Deer pedicle height, tissue interactions and antler evolution

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ANTLERS and their antecedent pedicles are deer cranial appendages. Each year, antlers are cast and fully regenerate from the apices of pedicles. Pedicles vary considerably in length, and antlers in both size and shape among deer species. This article attempts to put some seemingly unrelated characteristics, that is pedicle length, antler size and deer body size, together following the established deer evolutionary tree, and to seek a possible meaningful relationship for these phenomena. In order to do so, it is necessary to firstly introduce antler ontogeny. That is how deer pedicles and antlers are formed from deer heads.

1. Antler ontogeny

For a missing appendage to be replaced stem tissue must be left behind from which the new parts can develop. In case of antler, pedicles fulfil this role. Deer are not born with pedicles; these begin to develop when deer approach puberty (Fennessy & Suttie, 1985). When pedicles grow to their species-specific height, the first antlers transform spontaneously (Li *et al.,* 2000A). After the completion of first antler formation, development of the subsequent antlers enters a well-defined cycle: hard antler casting, new antler regeneration, antler maturation, and skin shedding. The first antlers grown by yearlings normally come in the form of simple spikes, but as the age proceeds deer develop large species-specific branched racks or palmate configurations. Maximum pedicle length in a deer species is achieved when they carry the first set of antlers. Thereafter, pedicles increase in diameter and decrease in length. In old adults, antlers seem to grow directly from the skull (Goss, 1983).

Pedicle formation

Deer pedicles are secondary sexual characters. As such, pedicle formation is under the control of androgen hormones. Prepubertal castration will stop pedicle initiation and administration of exogenous androgen hormones will overcome this abnormality of deer (Jaczewski, 1982; Goss, 1983). Suttie *et al.,* (1984; 1991) reported that pedicle formation was caused by increasing and subsequent elevated level of plasma testosterone. Therefore, androgen hormones are the prime stimulators for pedicle formation.

It has been convincingly demonstrated that the potential to form a pedicle and an antler is exclusively held in the periosteum overlying a crest of the deer frontal bone (Fig. 1A). Removal of this periosteum prior to pedicle initiation will abolish the future pedicle and any subsequent antler formation, and implantation of it elsewhere on the deer body will induce an ectopic pedicle and antler to grow (Fig. 1B). This periosteum has, therefore, been termed antlerogenic periosteum (Goss, 1983). Female deer also possess antlerogenic periosteum, but do not normally express this phenotype due to their androgen hormone level being insufficient to stimulate pedicles growth (Jaczewski, 1982).

First antler generation

Transformation of deciduous antlers from permanent pedicles is considered as a unique zoological phenomenon. Although it is known that androgen, which plays a critical role in pedicle formation, is not required for antler growth the factors driving antler growth are thus far elusive. In order to reveal the underlying mechanism of this unique phenomenon, we have systematically investigated pedicle formation and antler generation. Histologically, the axis of a pedicle and a growing antler mainly consists of two components, interior osseocartilage (mixture of bone and cartilage) and exterior skin.

Formation of the interior component results from the proliferation and differentiation of the cells residing in the antlerogenic periosteum (Li & Suttie, 1994). The exterior component of the axis, including pedicle skin and antler velvet, is a derivative of deer scalp skin (Li & Suttie, 2000B). Our histological results showed that the close contact between the apical pedicle tissue (antlerogenic tissue) and the overlying skin is associated with the initiation of deciduous antlers from the permanent pedicles (Li & Suttie, 2000B). This close contact is achieved by the substantial compression of subcutaneous loose connective tissue (Figs. 1C and 1D). This compression is caused by mechanical force, which is derived from the active augmentation of the interior component of a pedicle. The results from the both experiments of antlerogenic periosteum autograft (Goss, 1990) and xenograft (Li *et al.,* 2001) transplantation support the claim that the intimate association of antlerogenic tissue and the overlying skin is a prerequisite for antler generation.

