parts of the cortex.

Body mass estimation

The body mass during ontogeny was estimated from the bone diameters at the LAGs [61]. Body mass estimation formulas for both the femora and tibiae were obtained from [62] 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 diaphyseal 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 S15.

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), which had at least four data points including starting and terminal points. 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 [24]. Before fitting the growth curves, neonatal body mass data were obtained for both the extant and fossil taxa as the starting point (SI Appendix, Table S6, and Figure S7).

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:

$$W = aExp(-Exp(-b(x - c))$$

where a, b, and c represent the asymptote (i.e., the body mass when body growth ceases), growth rate, and inflection point, respectively. The Gompertz curve fitting was rationalized as this model showed the highest value of goodness of fit represented by the lowest AICc among the fitted growth curves (SI Appendix Table S7). 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 16.0 (SAS Institute Inc.).

Comparison of survivorship curves

As life history change is also represented by the change in mortality pattern, we estimated survivorship curves of both extinct and extant deer populations. Kubo et al. [38] 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 [63]. Variability of age estimation based on the molar wear rate was tested and discussed in previous studies [38, 46, 64], and we used the most appropriate age estimates. Age data of three sika deer populations that were used to investigate the long bone histology (Hokkaido, Honshu, and Yakushima Island populations) and an insular population free from hunting (Kinkazan Island) were collected from the literature [38, 65]. No reliable published age data were available for the Yakushima Island population, so instead histological analysis of 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, 74, and 264 individual deer in the Hokkaido, Honshu, Yakushima Island, and Kinkazan 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 (n = 65). Ozaki [66] previously estimated the age at death of the Ryukyu muntjac by applying the molar wear scoring method developed by Chapman et al. [67] 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 [24], age data have been reported for extant Reeves's muntjac from England [67]. Therefore, these age data were used to depict the survivorship curve of extant Reeves's muntjac (n = 84). 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 methodological descriptions, references, figures and tables.

- Supplementary file 2. Raw data of measurements in excel format (Table S15).

