

Morphogenetic aspects of deer antler development

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1. ABSTRACT

Deer antlers are unique mammalian appendages that have potential for application as a valuable model for biomedical research, such as organ regeneration, bone development and growth control. In this review we described the morphogenetic aspects of antler development, by summarizing relevant information accumulated over the course of our antler research in combine with other published papers. Antler development consists of two distinct phenomena: generation and regeneration. Generation starts with the initiation of a pair of pedicles, permanent bony protuberances on the skull of a male deer, when the animal approaches puberty; proceeds to transformation to antlers when the pedicles reach a species-specific height (around 5 cm in red deer); and terminates with total antler calcification and velvet (antler skin) shedding. Regeneration commences in the spring of the subsequent year with the initiation of new soft antlers growing from the pedicle stumps that remain following the casting of the previous hard antlers; proceeds to the elaboration of multi-branched antlers in summer; and ends up with total antler calcification and velvet shedding in autumn, resulting in the formation of hard bony antlers in winter. Therefore, this paper not only lays the foundation for further development of this valuable biomedical model, but also provides comprehensive background knowledge that will be of use to readers when reading the papers featured in this special issue.

2. INTRODUCTION

Deer antlers are unique mammalian organs (1). To hunters, they are desirable trophies; to animal lovers, they are magnificent head ornaments; to oriental doctors, they are a magical traditional Chinese medicine; and to biologists, they are zoological curiosities and a novel biomedical model. The unique annual renewal of antlers offers an unparalleled opportunity to explore how nature has bestowed full mammalian organ regeneration (1, 2). The unprecedented growth rate of antlers (up to 2.75 cm/day) provides a rare system for study in which fast cell proliferation is elegantly regulated without becoming cancerous (3). The atypical features of antler “stem” cells, such as expression of key embryonic stem cell markers (i.e. Oct4, Nanog and SOX2) and multi- or pluri-potency, serve as an invaluable model for understanding how some of the attributes of embryonic stem cells may be retained in postnatal animal cell populations (4-6). As a muscle- and joint-free bony cranial appendage, antler provides a singular system to investigate how bone tissue is formed without the influence of external forces (except for gravity) (7, 8). The formidable size and complexity of antlers are a powerful system to study how visual symbols can impact on animals’ social ranking status (9). All papers assembled in this special issue report scientific studies aimed at developing these models. The present account summarizes the data accumulated over the course of our antler research to describe how antlers form at the morphological level;

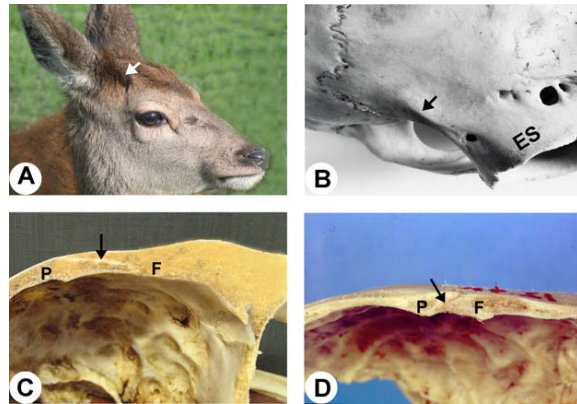


Figure 1. Future pedicle growth region and frontal crest. A. A future pedicle growth region (arrow) in a pre-pubertal male red deer. Note that the region is clearly distinguishable by a differential colour and growth direction of hair. B. A frontal crest (arrow) on a pre-pubertal male red deer skull. Note that the crest is located just behind and above the eye socket (ES). C. A sagittal cut through the centre of a frontal crest of a pre-pubertal male red deer. Note that in the centre, the frontal bone (F) overlaps the parietal bone (P) (arrow). D. A sagittal cut through the centre of a frontal crest of a pre-pubertal male Chinese water deer (an antler-less species). Note that in the centre, the parietal bone (P) overlaps the frontal bone (F) (arrow).

this not only lays the foundation for further development of this valuable biomedical model, but also provides background knowledge for readers before reading the other papers in this special issue.