As an organ, first antler generation must depend on the interactions between mesenchyme (M, apical pedicle stem tissue) and epithelium (E, epidermis of the overlying skin). This claim is supported by our histological findings (Li & Suttie, 2000B) that only fragmented pieces of the basement membrane were detected in the early antler generation stage (Fig. 1E), in contrast to the pedicle development stage, where a well intact basement membrane was encountered (Fig. 1F). It is known that an intact basement membrane between epidermis and dermis inhibits tissue regeneration (Neufeld *et al.*, 1986). The E-M interactions during antler generation may be via diffusible molecules as insertion of a thin layer of permeable membrane cannot stop antler generation, although the process is substantially delayed compared to the control side antler (Figs. 1G and 1H, unpublished).

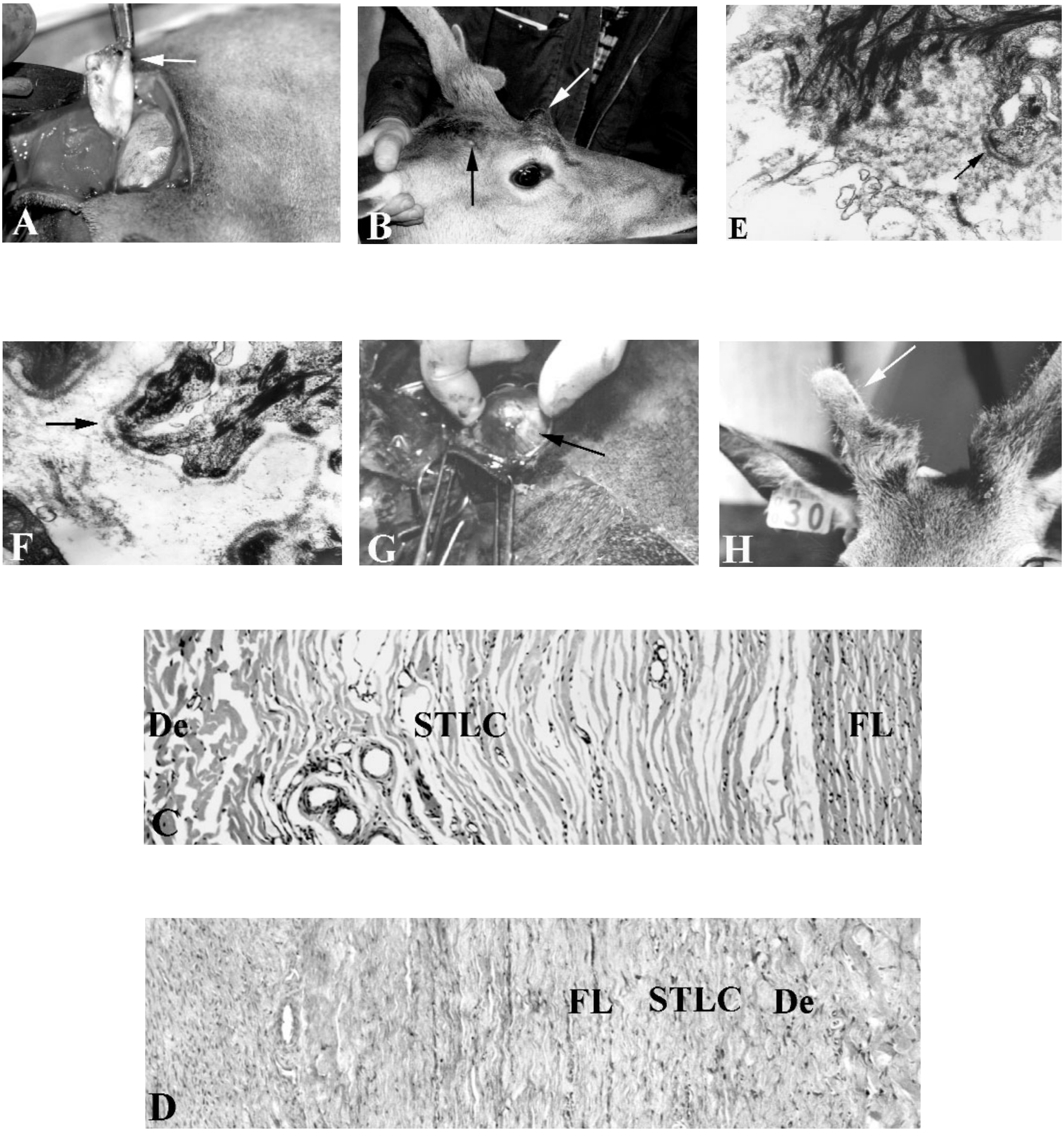

Figure 1. Tissue interactions and antler formation

A. A piece of surgically removed antlerogenic periosteum (arrow).

B. An ectopic antler formed on a male Red deer nasal bone (white arrow) by a piece of antlerogenic periosteum from the right side presumptive pedicle growth region (black arrow). Note that no pedicle and antler formed from the original region after the removal of antlerogenic periosteum.

C. Subcutaneous loose connective tissue (SLCT) between a frontal crest and the overlying skin at pre-pedicle stage. Note that the SLCT is a very thick and loose layer at this growth stage. Haematoxylin and eosin (H & E). De, dermis; SLCT, subcutaneous loose connective tissue; Fl, fibrous layer.

D. SLCT between an apical antler perichondrium and the overlying skin at incipient antler stage. Note that the SLCT is a very dense and narrow layer at this growth stage. H & E. De, SLCT and Fl are the same as in Figure 2C.

E & F. Electron micrographs of the apical skin basement membrane. E. From an early-growth stage antler. Note that a fragmented basement membrane (arrow) was found. F. From a mid-growth stage of pedicle. Note that a well intact basement membrane was in place (arrow).

G. A piece of permeable Teflon membrane was inserted into the space between a deer frontal crest and the overlying skin.

H. Formation of the pedicle and the antler following the permeable membrane insertion. Notice that although the pedicle from the membrane-inserted side formed in a similar way and to a similar height to the control side, the antler formation was substantially delayed.