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

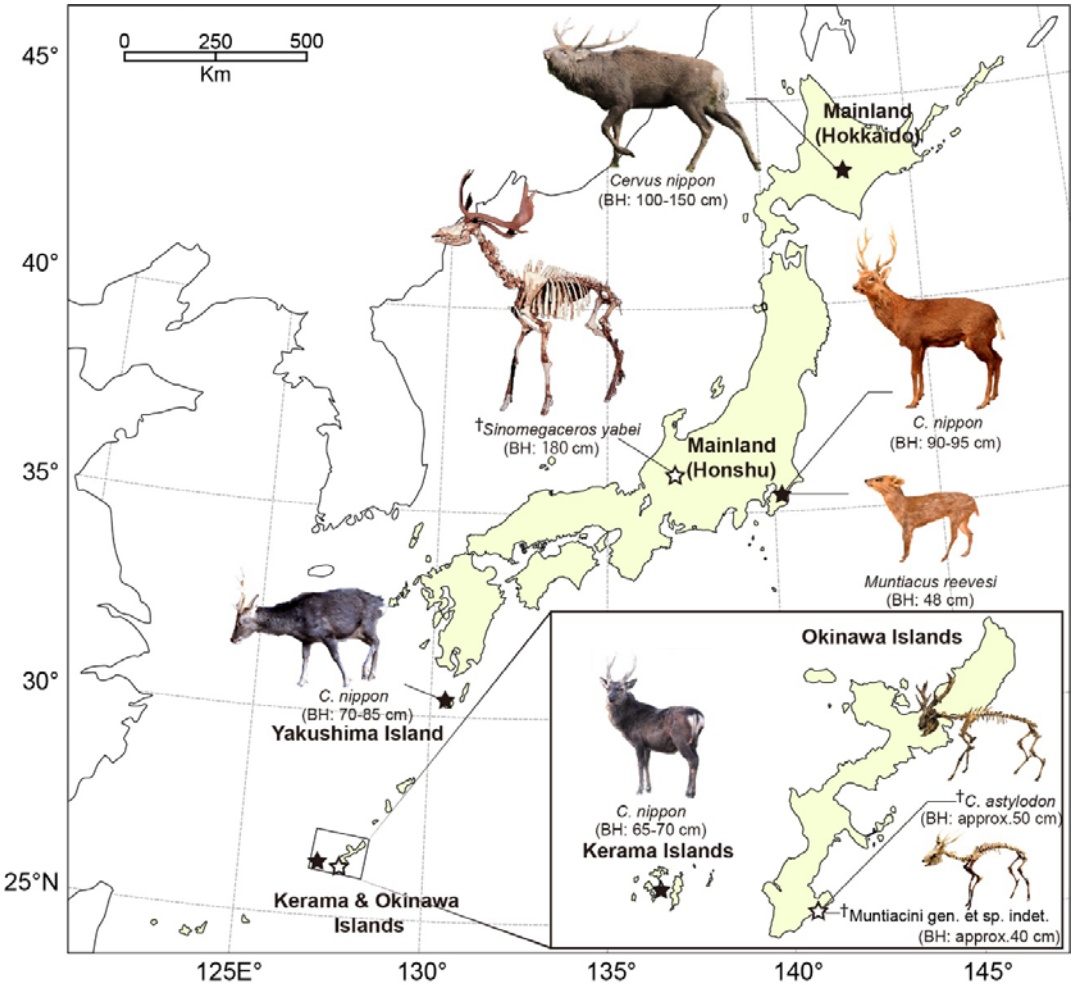

Fig. 1. Locations of the sampled cervids in Japan. †indicates fossils excavated from Late Pleistocene deposits. The localities of extant taxa are shown in closed stars and those of extinct taxa are in open stars. The approximate body height (BH) is indicated for each species/population. Photography of 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.).