3. GENERATION OF PEDICLES AND FIRST ANTLERS

3.1. Frontal crest

Deer antlers are bony organs which are cast and fully regenerate from permanent protuberances, or pedicles, each year. The presumptive pedicle growth region is obvious either visibly as a patch of differentially colored of hair (Figure 1A) or palpably as a bony ridge (behind and above the eye socket) on the frontal bone (Figure 1B). This bony ridge, prior to pedicle initiation, has been called the supraorbital process (10), frontal lateral crest (11), or the external frontal crest based on the Latin name (*crista frontalis externa*) (12). For the purposes of this review we have called it the frontal crest.

The sizes of frontal crests vary among individuals. The height of a crest can vary from 2.5 mm to 6.5 mm in red deer. Interestingly, frontal bone overlaps parietal bone in the presumptive pedicle growth region of red deer (Figure 1C), whereas in Chinese water deer (an antler-less species) the converse occurs (Figure 1D). Formation of the beveled coronal suture in red deer is thought to be due to the posterior migration of the cells responsible for future pedicle growth (13).

3.2. Pedicle and first antler

The development of deer pedicles is a once in a lifetime event. In most species, pedicles start to initiate (Figure 2A) when deer approach puberty (5-7 months of age in red deer) and reach a threshold body weight (about 56 kg in red deer), which normally occurs in late winter or early spring. Once visible, the incipient pedicles have transformed from an initial oval shape to a cylindrical configuration. When pedicles grow to their species-specific height (Figure 2B; around 5-6 cm high in red deer), the first antlers begin to generate spontaneously from the apices of these pedicles. This antler generation can easily be detected by a change in skin appearance from typical scalp-like to velvet-like (Figure 2C), which is called velvet skin or velvet.

It is known that all types of deer integument, except for three (tail ventral surface, back and nose snout), are capable of transformation into velvet skin if they are induced to do so by the antlerogenic periosteum (AP; for detailed information see the paper, “Histogenetic aspects of deer antler development”, in this special issue), the bone membrane that overlies the frontal crest. The lack of competence of the three exception skin types has been attributed to them either being unable to become closely associated with the grafted AP (ventral tail and back) or being devoid of hair follicles (nose snout) (14).

3.3. Antler growth and elongation

Once initiated, first antlers enter a rapid growth period in spring. First antlers normally form a single main beam and do not branch; hence they are called spikes (Figure 2D). However, first antlers can have tines and form to an appreciable size if deer are well nourished or mechanically wounded, although the pattern may not necessarily be species-specific. While growing, antlers are tender and sensitive to touch, and may bleed profusely when injured.

3.4. Calcification and velvet shedding

In autumn, as the reproductive season approaches, antlers gradually become calcified. It is clear that the antler calcification process starts from the base, which can be effectively demonstrated by the gradual appearance of pearling (Figure 2E); calcification proceeds upwards and finishes when the rounded configurations (Figure 2D) of the distal ends of spike antlers turn into sharp tips (Figure 2F).

Following the completion of antler calcification, velvet skin loses its shiny appearance and becomes dry (Figure 2E). Gradually the dried velvet starts to peel off to expose the underlying bone. There does not seem to be a particular place on the antler surface from where velvet shedding starts. However, one can observe that some shedding processes are lengthy and no obvious bleeding occurs (Figure 2G), whereas in others shedding is rapid and causes much bleeding (Figure 2H). Exposed hard antlers are firmly attached to their living pedicles for the entire winter season.

