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Residual antler periosteum holds the potential to partially regenerate lost antler tissue

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Abstract

Deer antlers are the only mammalian organs that can fully regenerate, which relies on pedicle periosteum (PP). Interestingly during the growing phase, antlers themselves can regenerate partially lost antler tissue. However, what tissue type in the growing antlers fulfills this role is not known. Following antler removal during the growing phase, a "second" antler regenerates from the stump. In this study, the "second" antler growing from the cut antler base (AB) was examined in both red and sika deer. The results showed that all regenerating antlers were formed from the peripheral edge of the AB, where the antler periosteum (AnP) is located. The growth center showed a clear demarcation from the AB bone in red deer. Therefore, it is highly likely that AnP is the tissue that possesses the potential. Factors that might affect this potential were explored and the main factor was found to be AB calcification, which was controlled by rising androgens. Thus, the ultimate antler regeneration potential of the AnP was assessed through castration and repeated antler removal. The results demonstrated that the regeneration potential of AnP was somewhat limited and inferior to that of the PP. The ability of AnP to achieve partial regeneration may be evolutionarily conserved, as the regeneration of partially lost antlers within the season is secured; whereas, with PP, a new set of antlers in the next season is guaranteed. This two-level mechanism may signify how evolutionarily important it is for deer to possess reasonably intact antlers.

KEYWORDS

antler periosteum, deer antler, pedicle, pedicle periosteum, regeneration

1 | INTRODUCTION

Epimorphic regeneration is generally considered as a luxury shared only among some lower level animals (Goss, 1983; Landete-Castillejos et al., 2019). However, deer antlers, the large mammalian bony appendages, are annually lost and fully regenerate from the permanent bony protuberances, known as pedicles. In spring, totally calcified (dead) antlers are cast from their pedicles and regeneration of new soft antlers immediately follows; in late spring and early summer, antlers grow very fast (up to 2 cm/day) and are enveloped with special pelage known as the velvet skin; in mid-late summer, the antlers become calcified and the velvet skin begins to shed before the rutting season starts; in late autumn and winter, exposed and dead bony antlers remain firmly attached to their pedicles until the next spring (Li et al., 2004). Hence, this unique case of full antler renewal offers the opportunity to explore how nature has bestowed this mammalian organ with its unique regenerative ability.

Previous work involving tissue deletion (Li, Mackintosh, et al., 2007) and membrane insertion (Li, Yang, et al., 2007) studies have demonstrated convincingly that it is the pedicle periosteum (PP) that gives rise to regenerating antlers. The pedicle skin and pedicle bone play only protective and supportive roles, respectively, during antler regeneration. These findings have laid the foundation for subsequent molecular studies on the PP tissue to unveil the mechanism underlying the initiation of antler regeneration (Akhtara et al., 2019; Ba et al., 2016; Dong et al., 2016, 2020; Guo et al., 2015; Li et al., 2012; Liu et al., 2018). Interestingly, antlers themselves can repair/partially regenerate if they are damaged/partially lost during their growth phase without the participation of PP tissue (Li & Man, 1990; Suttie & Fennessy, 1985). However, thus far, the question as to what tissue type(s) of the antler remnant initiates antler repair/partial regeneration is not known. The hypothesis is that the antler periosteum (AnP) is the prime candidate for fulfilling this role in the absence of involvement of the PP.

Deer antlers have been used for more than 2000 years as a traditional medicine in China and some Southeast Asian countries (Kong & But, 1985), and in Russia (Batchelder, 1999). For such use, antlers are harvested during their growing phase (known as velveting) to retain the maximal medicinal values. The cut antler is known as velvet antler (VA). Modern science has demonstrated that VA extracts can promote wound healing in vivo (Li, 2020) and inhibit cancer cell growth in vitro (Chonco et al., 2021). To protect the growth center for antler growth in the next season, VAs are removed at the level 2–3 cm above the antler–pedicle junction (Figure 1) on the commercial farms. Hence, an antler base (AB) of around 2–3 cm remains on top of the pedicle after velveting. In most cases, the residual AB still possesses partial antler regeneration potential, but this potential varies considerably according to the timing of velveting (C. Li et al., 1998). In New Zealand, VAs from red deer stags are normally harvested at around 55–60 days (Figure 1a,b) after casting of the previous fully calcified ABs. In China, VAs from sika deer are normally harvested when they form a specific shape (Li et al., 2004), such as two-branch VA (around 40–45 days of growth after the previous AB casting; Figure 1c) or three-branch VA (around 60–70 days of growth; Figure 1d). The time of harvest is chosen by experienced operators based on expected financial returns. Normally, two-branch antlers are harvested from younger stags (2–3 years old), whereas three-branch antlers are harvested from older stags. The practice of velveting on commercial deer farms offers a unique opportunity to observe and assess what tissue type(s) of ABs have initiated partial regeneration of the antler.

In the present study, I closely examined the candidate tissue type(s) of the residual ABs for their ability to regenerate within the same antler growth season on both red (Southern hemisphere) and sika (Northern hemisphere) deer farms. Overall, it is highly likely that the AnP of the residual ABs gives rise to the partially regenerated antlers. Subsequent functional testing in vivo (through castration and repeated antler removal in the castrated deer), demonstrated that the ultimate regenerative potential of the AnP was somewhat limited, and inferior to that of the PP.