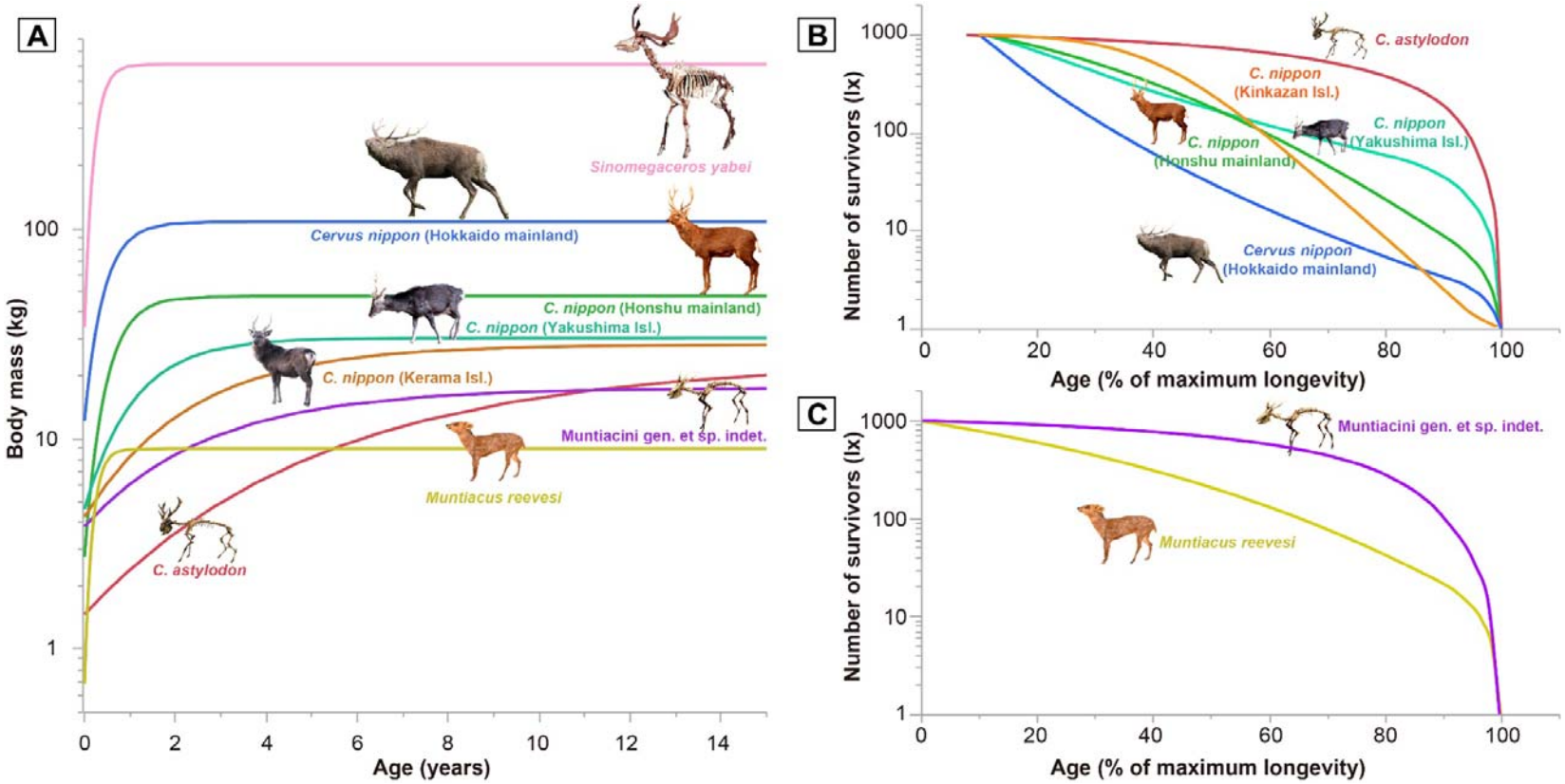

Fig. 2. Growth (A) and survivorship curves (B-C) for Japanese cervids. Insular deer have a significantly slower growth rate and a delayed growth plateau (somatic maturity) compared with mainland deer (A), and there is an ecological shift in mortality pattern between the insular and mainland deer (B, C). As the age estimation of *C. astylodon* was based on the lower third molar height, we confined the survivorship curves to juvenile and onwards for *C. astylodon* and the sika deer (B).