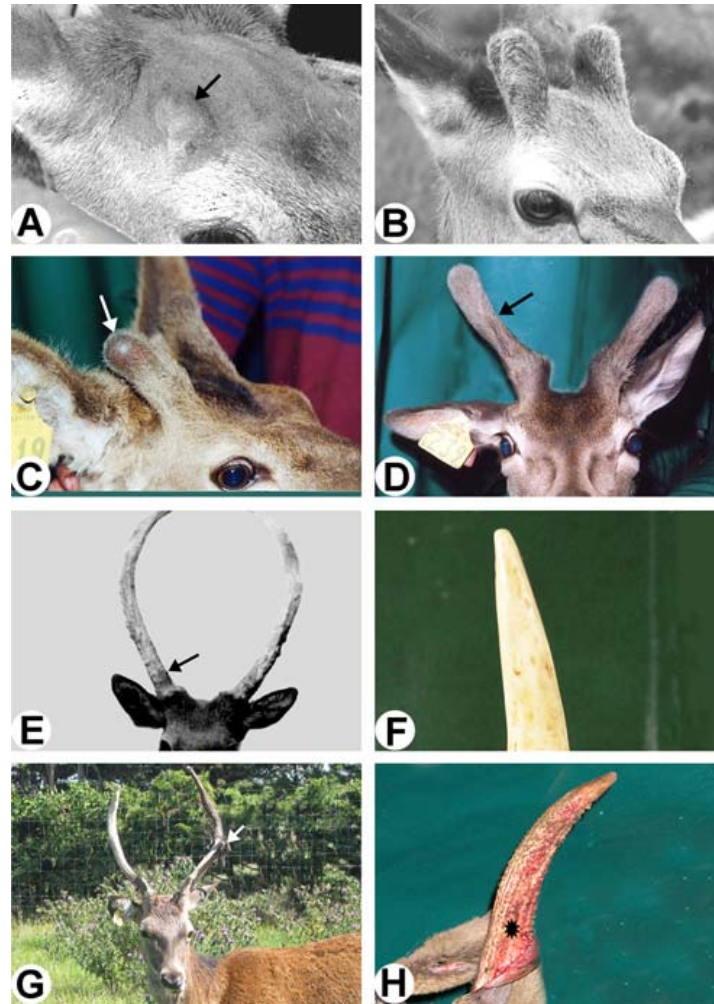


Figure 2. Formation of pedicles and first antlers. A. Initiation of a pedicle (arrow) from a pubertal male red deer. Note that a cylindrical pedicle bud had formed from an oval-shaped frontal crest. B. Fully formed pedicles from a pubertal male red deer. C. Generation of a first antler from a fully grown pedicle in pubertal male red deer. Note that this generation can be readily seen via the change in skin type from typical scalp to velvet-like (arrow). D. Late stage growing first antlers (arrow) in a young red deer stag. Note that these first antlers did not have branches. E. Fully calcified spike antlers from a young red deer stag. Note that velvet skin had become dry, and pearlation (arrow) was obvious on the antler surface. F. The tip of a hard spike antler. Note that this sharp tip was transformed from the rounded growing antler tip. G. Velvet skin shedding from spike antlers. Note that no bleeding (arrow) occurred during the shedding process. H. Velvet skin shedding from a spike antler. Note that obvious bleeding (arrow) occurred during the shedding process.

Velvet shedding is probably the least studied phase in an antler growth cycle, mainly due to antlers typically being cut off well in advance of velvet shedding as a part of standard deer farming practice; and also due to the fact that this phase does not seem to have an immediate link to medicinal applications. Goss (1) suggested that loss of velvet is causally and primarily related to the total ossification of antler bone since the ossification causes occlusion of blood vessels supplying antlers, but the course of shedding may be advanced by some secondarily located factors, such as fighting and rubbing velvet on tree bark. Other possible causes of velvet shedding have also been postulated by a number of antler biologists. Hypotheses include the stretching of the velvet covering a rapidly

growing antler causing occlusion of the blood vessels (15); the growth of the coronet (the coarse bony ring around the antler base and immediately above the pedicle) causing compression and subsequent death of the blood vessels passing through it (16); or blood vessels themselves closing due to increasing plasma testosterone levels (17).

3.5. Casting

The first antlers of red deer are cast in the spring approximately a year after initial generation, and regeneration of the second set of antlers begins immediately. However, in some species, such as white-tailed deer, antlers drop off in the late autumn or early winter season, and the initiation of the second set of antlers