Therefore, the intimate contact between the antlerogenic tissue and its overlying skin during antler generation may have been the prerequisite for the establishment of these M-E interactions between the two interactive tissues. The time length of this contact required for successful establishment of the M-E interactions may vary among deer species. For example, antlers can spontaneously generate from the exogenous-androgen-induced (EAI) pedicles in White-tailed deer by singular androgen hormone treatment (Wislocki *et al.,* 1947) because the M-E interactions between the interactive tissues in this species can be established within a short period of close contact. The latter is achieved by a surge of testosterone due to a singular treatment. On the other hand, the fact that antlers did not form from the EAI pedicles in Red deer by using the similar androgen treatment in the previous studies (Jaczewski & Krzywinska, 1974) is because the establishment of the M-E interactions in this species requires an extended period of close tissue association, which can only be sustained by continuous or regular administration of exogenous testosterone (Li *et al.*, in press).

Wounding to the apical pedicle tissue has been found to be the most powerful way to stimulate antler growth from the EAI pedicles in deer of the genus *Cervus*, so long as the wounding includes both apical pedicle skin and its underlying antlerogenic tissue (Jaczewski & Krzywinska, 1974; Jaczewski *et al.*, 1976). We propose that wounding can rescue the failure of antler generation because wounding can break the physical barriers (basement membrane, dermal tissue, subcutaneous loose connective tissue and fibrous layer of perichondrium) between the interactive tissues, thereby facilitating these interactions and hence promoting antler generation.

2. Antler phylogeny

Deer are the members of the order *Artiodactyla*, suborder *Pecora*, superfamily *Cervoidea*, and family *Cervidae* (Romer, 1966). Based on phylogenetic analysis, what families should be included in the superfamily *Cervoidea* is far from clear. Romer (1966) and Simpson (1945) classified this superfamily to include three families: *Giraffidae*, *Moschidae* and *Cervidae*.

According to molecular genetics (Cronin, 1998), *Cervidae* shares close ancestry with *Giraffidae*. *Moschidae* (Musk deer) and *Hydropotinae* (Chinese water deer) have been included in *Cervidae*, but appear to have evolved independently of the *Odocoileinae* and *Cervinae* (Janis and Scott, 1987). Therefore, they represent groups which may have evolved independently. This traditional phylogenetic relationship in deer has been confirmed by the approach of modern molecular evolutionary genetics (Cronin, 1998).