2 | MATERIALS AND METHODS

2.1 | Approach

In this study, AnP regeneration potential and pattern were closely examined in two species, red deer (*Cervus elaphus*, at the Invermay Agricultural Center, New Zealand, Southern Hemisphere) and sika deer (*Cervus nippon* at the Zuojia Research Deer Farm, China, Northern Hemisphere), by taking the opportunity at the time of commercial velveting on these farms. Subsequently, functional testing of the ultimate potential of AnP regeneration was carried out in sika deer on the Zuojia Research Deer Farm. The operation (using the procedure for commercial velveting) involved repeated removal of growing antlers. The procedure was approved by the Animal

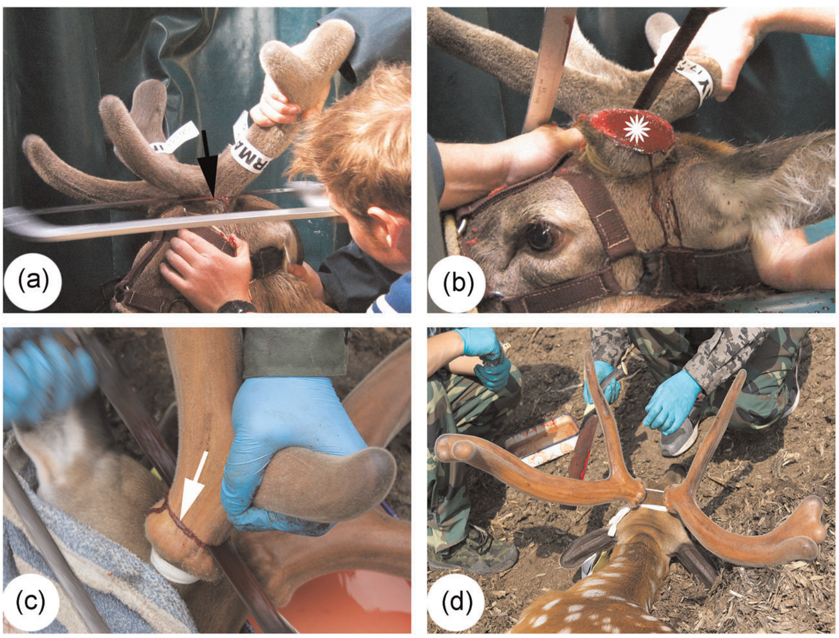

FIGURE 1 Antler bases (ABs) created through velveting of the first set of growing antlers (first cut ABs) at a level 2–3 cm above the junction with the pedicle. (a, b) Red deer; (a) velveting; arrow: cutting line; (b) first cut AB (asterisk) created after velveting. (c, d) Sika deer; (a) two-branch antler; arrow: cutting line; (b) three-branch antler, yet to be velveted [Color figure can be viewed at wileyonlinelibrary.com]

Ethics Committee of The Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agricultural Sciences (Permit Number: 2018-0029).

2.2 | Red deer

In total, 30 red deer stags (3–6 years of age) were used (mid-January), when they were yarded to remove their second set of antlers that had regrown from the first cut ABs. Each deer was placed in a specifically designed hydraulic deer handling cradle, and local anesthetic (bromacaine) injected around the junction of the pedicle and the skull before the VA was removed. Photographing of each AB was carried out before and after VA removal.

A head of a 3-year-old red deer stag was recovered from a local slaughterhouse in New Zealand in mid-January; this head was selected for the presence of a pair of the first cut ABs, each had a small single spike antler. The left-side AB was used for macroscopic examination to define the origin of internal antler bone by examining the longitudinal surface cut through the AB and attached antler. The right-side AB was used for testing the toughness of the PP: following removal of the pedicle skin, a small piece of PP (5 × 10 mm), including a sliver of underlying bone tissue, was collected for histology. A portion of VA (from a 3-year-old red deer) was obtained from the Invermay deer farm for comparative purposes. The proximal part of the velvet skin was surgically opened; the AnP toughness testing and histology were carried out following the same procedure used for the PP. The detailed histological process has been described elsewhere (Li et al., 2005). Briefly, tissue samples were fixed in 10% neutral buffered formalin, decalcified, embedded in paraffin wax, and sectioned at 5 μm. Hematoxylin and eosin were employed for staining the sections.

2.3 | Sika deer

In total, 41 sika deer stags (1–6 years of age) were selected for examination of the "first" (1–4 years old) or "second" (5–6 years old) cut ABs in early September (eight each of 1, 2, and 3 years; six each of 4 and 6 years; and five of 5 years) to determine the growth potential and partial regeneration pattern for the comparison with red deer. The approach followed the normal procedure for removal of the small or aborted VA which is conducted for safety reasons. The animals were captured using general anesthesia (xylazine hydrochloride, 1.5–2.0 ml/100 kg body weight) and the antlers removed using a handheld saw. Photographing of each AB was carried out before and after VA removal.

2.4 | Functional testing of ultimate regeneration potential of the AnP using castrated sika deer

Four 1-year-old male sika deer were selected for functional testing of the regeneration potential of the AnP. Antler removal and castration were carried out under general anesthesia (xylazine hydrochloride, 1.5–2.0 ml/100 kg body weight). The procedure was conducted in early December when the first VA reached 6–8 cm in length and the deer were around 6 months of age. Right-side antlers were cut at the level 2 cm above the junction with pedicle for testing the regenerative potential of AnP. By contrast, the left-side antlers were cut right through the junction with pedicle (for mechanically traumatizing the PP); these were used as a positive control. The resultant ABs were then allowed to heal and regrow. The timing for subsequent VA removal was determined as follows: both antlers on each stag were removed when the antler lengths on the right side taken 5 days apart were the same (no significant difference, $p > 0.05$) or when the positive control antler had reached 15 cm.

3 | RESULTS

3.1 | Evidence points to AnP being the tissue that gives rise to the partially regenerated antler in red deer

3.1.1 | Localized and discrete antler regeneration always takes place from the site where the AnP is located

Following removal of the VA, the "second" growth occurred at the periphery of the first cut ABs and was associated with the velvet skin. The degree of regeneration ranged from a single spike through to the tissue that completely encircled the wound site of the first cut AB. The different degrees of regeneration among the 30 red deer stags are summarized in Figure 2. Among the first cut AB in these red deer stags, 6 (20%) regenerated a localized single small spike antler (Figure 2a,b,o); 9 (30%) regenerated more than a single spike antler (Figure 2c,d); 3 (10%) regenerated a localized single spike and a quarter circular-shaped antler shafts (Figure 2e-g); 9 (30%) regenerated more than one localized single spike and a quarter circular-shaped antler shafts (Figure 2h-k); and 3 (10%) had a full circular-shaped antler shaft (Figure 2l,m).

