

# Breeding of Androgen Receptor (AR) Gene and Its Relationship with Antler Production in Sika Deer

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Abstract: Androgen receptor (AR) is a type I steroid receptor in nuclear receptor superfamily. It is a ligand dependent transcription regulatory protein located in cells. Antler production is the most basic link in deer industry. As a production index, its production level directly affects production efficiency. In this study, the androgen receptor gene was used as a candidate gene to affect the antler capacity. The molecular marker was mainly used to find the molecular marker to affect the antler production, and the correlation analysis was carried out with the antler characteristics of sika deer, and the genetic polymorphism of sika deer was analyzed. The results showed that when the saw blade type was 1, the fresh antler weight of sika deer was 1092g, the estimated value was 1124g; when the saw blade type was 2, the fresh antler weight of sika deer was 1421g, the estimated value was 1532g; the estimated antler yield of sika deer was 115g higher than that of AA type, the difference was significant (P < 0.05), the BB genotype was 18G higher than AB genotype, the difference was not significant (P > 0.05), and the mutation of androgen receptor gene would affect it The biological function of androgen and its receptor combination, the mutation of androgen receptor gene will play a biological role by affecting the combination of androgen and its receptor, thus affecting the production of antler of sika deer.

#### 1. Introduction

The breeding of sika deer is to obtain high-yield antler and realize its effective economic value. It is the main product of artificial deer breeding. In deer animals, except the male and female of reindeer, all other deer animals are in the growth process of antler, which is closely related to the change of androgen concentration. Only when androgen is combined with receptor can it to a great extent, androgen can play an effective role in regulating the growth and development of sika deer. Ar will be controlled by receptor gene coding to a certain extent. When AR gene mutates to a

certain extent, receptor gene coding will have a certain impact on the structure and function of AR and its complex to a certain extent, and will lead to certain changes in its biological effects, thus greatly affecting the growth and development of sika deer antler.

Antler is a kind of immature antler that grows on the forehead of male deer. Under natural conditions, all male individuals grow antler, while female individuals do not. According to the relevant scientific research in recent years, the growth and development of antler has a certain growth law. The growth and development of antler is related to many factors, but more closely related to the androgen in the blood of deer. Antler growth and plasma stimulation the relationship between the change of element content has important guiding significance for production practice.

In order to study the effect of insulin-like factor-I candidate gene on antler production, D ómhnall used polymerase chain reaction (PCR) and single strand conformation polymorphism (PCR-SSCP) technology to identify and genotype the single nucleotide polymorphism (SNP) of IGF-I gene in Shuanyang sika deer. Although this method is practical, it still lacks certain stability [1]. Noguchi studied the influence of temperature, humidity and light on the production of sika deer antlers in Guizhou and Jilin Province. The influence of temperature on the production of sika deer antlers was greater than that of humidity and light. The abscission of Guizhou sika deer antler plate was 15 days earlier than that of Jilin sika deer antler plate. The optimum growth temperature of sika deer antlers was about 15 °C, and the optimum growth temperature of antlers was about 15 °C the temperature is 10 °C - 24 °C, the relative humidity is 33% - 70%, and the antlers grow normally. Although this method has certain practicability, it still lacks certain accuracy [2]. Zhang evaluated the in vitro antitumor activity and in vivo biosafety of sika deer antler protein (sdapr). MTT method and plate cloning method were used to detect the anti-proliferation ability of prostate cancer cell (PC-3M), flow cytometry and Western blot were used .In addition, the biological safety of sdapr was evaluated by acute oral toxicity test, bone marrow micronucleus test and sperm aberration test. Although this method has certain effectiveness, it still lacks certain practicability [3].

Deer antler is a derivative of deer's frontal bone and scalp, and its products have high medicinal value. In this study, androgen receptor gene is used as a candidate gene to affect the yield traits of deer antler. Molecular biological methods are mainly used to mark the molecules that affect the yield of deer antler and analyze the correlation between them and the production traits of deer. Comparative genomics is used to analyze the genetic polymorphism of deer, To find the site, we can help to screen and clarify the molecular markers related to the growth of sika deer and provide some theoretical basis and technical support for the growth regulation mechanism of antler.

#### 2. Relevant Contents of Antler of Sika Deer

#### 2.1. Biological Characteristics of Sika Deer

Cervus Nippon Temminck belongs to mammal and deer family in classification. It is an ancient species with a long history. At present, the sharp decrease of its distribution area in China is related to the strong uplift of the Qinghai Tibet Plateau and climate change and other natural factors, but it is mainly affected by human activities after the ice age. The disappearance of sika deer in Northeast China, North China, central China, Taiwan and other regions is closely related to over hunting. At the same time, the eastern part of China has a long history of development. However, long-term large-scale deforestation and land reclamation have caused destructive damage to the habitat of sika deer, which is the most fundamental reason for the extinction of sika deer in China [4].

In order to protect the endangered species of sika deer, all subspecies of Wild Sika Deer in China are listed as endangered species in the red book compiled by the international union for conservation of nature, which is also listed as endangered by the Chinese government and listed as a

key protected animal at the national level [5]. There are 13 subspecies of sika deer in East Asia, 6 of which are distributed in Chinese history, including Northeast subspecies, South China subspecies, Sichuan subspecies, Taiwan subspecies, Shanxi subspecies and Hebei subspecies.