Pedicle height and deer evolution

It is generally agreed that throughout their evolution, deer have gradually increased in body size, and in the size and complexity of their antlers, as well as displaying a tendency toward a more gregarious habit (Putman, 1988). These have been used as important parameters for constructing the deer evolutionary tree along with modern molecular genetics (Cronin, 1988).

Musk deer (*Moschus*), with their more simple stomach structure, and lacking pedicles and antlers, but with enlarged canines (Fig. 2A), show striking similarities to Mouse deer (chevrotains). Such a relationship suggests that these deer may have developed as a relatively early side-shoot of the line leading to the more advanced groups of deer. Lack of pedicles and antlers, and the possession of tusk-like upper canines also seem to cast the Chinese water deer (*Hydropotes inermis*; Fig. 2B) as a another primitive group. However, other characters of Chinese water deer are more certainly cervid, implying that these deer may originate from a somewhat later offshoot from the major evolutionary line.

Muntjacs (*Muntiacus*) are small-sized deer with small antlers, raised up on long pedicles (Fig 2C). Possessing a more advanced stomach structure, they have clearly progressed further along the main evolutionary line. However, the relatively elongated pedicles (5-8cm), the simplicity of the antlers (6-9cm in length) and the retention of an upper canine tusk cast these species as a relatively early offshoot. Molecular genetic analysis, including fibrinopeptides, mitochondria DNA and repetitive DNA, place Muntjacs within *Cervinae* (Cronin, 1998).

Eurasian Cervinae and New World *Odocoilinae* represent highly successful and relatively independent radiations among the more advanced deer. Among the deer in these two subfamilies, Red deer (*Cervus elaphus*) which grow 4-5cm high pedicles (Fig. 2D) for *Cervinae* and White-tailed deer (*Odocoileus virginianus*) which form 2-3cm high pedicles (Fig. 2E) for *Odocoilinae* are the two most studied deer species in the world.

Reindeer (*Rangifer*) are the most peculiar species in the deer family as they are the only deer to possess antlers in both sexes and do not form visible pedicles (<0.5cm) before proceeding to antler formation. These animals are the most gregarious species, forming massive herds that dominate the tundra. Within weeks of being born, Reindeer calves begin to grow their first antlers without visibly passing through a pedicle stage. Like the other species, the first antlers of Reindeer are also usually unbranched (Fig. 2F). Reindeer and Caribou appear to have evolved in more recent times (Goss, 1983; Cronin, 1998).

From the forgoing accounts, there clearly exists a relationship between the degree of deer evolution and pedicle height across all deer species: the more advanced the deer in evolution, the shorter the pedicles. To the best of our knowledge, this relationship has never been described in previous literature. Based on pedicle height, Muntjac (pedicle: 5-8 cm in height), Red deer (pedicle: 4-5cm in height), White-tailed deer (pedicle: 2-3cm in height) and Reindeer (pedicle: <0.5cm and not easily visible) can be readily placed in an order.

This order fits the established deer evolutionary tree (Putman, 1988) well and may represent a refinement in relation to advancing evolutionary mechanisms. In addition, there exists a trend during deer antler evolution whereby the shorter the pedicles, the bigger the antlers they produce. Along this trend, we can extrapolate that preceding Muntjac, less-advanced species would carry even longer pedicles and smaller antlers. At a certain point, there must be a species that carries exceedingly long pedicles but no antlers.