In the cases of full encirclement (Figure 2l,m) of the first cut AB, exposed bone tissue on the centrally encircled surface had died back proximally (3–5 mm), become desiccated due to long-term exposure, and sometimes detached from the first cut AB during the process of velveting (Figure 2n). Overall, the examination of partial antler regeneration in red deer indicates that, morphologically, regeneration of the localized discrete VA was always situated peripherally and derived from the AnP. The regenerated VAs were enveloped by the velvet skin and showed clear demarcation from the first cut AB bone (Figure 2o). Observation of the longitudinal surface cut through a first cut AB and the attached small regenerated spike VA (Figure 2p) further supports the proposition: It is the distal AnP that forms the localized regeneration center that gives rise to the regenerating antler.

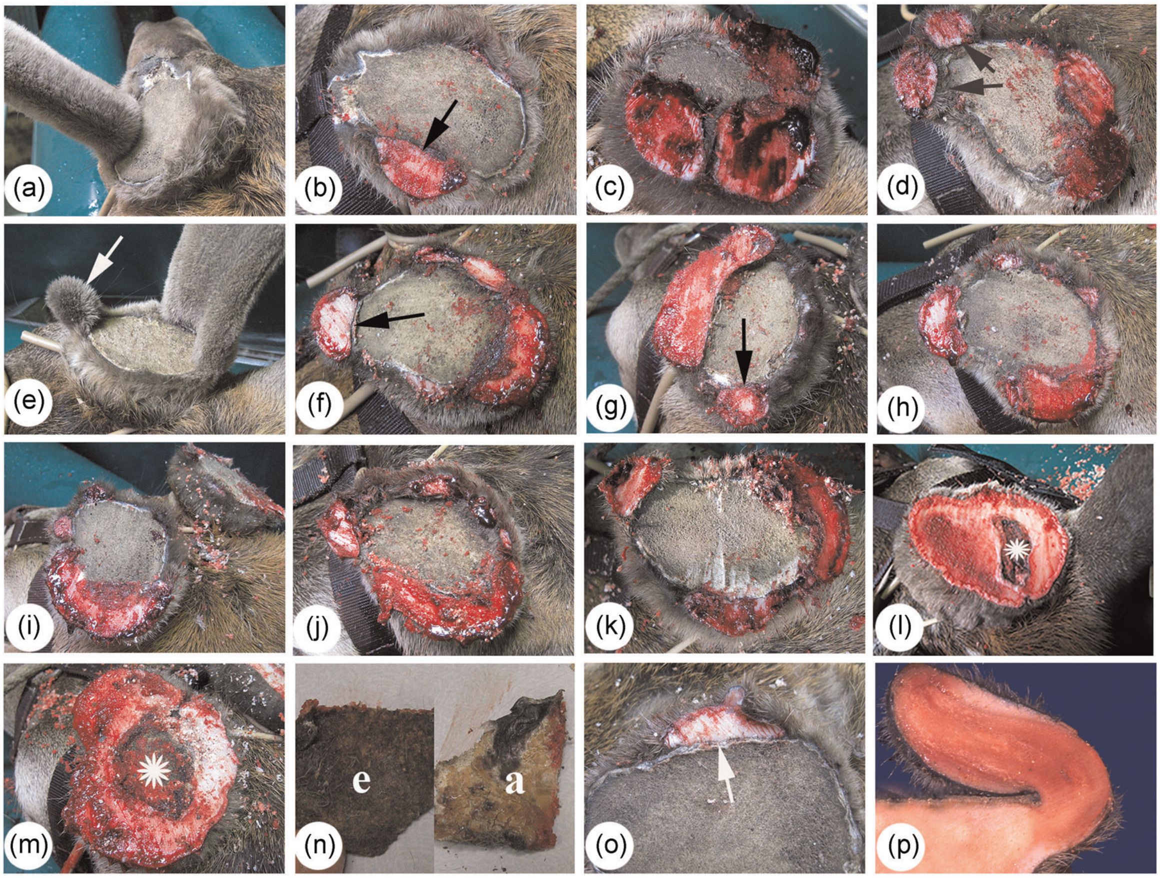

FIGURE 2 Fresh second cut antler bases (ABs) beside the first cut ABs, created after removal of the second set of regenerating antlers in red deer. (a, b) A second cut AB after removal of a singly regenerated spike antler; (a) before and (b) after velveting. Note that the antler was positioned at the edge of the first cut AB and its growth center had clear demarcation with the first cut AB bone (arrow). (c, d) Second cut ABs after removal of multiregenerated singly spike antlers surrounding the first cut ABs. Note that all the singly discrete growth centers were situated peripherally where the AnP locates, particularly the two centers located anteriorly in (d) even had gaps with the first cut AB bone (arrows). (e–k) Second cut ABs after removal of singly spike-shaped and quarterly circular-shaped shafts of regenerated antlers surrounding the first cut ABs. The common feature of these regenerated antlers is that their growth centers were all situated peripherally and had demarcations with the first cut AB bone. (l, m) Second cut ABs after removal of full circular-shaped shafts of the regenerated antlers. Note that the central regions of the first cut ABs were not healed and the exposed tissue of centrally located cut surface was dead and became desiccated (asterisk; dark gray color). (n) A detached dead tissue slice from the centrally located cut surface during velveting of second set regenerating antler; e, exposed side; a, attached side. (o) A second cut AB after removal of a singly regenerated spike antler to demonstrate again its growth center was clearly separated (arrow) from the first cut AB bone. (p) A longitudinal cut surface through a first cut AB and the regenerated small spike antler, indicating that the regenerated antler is derived from the AnP of the first cut AB, evidenced by the position and tissue color of the regenerated antler, the color is closer to the AnP (reddish) than to the AB bone (whitish) [Color figure can be viewed at wileyonlinelibrary.com]

3.1.2 | AnP morphologically and histologically resembles PP

It is known that the AnP is anatomically an extension of the PP (Li & Suttie, 1994). The results of the present study showed that, morphologically and histologically, AnP resembled the PP (Figure 3). However, AnP appeared to be more brittle than the PP, which was apparent when being peeled from the attached bone: the former was frequently broken (Figure 3a), whereas the latter always remained intact (Figure 3b). Histologically, both AnP and PP consisted of two layers: the fibrous layer and the cambium layer, but the cambium layer of AnP (Figure 3c) was significantly thinner than that of the PP (Figure 3d).