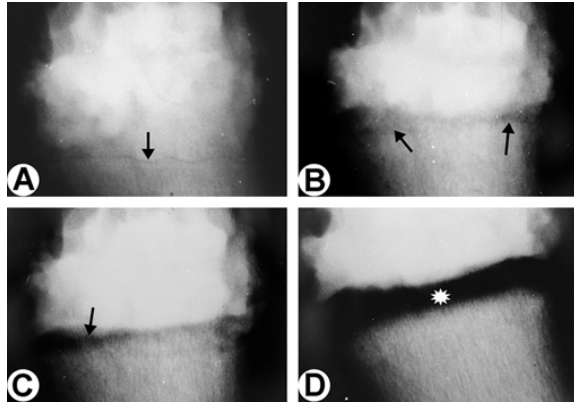


Figure 3. Serial radiographs of the junction between a pedicle and a hard antler taken 3, 7, 11, and 17 days after castration in a young stag. A. A fine line (arrow) between the pedicle and the hard antler became visible three days after castration. B. Two clear wedge-like spaces (arrows) at each side of the junction, formed seven days after castration. C. Bone resorption space (arrow) along the junction became larger on day 11 as the casting date approached. D. Complete bone separation (asterisk) of the dead antler bone from the living pedicle bone was evident on day 17, four days before casting.

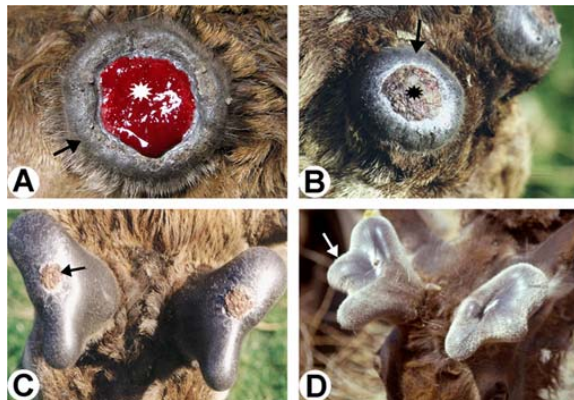


Figure 4. Early regeneration stages of red deer antlers. A. Casting plane on a red deer pedicle stump. Note that blood (asterisk) resulting from antler casting was profuse but well contained in the centre of the plane by the distal pedicle skin rim (arrow). B. A scab was formed from the blood two days after antler casting. Note that the scab (asterisk) was surrounded by a ring of shiny skin (arrow). C. Early regeneration stage of red deer antlers. Note that at this stage the antler main beam and brow tine were of a similar length, which makes them resemble a saddle (arrow). D. Early regeneration stage of red deer antlers with a bezel tine (arrow). Note that the shapes of the antlers at this stage resemble a "silver ingot", a type of ancient Chinese money.

does not occur until the following spring, so the gap between hard antler casting and new antler regeneration is around 3-4 months.

Radiographs of the antler and pedicle junction, taken using a portable X-ray machine between castration

(conducted to experimentally induce antler casting) and antler casting, clearly revealed the casting process inside the bone tissue (25). The results showed that at the time of castration no obvious demarcation could be detected between the pedicle and the antler. However, within 3 days of castration, a thin line became visible below the coronet indicating bone resorption having started (Figure 3A). On day 7, two clear wedge-like spaces had formed at each side of the junction, which were two dimensional manifestations of a resorption band around the circumference of the antler and pedicle junction (Figure 3B). The bone resorption spaces became larger as actual casting was approached (Figure 3C), and eventually appeared to completely separate the dead antler bone from the living pedicle bone 4 days before casting (Figure 3D). The hard antler dropped off around 21 days after castration. This work not only clearly demonstrated the hard antler casting process, but also highlighted the importance of sex hormones in controlling antler casting.

4. REGENERATION OF SECOND AND SUBSEQUENT ANTLERS

Following first antler casting, the annual development of subsequent antlers occurs in a well-defined cycle: previous hard antler casting and new soft antler regeneration take place in early spring, rapid growth and maturation in late spring and early summer, calcification and velvet shedding in autumn, and bare bony antlers in winter (18).

4.1. Around the time of antler casting

Immediately prior to hard antler casting, the distal end of the pedicle skin becomes swollen and shiny. Following hard antler casting, bleeding takes place from the cast surface of the pedicle stump. The extent of blood lost from each antler casting varies among individuals. In some deer, bleeding is profuse, although blood rarely flows from the distal pedicle skin rim (Figure 4A), whereas in others bleeding is very limited. The variation in the blood loss may solely depend on the length of time required to complete the casting process. The quicker the casting process can be completed, the greater the blood loss that occurs from the cast surface of a pedicle stump. This is reminiscent of velvet shedding. The length of time for completing a casting process may be determined by the rate at which the endogenous testosterone level falls (not experimentally proven). This is because the activity of osteoclasts, the cells responsible for dissolving the bone tissue along the pedicle (live) and antler (dead) junction (see the paper "Histogenetic aspects of deer antler development", in this special issue), is triggered by a decrease in testosterone to the sub-threshold level.