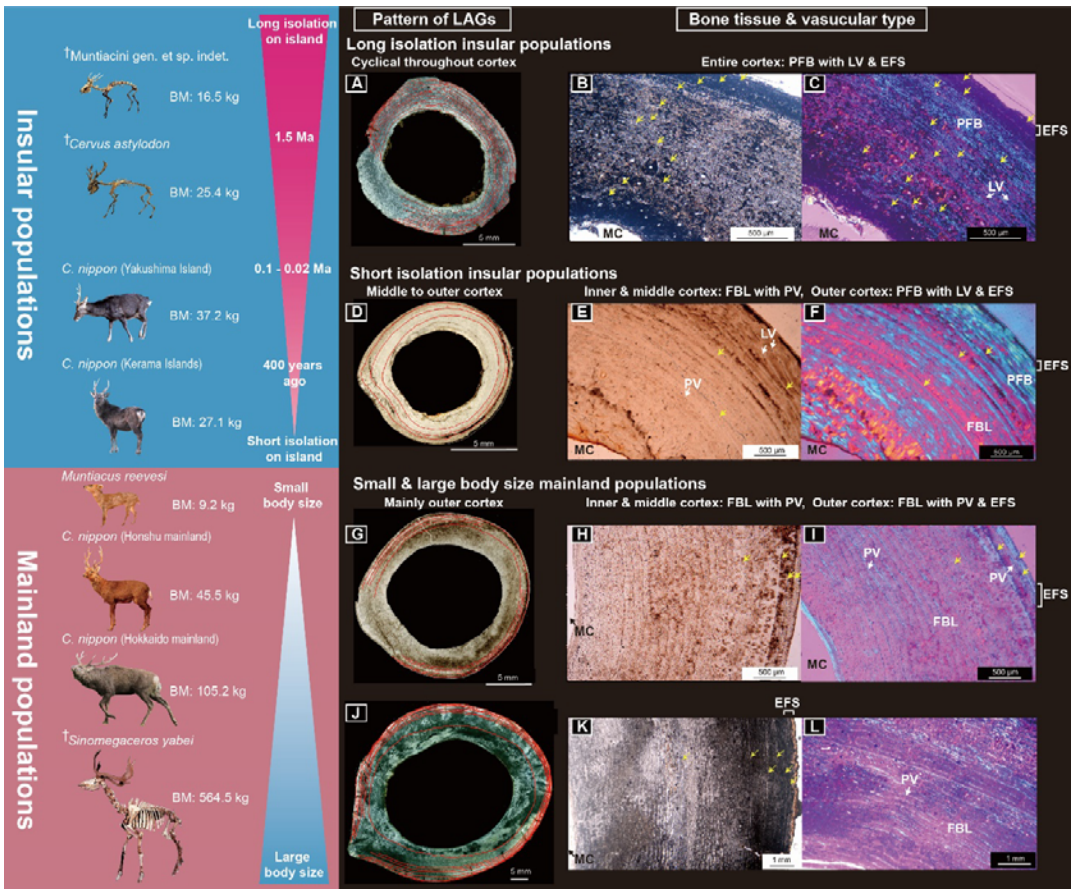

Fig. 3. Bone histological features of the Japanese cervids. (A–C) Midshaft cross section (A), cortex in normal light (B), and cortex in polarized light (C) of a femur of fossil *Cervus astylodon* (OPM-HAN07-1603). (D–F) Midshaft cross section (D), cortex in normal light (E), and cortex in polarized light (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 [29].