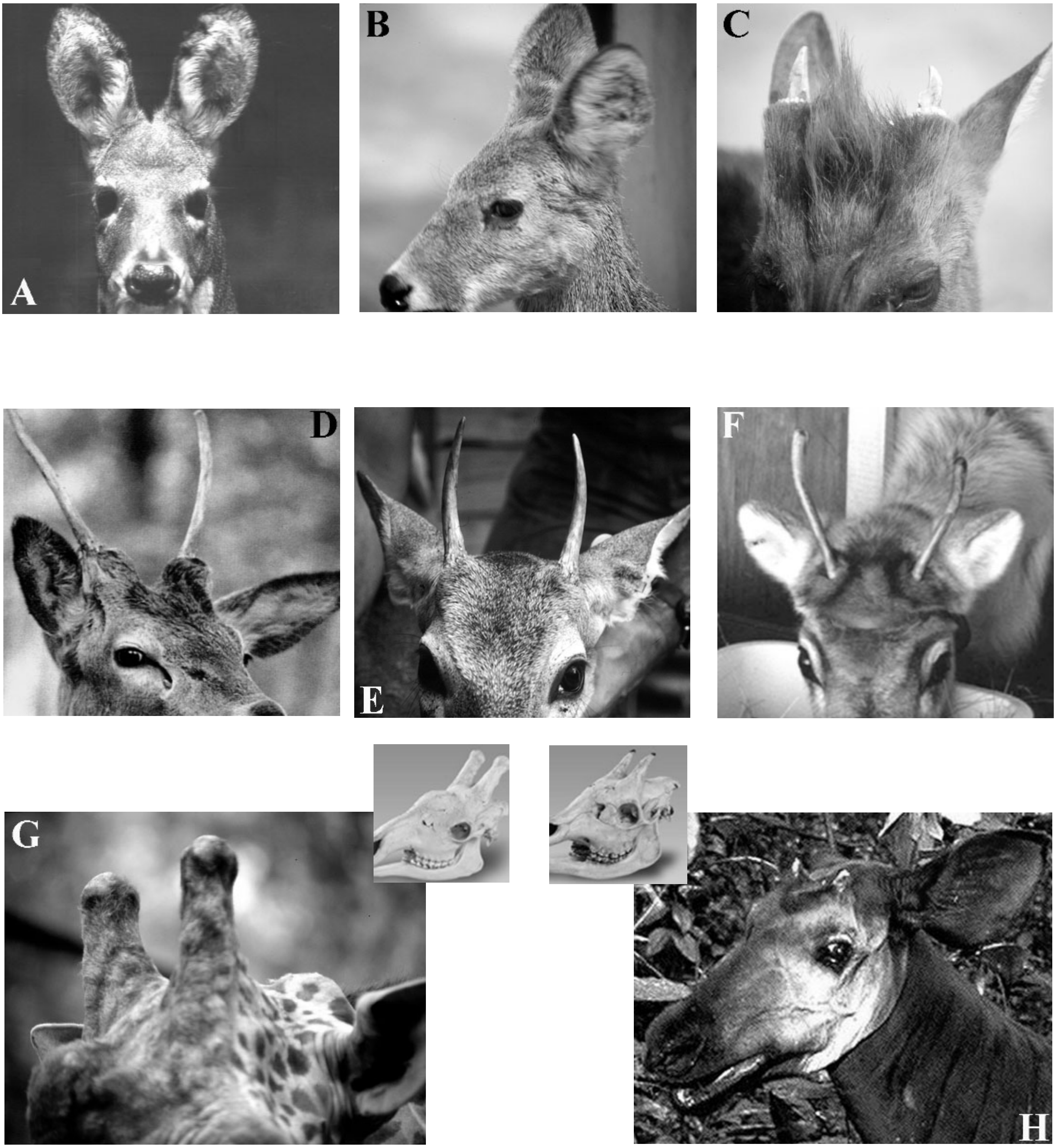

Figure 2. Pedicle height and deer evolution

A. Male Musk deer. Notice that Musk deer lack pedicles and antlers, but have enlarged canines.
B. Male Chinese water deer. Like Musk deer, Chinese water deer do not possess pedicles and antlers, but have upper canines.
C. Male Muntjac. Muntjacs have small antlers, which are surmounted on long pedicles (5-8cm).
D. Male Red deer. Red deer grow 4-5cm high pedicles before forming the first set of antlers.
E. Male White-tailed deer. White-tailed deer carry short but obvious pedicles (2-3cm) at the time when they give rise to first set of antlers. (Both the photo and the pedicle length are kindly provided by Dr. G. A. Bubenik).
F Male Reindeer. Reindeer grow antlers within weeks after born without visibly passing through a pedicle stage (<0.5cm in height). (The photo is kindly provided by Dr Eva Wikiund).
G. Male giraffe. Giraffes carry very long pedicle-like horns (about 18cm), but no antlers. The inset, a male giraffe skull with bony protuberances.
H. Male Okapi. Okapis also carry long pedicle-like horns (about 8.5cm). Interestingly, the tips (black dots on the bony protuberances, refer to the inset) of these pedicle-like horns are polished bare bone, which is separated from the horn body by a small suture. These bony tips are considered as rudimentary antlers (see text).