Bubenik (1966) observed a change in the configuration of the antler seal surface (the shape of the base of the cast antler) throughout the life of a red deer stag: convex from yearling up to prime-age, flat during the transitional years, and concave in older stags. A possible explanation for this phenomenon is that the interface between living pedicle and dead antler undergoes

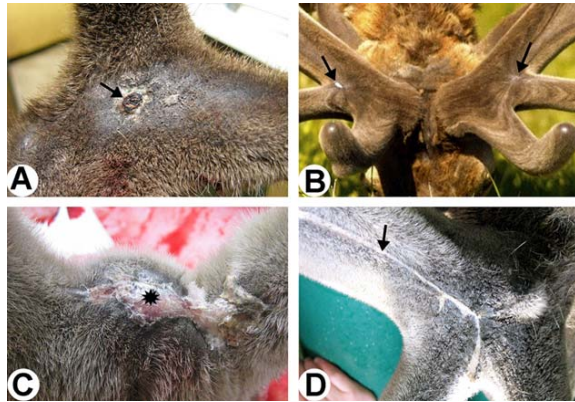


Figure 5. Scar formation over the casting plane of a pedicle stump/regenerating antlers. A. A centrally located scab (arrow) was displaced by the eccentrically formed main beam and the brow tine. B. Scars (arrows) revealed after the scabs flaked off were small and located on the medial surface of the region where antler main beam, brow tine and bez tine (if it existed) branch. C. A large scar (asterisk) formed due to imperfect wound healing on the area where antler main beam and brow tine bifurcate. D. A scar had long thread-like shape (arrow) due to imperfect wound healing and was located markedly away from the normal place, the bifurcation region.

continuously dynamic changes. The higher the level of circulating androgen hormones (such as in stags approaching prime-age), the later the hard antlers will cast. The longer the hard antlers remain on their pedicles, the further the casting line will move proximally as the distal pedicle tissue constantly dies back. Therefore, antler seals will become convex. Alternatively, a less complex explanation is that the pedicle simply gets shorter as the stag ages and there is less bone tissue available for subsequent antler regeneration.

4.2. Wound healing

Shortly after antler casting (one or two days), the blood remaining in the depressed centre of the casting surface on a pedicle stump dries and a scab is then formed. At this stage, the scab occupies approximately half of the entire casting surface and is surrounded by a ring of shiny skin (Figure 4B). As the wound healing process advances, the skin ring migrates further centripetally and the scab and underlying scar become smaller compared to the previous stage. The wound healing process over a pedicle stump is considered as completed when the peripheral healing skin converges in the centre of the casting plane. In some cases, before wound healing is fully complete, the anterior and posterior portions of a pedicle stump have already bulged out for antler brow tine and main beam formation, which grow beyond the central scab/scar region due to differential tissue growth rate. Therefore, this period can be considered as the transition between pedicle wound healing and antler regeneration.

4.3. Regeneration

Morphologically, the antler main beam and brow tine are simultaneously formed posteriorly and

anteriorly, respectively. During the initial stages, these two are similar in length, which makes an incipient antler resemble a saddle (Figure 4C), and an incipient antler having a bez tine at the same stage of regeneration resemble a “silver ingot”, a type of ancient Chinese money (Figure 4D).