3.2 | Exploration of factors that may affect regenerative potential and pattern of AnP in sika deer

3.2.1 | Deer species

To determine whether the formation of a localized discrete growth center in ABs in red deer is a general phenomenon or species-specific, a close examination of the pattern of partial antler regeneration in sika deer was performed. An AB was created following velveting of the first (Figure 4a-c; first cut AB) or second (Figure 4d-f; second cut AB) sets of VA. In contrast to the response of the AB to velveting in red deer (i.e., forming localized discrete antler growth centers), healing over an AB

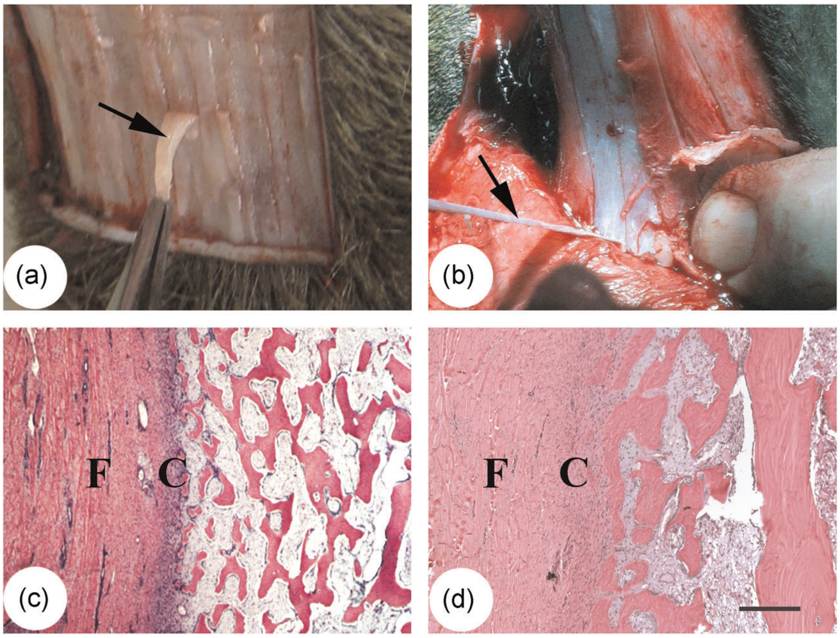

FIGURE 3 Comparisons between antler periosteum (AnP) and pedicle periosteum (PP). (a, b) At macroscopic level; (a) AnP (arrow); (b) PP (arrow). Note that AnP was somewhat more fragile than the PP evidenced by the fact it was frequently broken when being peeled off from the attached bone. (c, d) At a microscopic level. Note that histologically AnP and PP were comparable as both consisted of a fibrous layer (F) and cambium layer (C), however, the cambium layer of PP was much thicker than AnP, which may partially explain why PP has greater regenerative potential than AnP. Scale bar = 0.1 mm [Color figure can be viewed at wileyonlinelibrary.com]

wound in sika deer preferentially took place from the distal end of the AnP peripherally surrounding the AB wound (Figure 4b). Subsequently, a two-branch antler was regenerated from the completely healed first cut AB (Figure 4c). To make comparisons with the situation of red deer (i.e., regeneration of discrete and singular antler from an AB), the second cut ABs were created by removing the regrown antlers in early July. These second cut ABs were overlying the first cut ones, but somewhat smaller in size. Healing of the second cut ABs in the early cases was completed, but no sign of regeneration was observed (Figure 4d). In the latter cases, even wound healing did not occur over the second cut ABs when the regrown antlers were removed in late July/early August (Figure 4e). Interestingly, a localized antler growth from the second cut AB was observed (<1%) in sika deer; however, when that occurred, wound healing was always completed first (Figure 4f). Overall, healing the wound over the cut surface of an AB takes the priority over forming discrete spike antlers in sika deer, although the healing also initiates from the AnP of ABs. Therefore, AnP is the tissue that heals the AB wound.

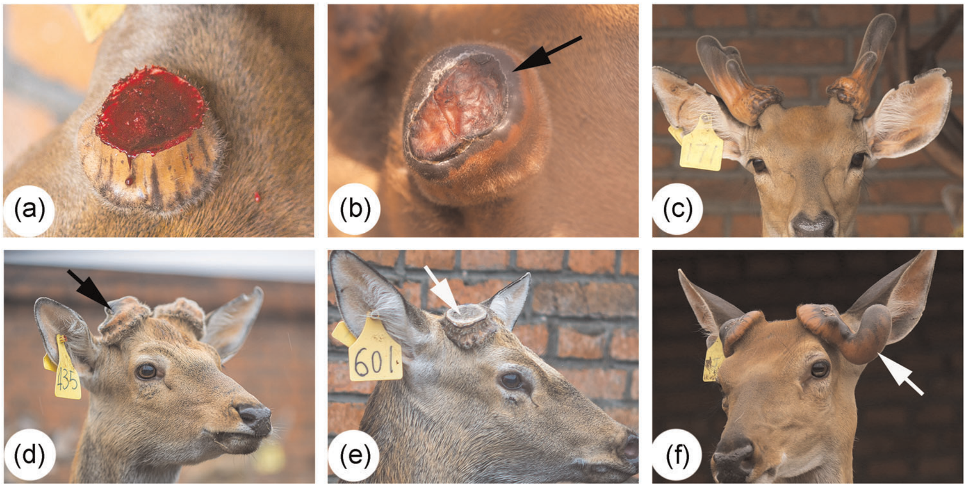

FIGURE 4 Antler bases (ABs) created after the removal of regenerating antlers in sika deer. (a) A fresh first cut AB created in early May. (b) A first cut AB at early wound healing stage (4 days after cutting). Note that the newly regenerated healing skin (black color) and underlying bony tissue were migrating centripetally from the peripheral edge. (c) Second set of two-branch antlers regenerated from the first cut ABs. (d) Second cut ABs created in early July reached the stage of wound healing completion. Note that the totally healed ABs did not show any sign of regeneration potential as they reached the final stage of calcification. (e) Second cut ABs created in late July reached the final stage of calcification before wound healing took place. (f) Second cut ABs created in late June still showed some regeneration potential and regenerated a spike antler at the peripheral edge of the second cut AB after the completion of wound healing, as the AB was only at the mid-late stage of calcification [Color figure can be viewed at wileyonlinelibrary.com]