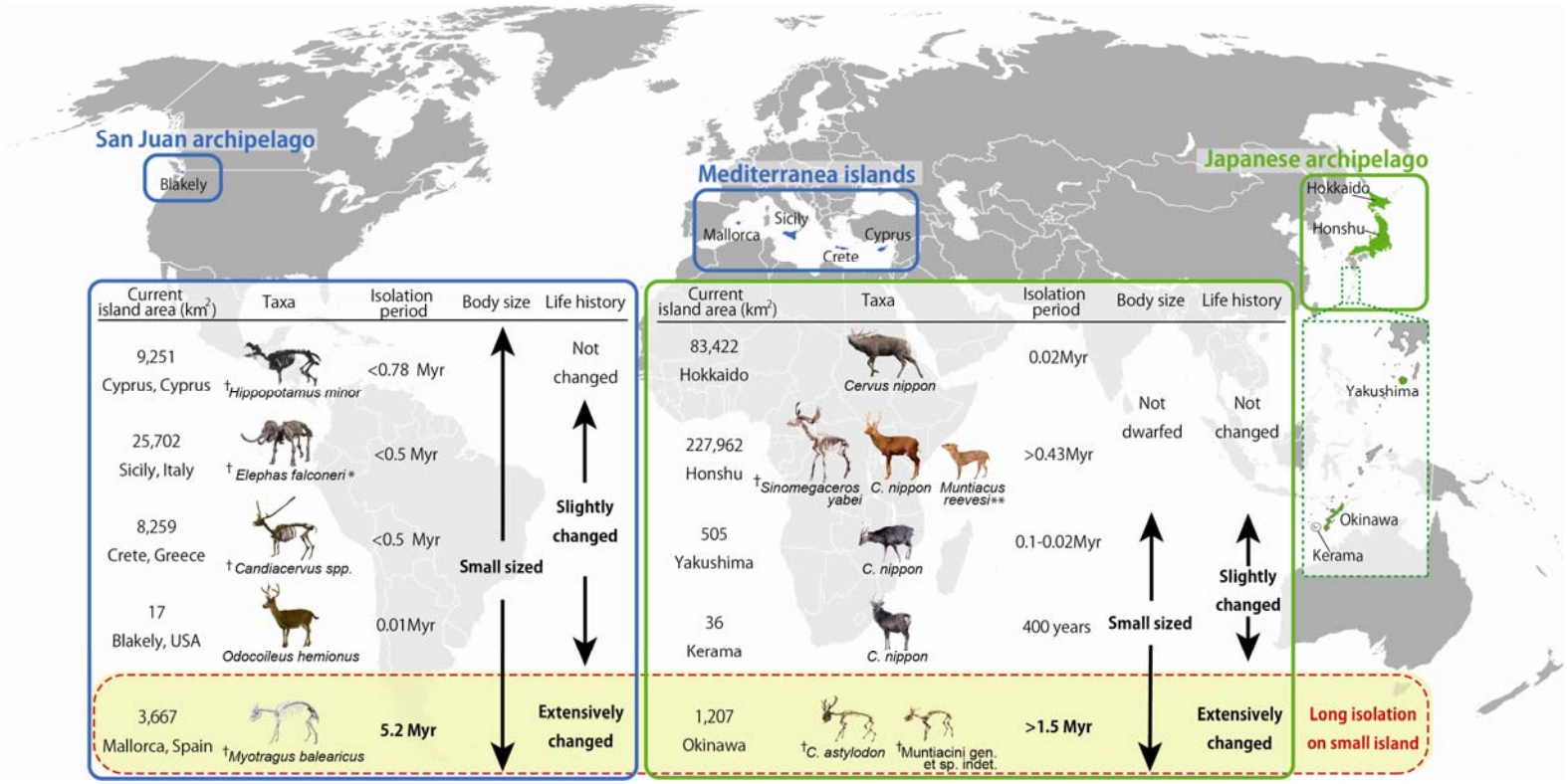

Fig. 4. Summary of the relationship between insularity, body size and life history found in extant and fossil large mammals. The cervid species/populations of Japanese Archipelago were investigated in the present study and information of animals in other islands were obtained from references: *Odocoileus hemionus* [8], *Hippopotamus minor* [2], *Candiacervus* spp. [2], *Elephas falconeri* [2, 68], *Myotragus balearicus* [6]. Photographs of fossil mammals of Mediterranean islands courtesy of Gunma Museum of Natural History (*Elephas falconeri*), Alexandra van der Geer (*Hippopotamus minor* and *Candiacervus* spp.), and Meike Köhler (*Myotragus baleanicus*). † Fossil cervids. * Life history of *Elephas falconeri* is disputed [53, 54]. ** *Muntiacus reevesi* is an artificially introduced animal in 1980's, therefore the isolation period of > 0.43 Myr is not applicable to it [24].

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) [69]	Subarctic coniferous forests or mixed forests	105.2	F: 7, T: 7	1060 [65]
Cervus nippon (Honshu mainland)	Honshu (227,962 km^2)	Middle Pleistocene (0.43 Ma) [69]	Temperate evergreen broadleaved forests	45.5	F: 8, T: 8	594 [38]
Muntiacus reevesi		Recent introduction (1960–1980) [24]		9.2	F: 5, T: 5	84 [67]
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) [42]	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) [18]	NA	564.5	F: 2, T: 2	NA
† *Cervus astylodon*	Okinawa Island (1,207 km^2)	Early Pleistocene (ca. 1.5 Ma) [17]	NA	25.4	F: 6, T: 5	45 [38]
†Muntiacini gen. et sp. indet.				16.5	F: 4, T: 2	65 [66]

* Habitat type was obtained from a reference [56]. † Fossil cervids. ‡ The estimated body mass (EBM) was obtained from fitted growth curves (SI Appendix, Fig. S8, S9) by averaging asymptotes for each species/population (SI Appendix, Table S8).