To our knowledge, there are no any living species from *Cervidae* can fill in this gap. If some deer species were evolved but became extinct during the course of evolution, these species would look like today's Giraffe (*Giraffa*) a member of the *Giraffoidae*, which is closely related to *Cervidae*. Giraffes carry around 18cm long cranial protuberances (Fig. 2G and the inset). These protuberances are permanent and unbranched. Giraffe protuberances are different from the horns of *Bovidae* in that they lack a keratinised epidermal sheath, but are more like the pedicles of the *Cervidae* as both of them are permanent and viable structures that are enveloped by skin. The apex of giraffe pedicles becomes cornified after completion of basic ossification. The terminal hair is worn away and the keratinised epidermis is anchored to the bone by long connective tissue filaments that extend into the bone.

Like giraffe, Okapi (*Okapia*), another member of *Giraffoidae*, also bears pedicle-like protuberances (Fig. 2H and the inset). These structures are about 8.5cm long, which is longer than that of Muntjac (5-8cm) but shorter than giraffe (18cm). Interestingly, the tips of these pointed pedicles of Okapi are free of skin, i.e. polished bare bone (the inset of Fig. 2H). The bare bone tip appears separated from the body of the pedicle by a small suture, forming a small terminal cap of bone 8mm in depth, which resembles the tiny hard antlers on the top of their pedicles. Lankester (1907) suggested that the bony tips in the okapi may be called rudimentary antlers. The transverse fissures are caused by the ingrowth of the living tissue after the protrusion of the dense polished cap, acting to cut off the protruding portion and provide for its breaking off – just as an antler is cut off. A small conical piece is thus thrown off from the end of the pedicle. Therefore, Okapi or Okapi-equivalent deer species may quite well be the transition between pedicle-only and true deciduous-antler.

Although the existing deer species can be used to explore possible clue as to how deciduous antlers have evolved, the antler evolution puzzle can never be satisfactorily completed without using fossil samples from extinct deer. This is particularly true in the early stage of antler evolution. During the Miocene epoch, the *Merycodon tidae* grew branched appendages that lost their skin but remained permanently attached thereafter as dead bony structures (Frick, 1937) The world's earliest known deer are believed to be *Dicrocerus* and *Stephanocemas* (Goss, 1983). These animals appeared in the Miocene epoch and survived to the Pliocene epoch. They not only shed their velvet but also renew their antlers each year. Both of them were small and had long pedicles surmounted by antlers, like that of today's Muntjac. These extinct deer could well have evolved between giraffes and Muntjacs, but for some unknown reason did not survive to the present.

With the establishment of a correlation between pedicle height and antler evolution, one would inevitably ask what mechanism underlie this relationship?

Pedicle injury and antler evolution

To be effective as combat weapons, deer antlers have evolved as hard durable structures. In order to evolve hard bony appendages, ancestral deer would have to invent a way for their antlers to enlarge with increasing body size, to repair broken branches, yet not be vulnerable to freezing should they inhabit temperate zones. Annual regeneration would be the only way to meet these conditions. This necessitated a velvet phase for new bone to develop by appositional growth, a process that enables antlers to elaborate extensive systems of branching or palmation (Goss, 1995). How these events might have occurred, however, is only a matter of conjecture.

Goss (1995) reasoned that if the earliest perennial antler antecedents were enveloped in viable integument year-round like giraffid horns, the deciduous antlers might have evolved through either or both of the following two pathways:

1) Rutting season fighting would have frequently injured the combatants' viable antlers
2) The perennial and viable antlers may have been subject to frost-bite if the climate changed or if the deer invaded temperate latitudes.