3.2.2 | Deer age and timing of velveting

Next, I explored some other variables that may affect AnP regenerative potential, namely age of the stag and timing of velveting in sika deer. Deer from 1 to 6 years of age were selected for the age study. For the timing of velveting, three stages of VA removal were assessed: spike in 1-year-old stags (VA removal at around 15–25 days of growth at about 6–8 cm length); two-branch in stags aged 2–3 years (VA removal at around 40–45 days of growth at about 35 cm length); and three-branch in stags aged 4–6 years (VA removal at around 60–70 days of growth at about 60 cm length). In the cases of 5–6 years old, the regrown antlers were removed to create a second cut AB to observe partial VA regeneration pattern in sika deer for comparison with red deer, as the regeneration from all the first cut AB was complete (earlier in the season) although smaller than the predecessors, thus it was not possible to discern any differences in the degree of regeneration (i.e., it exceeded partial regeneration). The results were summarized in Figure 5 and Table 1.

Overall, the observations suggest that the regeneration potential of the AB is more related to the timing of VA removal, than to the age of the deer.

3.3 | Regeneration potential of the AnP is limited and inferior to that of PP

The reason why the growth of all VA finishes at around the same time is that the antlers start to calcify at a similar time, which is controlled by photoperiod through induction of a sharp increase in the level of circulating androgen hormones. Consequently, the ultimate regenerative potential of the AnP can only be properly tested when the androgenic factor is removed. To assess this aspect, the approach of castration was taken when the first VA reached 6–8 cm as per the methods (Figure 6). Thereafter, removal of the VA was completed at the same level (about 2 cm above the junction with the pedicle) at predetermined intervals (see Section 2).

FIGURE 5 Antler regeneration status from the first cut antler bases (ABs) created after removal of the first set of regenerating antlers (6–8 cm) in sika deer. (a, b) Second set of antlers regenerated from the first cut ABs in the sika deer calves. (a) Antlers from the first cut ABs created in mid-December (deer—6 months old); (b) antlers from the first cut ABs created in early June (deer—12 months old). Note that antlers from the ABs created in December were much bigger (also with multibranches) than the ones in early June, due to the longer period of growth in the former. (c, d) Antlers (two branches) from the first cut ABs in 2-year-old deer; (c) antlers from the ABs created in mid-June; (d) antlers from the ABs created in mid-July. Note that antlers from the ABs created in mid-June were much bigger than the ones in mid-July. (e, f) Antlers from the first cut ABs in 3-year-old deer. (e) Antlers from the ABs created in mid-June; (f) antlers from the ABs created in mid-July. Note that antlers from the former were much bigger than the ones from the latter. (g, h) Antlers from the second cut ABs in 4-year-old deer. (g) Antlers from the ABs created in mid-June; (h) antlers from the ABs created in mid-July. Note that antlers from the former are much bigger than the ones from the latter. (i, j) Antlers from the second cut ABs in 5-year-old deer. (i) Antlers from the ABs created in late-June; (j) antlers from the ABs created in mid-July. Note that antlers from the former are much bigger than the ones the latter. (k, l) antlers from the second cut ABs in 6-year-old deer or older. (k) Antlers from the ABs created in mid-July; (l) antlers from the ABs created in late-July. Note that antlers (arrow) from the former were much bigger than the ones (arrow) from the latter, and the ABs in the latter case even did not complete the wound healing. All these comparisons suggest that timing of velveting is the main factor that influences the growth potential of the ABs [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Antler growth status at first cut (primary antler) and the second cut (regrown antler) in sika deer

	First set antler		Second set antler		Third set antler	
Age of stag	Shape	Timing of removal	Status of regeneration	Timing of removal[a]	Status of regeneration	Number of stags
6 months	Spike	December	Large multibranch	–	–	5
12 months	Spike	June	Small two-branch	–	–	3
2 years	Two-branch	Early June	Standard two-branch	–	–	3
	Two-branch	Late June	Small two-branch	–	–	5
3 years	Two-branch	Mid May	Standard two-branch	–	–	4
	Two-branch	Late June	Aborted two-branch	–	–	4
4 years	Three-branch	Mid May	Standard two-branch	–	–	3
	Three-branch	Early June	Aborted two-branch	–	–	1
5 years	Three-branch	Early May	Standard two-branch	Late June	Standard two-branch	3
	Three-branch	Late June	Standard two-branch	Mid July	Aborted one or absence	2
6 years and older	Three-branch	Late April	Standard two-branch	Mid July	Aborted two-branch	4
	Three-branch	Early May	Standard two-branch	Late July	Failed to grow	2

Abbreviation: AB, antler base.

[a]To create ABs that only have partial regeneration potential to make a comparison with those in red deer.

The regeneration status of antlers either from the AnP of the AB or from the PP of the pedicle stumps in castrated sika deer is summarized in Table 2 and Figure 6. Overall, the regenerative potential of the AnP was completely lost after the fourth cut, whereas that of the PP was essentially maintained at a similar level to that of the original regeneration potential (around 15 cm). Therefore, the ultimate regenerative potential of AnP was limited and inferior to the PP.