The centrally located scab is displaced by the eccentrically formed main beam and brow tine (Figure 5A), and eventually flakes off to reveal the underlying scar. The size and shape of the scar varies among individuals, so does the scar location on a regenerating antler. Usually, the resultant scar is small, round and hardly visible, and is located on the medial surface of the region from which the main beam and brow tine (and bez tine, if it exists) of a regenerating antler bifurcate (Figure 5B). Sometimes the scar can be large and rod-like, and positioned outside the bifurcation region. In cases of imperfect wound healing, the scar can be extremely large (Figure 5C), or exceedingly long and thread-like and located markedly away from the normal position (Figure 5D). However, no causal relationship has thus far been detected between antler deformation and wound healing quality over a pedicle stump. Consequently, wound healing status may play only a minor role, if any, in antler regeneration in terms of final antler size and shape. Goss (19) concluded that wound healing over the top of a pedicle stump is prerequisite for the initiation of antler regeneration. However, our recent membrane insertion experiment demonstrated that antler regeneration could take place without pedicle skin participation resulting in a skin-less antler (20).

4.4. Elongation and bifurcation

Growth of regenerated antlers is comparable to, but faster than, that of the first antlers, branching also takes place. Regenerating antlers grow rapidly in the late spring and early summer, with growth rates comparable to or exceeding that of embryonic tissues. The elongation of an antler shows a typical S-shaped growth curve. It starts out slowly in the spring, accelerates exponentially during the summer, and slows down its growth rate as autumn approaches (1). However, the antler growth pattern in red deer showed the S-shaped curve with a period of about 40 days of approximately linear increase (from about day 30 to day 70) (21). If the growth of all tines was included, the growth rate in length over this linear period was about 1.8 cm/day in red deer.

Undoubtedly antler branching is under the control of genetic programming, as antler shape is species-specific. However, thus far no research is available on the antler branching process. We found that well before being externally visible, the branching process has been underway internally. The growth centre for a new branch was clearly revealed on the longitudinal cut surface of a growing antler tip (Figure 6A). Another interesting finding (22) was that blood vessel branches supplying the brow, bez and/or trez tines from the superficial temporal artery were already present prior to the initiation of these antler tines (Figure 6B). In contrast, the arteries supplying the terminal fork (royal tines) only began to form just before

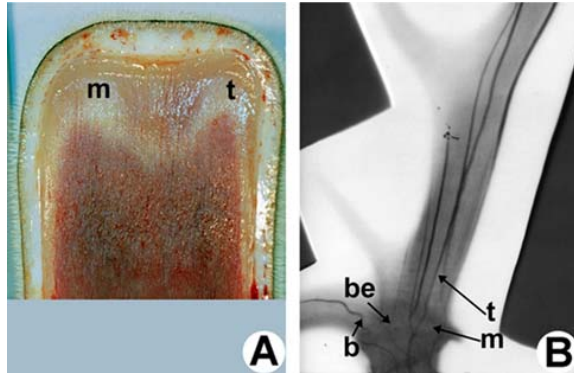


Figure 6. Antler growth centres and blood vessels associated with antler branching. A. A new growth centre (t) for a trez tine had formed and separated from the antler main beam growth centre (m) well before the tine could externally be seen. B. Blood vessel branches supplying the brow (b), bez (be) and/or trez (t) tines were already present well prior to the initiation of these antler tines.

these structures started to take the shape. This might be one of the reasons why normally the first two to three tines are predestined to form in regenerating antlers independent of environmental factors, whereas the number of royal tines that form depends at least in part on the level of nutrition.

Interestingly, Lake *et al* (23) found that antler growth rate was positively and significantly correlated with electronegativity; and application of direct current to a growing antler could result in abnormal branching patterns. Speer (24) considered that these bioelectric potentials associated with growing antlers may be related to the enveloping collagen fibre systems. The axially-oriented collagen fibres in both velvet and perichondrium of a growing antler are under tension provided, apparently, by the proliferating cell masses at the tips of the antler tines. Tension in the oriented collagen fibre bundles may be responsible for maintenance of these observed electrical charge differentials (23) and may mediate local morphogenetic effects including elongation and branching. Electronegativity and antler growth and patterning are a fertile research field and worth further study.

Calcification and velvet shedding of a regenerating antler are morphologically identical to those of the first generating antlers.

The study of organ morphogenesis is, by its narrow definition, to observe the formation of an organ at the morphological level; from this more sophisticated mechanistic studies can be built. The morphogenesis of deer antler generation and regeneration described here, together with the companion paper “Histogenetic aspects of deer antler development”, form the background for the subsequent papers in this special issue that address other individual aspects of deer antlers.

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