Either way it seemed that mechanical injury and wound healing would have played a crucial role in the evolution of regenerative antler. However, thus far a hypothesis has never been advanced as to why mechanical injury and wound healing are so crucial for this particular event.

3. Recapitulation

It is known that in the animal kingdom ontogeny in some cases recapitulates phylogeny. If this applies to deer antler, the course of deciduous antler evolution may be a reflection of the gradual establishment of the interactions between antlerogenic tissue and its overlying skin. The frontal periosteum of Musk deer or Chinese water deer is incapable of reaction to androgen hormone stimulation; hence they do not carry pedicles. Without the formation of pedicles, close association between frontal periosteum and the overlying skin cannot be created, therefore no antler generation can take place, even if the two tissue types are competent to interact. That "pedicles" of giraffe or giraffe-equivalent deer species cannot give rise to antlers could well be because the bony apical pedicle tissue cannot interact with the overlying skin, therefore the interactions between these two tissues cannot be established. For antlered deer, there exists an obvious trend from primitive to more recently evolved deer (Section 2: Pedicle height and deer evolution) – that is: pedicles are getting shorter and shorter, whereas antlers are becoming bigger and bigger. For example, it would take long time (data not available in the literature) for a primitive deer species like Muntjac, to complete the formation of their lengthy pedicles before small antlers can generate. In contrast, calves of more recently evolved deer species like reindeer, begin to grow their first sets of antlers within weeks of being born, without passing through an obvious stage of pedicle formation. This could well be because the time length required for the two interactive tissues to remain in close contact to trigger the successful interactions is getting shorter during antler evolution. If this hypothesis is right, the high androgen level that is so important to sustain continuous pedicle growth, and subsequently maintain the close association between the interactive tissues, may become less critical. This is because in the more recently evolved antlers, the establishment of the tissue interactions is so easy that a quick contact between the interactive tissues would initiate the interactions, and hence drive antler formation.

Consequently, Reindeer do not have long pedicles and female reindeer can grow antlers.

We can further extrapolate along this line. In order to grow antlers, Muntjacs have to have not only high levels of androgens, but also this high level would have to remain for an extended period (data not available in the literature) to sustain the continuous growth of pedicles, which in turn will maintain the close contact between the two interactive tissues. In the extreme cases, male Muntjac might fail to achieve this daunting task, and would have to carry giraffe horn-like pedicles for their life, although this has not been reported so far.

Besides these two extreme deer species, there is a tendency that the shorter the pedicles, the higher the incidence of females carrying antlers. For example, White-tailed deer pedicles are shorter than those of Red deer, the frequency of females carrying antlers in White-tailed deer naturally is much higher than in Red deer (Goss, 1983). In addition, the majority of these antlered females are encountered in the late autumn, which is the deer rutting season when males have highest plasma testosterone level and normally carry their hard antlers. Therefore, a minimal level of androgen hormone may help females grow their rudimentary pedicles, hence promoting a short period of close tissue contacts. Conversely, the higher the pedicles, the higher the incidence of males not possessing antlers. For example, Red deer pedicles are higher than White-tailed deer, antlerless-males are more frequently encountered in Red deer, which are called hummels (Li & Suttie, 1996), than in White-tailed deer.

Wounding has played a pivotal role in the evolution of deciduous antlers (see section 2: Pedicle injury and antler evolution). Wounding during antler evolution is reminiscent of the mechanical injury on the pedicles of EAI and hummels, or annual antler casting, which facilitate antler generation and regeneration. This may be because tissue injury can break the barrier (dermis, subcutaneous loose connective tissue and fibrous layer of antlerogenic tissue) between the two interactive tissues, hence facilitate the critical E (epidermis)-M (antlerogenic tissue) interactions.

Overall, tissue interactions could have been critically involved in both antler ontogeny and phylogeny. The eventual identification and isolation of the putative interacting molecules will undoubtedly help for a better understanding of antler generation and regeneration in both ontogeny and phylogeny.

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