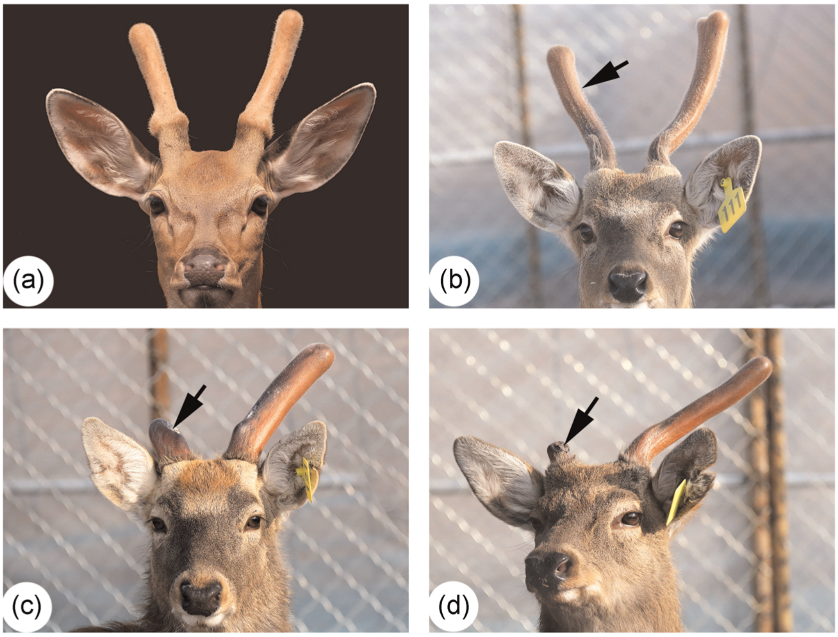

FIGURE 6 Antlers grown from the antler bases (ABs) created after repeated removal through the same cutting line in the castrated sika deer. (a) A sika deer calf with a pair of growing antlers (6–8 cm). (b) Antlers regenerated from the first cut ABs/pedicle stumps. Note that antlers (arrow) from the AB side (right) were significantly shorter than those from the pedicle stump side (left; around 85%). (c) Antlers regenerated from the second cut ABs/pedicle stumps. Note that antlers (arrow) from the AB side (right) were significantly shorter than those from the pedicle stump side (left; around 60%). (d) Antlers regenerated from the fourth cut ABs/pedicle stumps. Note that antlers (arrow) from the AB side (right) were significantly shorter than those from the pedicle stump side (left; around 8%) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Status of antlers regenerated from either the AnP or the PP in castrated sika deer

Cut number	Regeneration status (based on the length, cm) of the regrown antler			
	AnP (±*SD*)	PP (positive control)	Relative length of the AnP/PP antler	Figure
1	12.8 ± 1.8	15.0	0.85	6b
2	8.7 ± 0.8	14.5	0.60	–
3	4.6 ± 0.5	15.2	0.30	6c
4	1.2 ± 0.2	14.8	0.30	6d

Abbreviations: AnP, antlerogenic periosteum; PP, pedicle periosteum.

4 | DISCUSSION

Antlers are male secondary sexual characters and fully regenerate from their pedicles, the permanent bony protuberances from the deer skull (Goss, 1983). It has been demonstrated convincingly through tissue deletion that PP is the tissue that gives rise to regenerating antlers (Li, Mackintosh et al., 2007). Interestingly during the growth phase, antlers themselves can also repair damaged tissue or regenerate that which is lost or partially lost (Li & Man, 1990; Suttie & Fennessy, 1985). However, it appears that the question as to what tissue type in the antler remnants (ABs) initiates repair of damaged tissue/regeneration of that which is lost is not known.

Through close examination of the residual AB in red deer after removal of the second set of aborted VA in the same antler growth season, all the small regenerating antlers on the residual ABs were formed from the peripheral edge of the AB where AnP is located. Therefore, it is highly likely that AnP is the tissue that possesses the potential to repair or partially regenerate antler tissue. The results of the in vivo manipulation via castration and the repeated removal of the regrown VA in sika deer demonstrate that the ultimate regenerative potential of the AnP is somewhat limited, and inferior to that of the PP.

Here, one may argue whether the best approach has been taken to demonstrate the role of AnP in partial antler regeneration. Logically, the most convincing way to test whether AnP is the tissue responsible for this role would be to ascertain whether antler regeneration can still take place in the absence of AnP. However, practically this is not straightforward.

Deletion of the AnP from the residual AB at the time of removal of the first set of VA would be unlikely to provide a satisfactory approach for the following reasons: (1) by the time that an AnP-less AB regathers momentum (including cutaneous wound healing, reestablishment of the regeneration center in the absence of the AnP, and excluding the possible regeneration of the AnP) to launch antler regeneration (if it can do so), intensive calcification will have commenced, with the consequence that it is most unlikely that there would be sufficient time to initiate a regenerative antler tissue response; (2) should deletion of the AnP be performed sufficiently early in the season when there is sufficient time to develop a regenerative response, it is likely that the AnP would regenerate from the distal end of the PP (Li et al., 2007). Consequently, it would not be possible to discriminate as to whether successful antler regeneration from the residual AB is independent of AnP, or whether it was due to the regeneration of AnP from the distal end of the PP. Therefore, close examination of the cut surface of ABs after the second set VA removal can be considered as a valid, although imperfect, approach to address this question.

The reason why the timing of velveting is the most influential single factor affecting the size of regrowth antlers is because the timing of velveting determines the length of subsequent antler regrowth period: The earlier in the season that velvet removal is performed, the longer the regrowth period, and the larger the antler can grow. This is because antler growth finishes at virtually the same time, which is caused by the synchronized intensive antler calcification (controlled by photoperiod), irrespective of the timing when the initiation of antler growth starts in different-aged deer. Based on the records on Zuojia Experimental Deer Farm, velveting of two-branch antlers in 2-year olds was carried out in early June, and in 3-year olds in late May. In contrast, velveting of three-branch antlers in 4-year olds was completed around mid May, in 5-year olds in early May, and in 6-year olds or older in mid- to late April. The complication due to the calcification of the VA (and hence the residual AB) with the approach of the rutting season means that it is not feasible to test the ultimate regenerative potential of AnP.

The observation that 1-year-old male sika deer in the present study could grow large multibranched antlers (not species-specific shape) from the ABs created after the first set antler removal at early growth stage (6–7 cm) may not be surprising. This is because the timing of the growth of the first antler is more a function of body weight (nutrition) rather than photoperiod as for the subsequent regenerating antlers (Suttie & Kay, 1982). The age at which male sika deer calves initiate pedicle growth and that of their first antlers can vary considerably ranging from 6 to 12 months of age (Li et al., 1988). Thus, early initiation provides a longer growth period for the first antler, and hence a longer period for regeneration if the spike antler is removed at about 6 months of age.

It is known that calcification of the antler is caused by the sharp increase in circulating androgen hormones (Bubenik, 1982; Suttie et al., 1995). Therefore, to overcome this problem, in a previous study we used castration and repeated removal of regenerating antlers at the same level using two red deer. In that study, we found that the regenerative potential of the AB was lost gradually over five consecutive removal events, whereas the pedicle stumps created by cutting through the antler/pedicle junction maintained their antler regenerative potential (Li et al., 2004). In the present study, this experiment was repeated using four 1-year-old sika deer and achieved similar results. Overall, the regenerative potential of the ABs was diminished after four consecutive cuts, whereas the pedicle stumps maintained their regenerative potential over the experimental period. Therefore, the regeneration potential of the AnP was inferior to that of the PP.

Recently, Dong et al. (2020) compared messenger RNA and protein levels of the AnP and PP, and found that proline- and

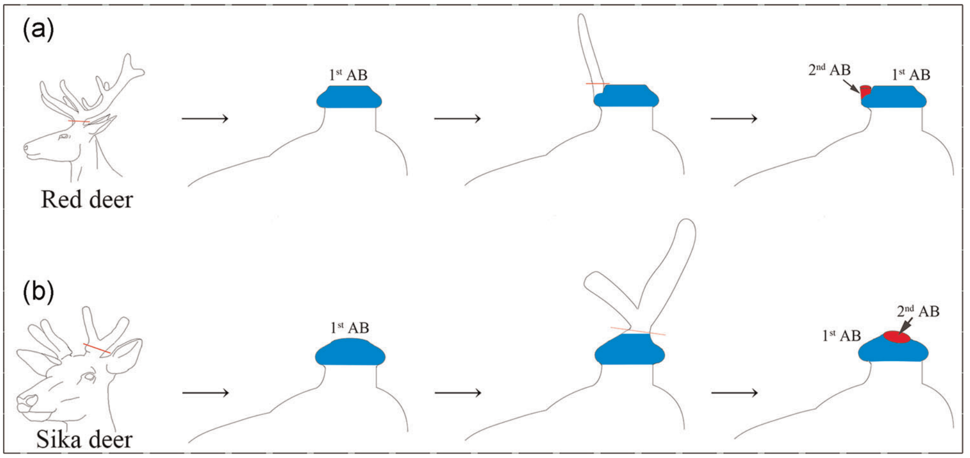

FIGURE 7 Schematic drawing of creation of the first (blue) and second (red) cut antler bases (ABs) and antler regeneration patterns from the first cut ABs. (a) Red deer. Note that a single and peripherally located antler was regenerated from the first cut AB, and the second cut AB was independent of the first cut AB. (b) Sika deer. Note that a small two-branch antler was regenerated from the first cut AB after total heal of the first cut AB wound, and the second cut AB was situated on top of the first cut AB [Color figure can be viewed at wileyonlinelibrary.com]

arginine-rich end leucine-rich repeat protein (PRELP) was more highly expressed in the PP than in the AnP (14-fold). Because PRELP is known to function in the anchoring of basement membranes (Bengtsson et al., 2002), they inferred that PRELP may be involved in the maintenance of PP dormancy by decreasing cell motility. However, the AnP used in their study was also in an inactive state at the time of sampling, and thus their suggestion must be considered inconclusive. Therefore, the phenomenon of the differential regenerative potential of the AnP and the PP must be addressed through mechanical traumatization. By doing so, we can expect that the potent mitogenic stimulating factors that provide the PP with full antler regenerative potential could be identified.

The observation that red deer and sika deer exhibited different antler regeneration patterns from the ABs is intriguing. Singular, discrete, and edge-situated spike antlers were regenerated in red deer, thus the second cut ABs were independent of the first cut ABs (Figure 7a), clearly indicating that AnP is the tissue that forms the localized growth center to form the spike antlers. In contrast, centrally positioned bifurcated antlers grew in sika deer; thus, the second cut ABs were overlying the first cut ABs (Figure 7b), not so clearly the tissue type that forms the second regenerated antlers. Nevertheless, the wound over the first cut ABs in sika deer was healed by the centripetally migrated tissue, which was initiated peripherally where the AnP locates (Figure 4b). It is not known, thus far, why the limited regenerative potential of the AnP during the permissive period was preferentially channeled to form singly discrete spike antlers in red deer, but spent to heal the entire AB wound and then to regenerate an antler if conditions (regenerative potential and permissive environment) were still permissive in sika deer. The pattern in red deer might be regarded as supporting a defensive/offensive weapon strategy, where the antler could possibly be utilized in the rutting season or whenever needed. In contrast, the pattern in sika deer might be more supportive of sealing the open wound, a strategy that would provide an effective means for reducing the risk of infection, as a consequence no infection has been encountered in sika deer. Interestingly, infection of the unsealed wound over the cut surface of an AB in red deer has not been reported either, so that red deer may have evolved an effective way to prevent that occurrence. Indeed, the exposed bone tissue on the cut surface in red deer was found to die back proximally to 2–4 mm (Figure 2n) and stopped when meeting the front line of intensive calcification process moving distally. Therefore, an intensive calcification process that occurred in the ABs in red deer may have stopped possible infection to go deeper.

Irrespective of the reason why AnP of the AB in red deer and sika deer were different in their pattern of regeneration, the fact that the AnP retains partial regeneration potential during evolution would guarantee that deer will have a reasonably intact set of hard antlers in the coming rutting season, as damaged antler tissue can be effectively repaired or partially lost antler be regenerated if these occur in the growth phase. With the presence of PP, a new set of antlers in the next season is guaranteed, and with the presence of AnP, the repair/regeneration of damaged/partially lost antler tissue in the same growth season is secured. Such twin reassurances may signify how evolutionarily important it is for deer to possess a reasonably intact set of antlers for their survival, social status, and ultimately for passing on their genes, although the possibility cannot be ruled out completely that the repaired antlers maybe just a nonfunctional by-product of the physiological process of antler regeneration.

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CONFLICT OF INTERESTS

The author declares that there is no conflict of interests.

DATA AVAILABILITY STATEMENT

All the data presented in the paper are available upon request.

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REFERENCES

Akhtara, R., Liu, Z., Wang, D., Ba, H., Shah, S., & Li, C. (2019). Identification of proteins that mediate the role of androgens in antler regeneration using label free proteomics in sika deer (*Cervus nippon*). *General and Comparative Endocrinology*, *283*, 113235. https://doi.org/10.1016/j.ygcen.2019.113235

Ba, H., Wang, D., & Li, C. (2016). MicroRNA profiling of antler stem cells in potentiated and dormant states and their potential roles in antler regeneration. *Molecular Genetics and Genomics*, *291*, 943–955. https://doi.org/10.1007/s00438-015-1158-8

Batchelder, H. (1999). Velvet antler: A literature review. *Health Supplement Retailer*, *5*, 40–51.

Bengtsson, E., Morgelin, M., Sasaki, T., Timpl, R., Heinegard, D., & Aspberg, A. (2002). The leucine-rich repeat protein PRELP binds perlecan and collagens and may function as a basement membrane anchor. *Journal of Biological Chemistry*, *277*(17), 15061–15068.

Bubenik, G. (1982). Endocrine regulation of the antler cycle. In R. D. Brown (Ed.), *Antler development in Cervidae* (pp. 73–107). Caesar Kleberg Wildlife Research Institute.

Chonco, L., Landete-Castillejos, T., Serrano-Heras, G., Serrano, M., Pérez-Barbería, F., González-Armesto, C., García, A., de Cabo, C., Lorenzo, J., Li, C. & Segura, T. (2021). Anti-tumour activity of deer growing antlers and its potential applications in the treatment of malignant gliomas. *Scientific Reports*, *11*, 42. https://doi.org/10.1038/s41598-020-79779-w

Dong, Z., Ba, H., Zhang, W., Coates, D., & Li, C. (2016). iTRAQ-based quantitative proteomic analysis of the potentiated and dormant antler stem cells. *International Journal of Molecular Sciences*, *17*, 1778. https://doi.org/10.3390/ijms17111778

Dong, Z., Haines, S., & Coates, D. (2020). Proteomic profiling of stem cell tissues during regeneration of deer antler: A model of mammalian organ regeneration. *Journal of Proteome Research*, *19*, 1760–1775. https://doi.org/10.1021/acs.jproteome.0c00026

Goss, R. (1983). Regeneration. *Deer antlers: Regeneration, function and evolution*. (172–192). New York: Academic Press.

Guo, Q., Wang, D., Liu, Z., & Li, C. (2015). Effects of p21 gene down-regulation through RNAi on antler stem cells in vitro. *PLOS One*, *10*(8), e0134268. https://doi.org/10.1371/journal.pone.0134268

Kong, Y., & But, P. (1985). Deer: The ultimate medicinal animal (antler and deer parts in medicine). In P. F. Fennessy & K. R. Drew (Eds.), *'Biology of deer production'. Bulletin 22* (pp. 311–324). Royal Society of New Zealand.

Landete-Castillejos, T., Kierdorf, H., Gomeze, S., Lunae, S., Garcíaa, A., Cappellia, J., Pérez-Serranoa, M., Pérez-Barberíaa, J., Gallegoa, L., & Kierdorf, U. (2019). Antlers—Evolution, development, structure, composition, and biomechanics of an outstanding type of bone. *Bone*, *128*, 115046. https://doi.org/10.1016/j.bone.2019.115046

Li C. (2020). Antler Stem Cells Sustain Regenerative Wound Healing in Deer and in Rats. *Journal of Regenerative Biology and Medicine*, *2*(1), 1–8. https://doi.org/10.37191/Mapsci-2582-385X-2(1)-016

Li, C., Harper, A., Puddick, J., Wang, W., & McMahon, C. (2012). Proteomes and signaling pathways of antler stem cells. *PLOS One*, *7*(1), e30026. https://doi.org/10.1371/journal.pone.0030026

Li, C., Mackintosh, C., Martin, S., & Clark, D. (2007). Identification of key tissue type for antler regeneration through pedicle periosteum deletion. *Cell and Tissue Research*, *328*, 65–75.

Li, C., & Man, T. (1990). Observation of antler growth following removal of its growing tip. *Biology and Native Products*, *6*, 29–30.

Li, C., & Suttie, J. (1994). Light microscopy studies of pedicle and early first antler development in red deer (Cervus elaphus). *The Anatomical Record*, *239*, 198–215.

Li, C., Suttie, J., & Clark, D. (2004). Morphological observation of antler regeneration in red deer (*Cervus elaphus*). *Journal of Morphology*, *262*, 731–740.

Li, C., Suttie, J., & Clark, D. (2005). Histological examination of antler regeneration in red deer (*Cervus elaphus*). *Anatomical Record*, *282A*(2), 163–174.

Li, C., Yang, F., Li, G., Gao, X., Xing, X., Wei, H., Deng, X., & Clark, D. (2007). Antler regeneration: A dependent process of stem tissue primed via interaction with its enveloping skin. *Journal of Experimental Zoology*, *307A*, 95–105.

Li, C., Zhao, S., & Wang, W. (1988). Morphology of Deer Antler. *Deer antlers*. (7–28). Beijing: Chinese Press of Agriculture Science and Technology.

Liu, Z., Zhao, H., Wang, D., McMahon, C., & Li, C. (2018). Differential effects of the PI3K/AKT pathway on antler stem cells for generation and regeneration of antlers in vitro. *Frontiers in Bioscience (Landmark Edition)*, *23*, 1848–1863.

Suttie, J., & Fennessy, P. (1985). Regrowth of amputated velvet antlers with and without innervation. *Journal of Experimental Zoology*, *234*, 359–366.

Suttie, J., Fennessy, P., Lapwood, K., & Corson, I. (1995). Role of steroids in antler growth of red deer stags. *Journal of Experimental Zoology*, *271*, 120–130.

Suttie, J., & Kay, R. (1982). The influence of nutrition and photoperiod on the growth of antlers of young red deer. In R. D. Brown (Ed.), *Antler development in Cervidae* (pp. 61–71). Caesar Kleberg Wildlife Research Institute.

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