## 2.2. Related Pharmacological Effects of Pilose Antler

#### (1) Improve sexual function

Pilose antler has the function of similar sex hormone. The alcohol extract of pilose antler can increase the number of testicular spermatogonia and spermatogenic cells. To a large extent, it reflects the increase of acetone content in human body, and when the estradiol in pilose antler reaches a certain level, it shows that estradiol is the main active component of pilose antler estrogen [6]. The low content of testosterone in pilose antler indicates that testosterone is not an effective component of pilose antler. Pilose antler extract can significantly increase the weight of testicles, prostate, seminal vesicles and other gonads in young male animals to a large extent. Pilose antler extract can increase the weight of testicles, epididymis, prepuce, prostate and seminal vesicles related to Yang deficiency and bone marrow injury.

# (2) Antioxidant and anti-aging effects

At the same time, antler can significantly inhibit the activity of MAO-B in brain and liver, increase the content of 5-HT and DA in brain, indicating that antler has certain anti-aging effect, and can significantly reduce and improve the activity of SOD in brain and liver [7].

#### (3) Antitumor effect

The protein peptide with molecular weight more than 10000 extracted from pilose antler can promote the differentiation of tumor cells and inhibit the proliferation of tumor cells in adrenal pheochromocytoma (PC-12) to a certain extent. Oral pilose antler protein extract can significantly prolong the survival time, which shows that pilose antler protein has anti-tumor effect. Pilose antler polysaccharides are beneficial to the treatment of tumors, because they can kill tumor cells by activating the immune system and have more obvious differentiation induction effect when the gas immune function is reduced [8].

## (4) Effect on cardiovascular system

In the case of reperfusion injury, antler can reduce the damage of myocardial cells to a certain extent, expand the coronary artery, increase the energy supply of ischemic myocardium, increase the activity of calcium pump and sodium pump on the cell membrane, and avoid the formation of microthrombus [9].

## (5) Promote growth and repair fractures

Pilose antler extract passes through sephmaeyls-200 HR gel can promote the proliferation of osteosarcoma cells to a certain extent. Velvet antler has the function of promoting growth and anti-fatigue. It can promote mitosis activity, epidermal cell mitosis, and anti-inflammatory and promoting growth of chondrocytes. It can stimulate the proliferation of chondrocytes and osteoblast like cells to a large extent, and can also differentiate bone and cartilage cells. Fracture and fracture repair have obvious effect, which greatly improves the quality of fracture healing.

#### (6) Other functions

To some extent, deer antagonistic peptide can significantly promote the division of brain neurons into neurons in vitro, which means that deer antler peptide can be used to treat neurodegenerative diseases, can also promote the proliferation of human bone marrow mesenchymal cells (mscb) in vitro, and prevent MSC from producing life in the process of continuous culture in vitro [10].

## 2.3. Quality Evaluation Grade of Sika Deer Antler

The antlers can be divided into saw antler and chopped antler according to the way of antler

selection. According to the shape of antler, antler can be divided into antler with blood and antler with blood discharge. According to the growth stage of antler, antler can be divided into initial antler, first stubble antler and regenerated antler. Different types of antler, residual stubble antler and regenerated antler have different quality standards the body standard is as follows:

## (1) Two bar sawdust

#### 1) First class

It has typical branches and trunks, all parts are in harmony, short and thick, full mouth, full texture, fine texture, not fragile, no passbook, no smell, no worm, the weight of dry product is not more than 18%, the color of blood antler is orange red, shiny, broken white, blood antler skin is dark brown, shiny, blood section is full and even, dark red, single weight is not small at 125g [11].

#### 2) Second class

The branch is normal, the proportion is appropriate, the main body is slightly lean, the whole body is slightly thin, the mouth is not twisted, the root is slightly covered by ribs and bone pox, the processing is continuous, the eyebrow branch is slightly broken and not exposed, no peculiar smell, no insect damage, the skin is orange red, shiny, the flour is white or pink, the skin with blood antler is black brown, the cross section with blood deficiency, dark purple, the moisture content of dry product is not more than 18%, and the weight of single branch is not low at 100g.

#### 3) Third class

The branch is normal, the proportion is not harmonious, the main stem is not horny, not broken, the mouth is twisted, the branch is not branched, the root is covered, the bone pox is obvious, there is no bug, no insect, the blood color is not bright, the cross section contains residual blood, the blood stained skin color is deep, the cross section contains less blood, the blood color is dark purple, the moisture content of the dry product is not more than 18%, and the weight of a single branch is not less than 75g.

#### 4) Fourth class

The sawdust of dry, tasteless and moth free ergot below the third grade belongs to the fourth grade.

## (2) Two bar chopped mushroom

The two bar antler with part of skull and scalp is called two bar antler. The requirements for its quality are: white skull, no meat, intact, stable position, beautiful appearance, thick and soft body, round root, full mouth, symmetrical eyebrow off the main branch, no skin damage, no hair loss, no bottom leakage, no odor, no moth eaten, water content no more than 18% of the dry product is the top product.

## (3) Three pronged sawdust

#### 1) First class

The branches are typical, the proportion of each part is harmonious, the antler body is thick, the stem is thick and round, the root is neat, the mouth is full, the fur is complete, the feel is soft, there is no broken skin, no passbook, no peculiar smell, no insects, the blood skin is orange red or brown yellow, shiny, the longitudinal section is pink or pink, the blood skin is black brown, shiny, the longitudinal section is full of blood, uniform and bright, the water content of the dry product is not more than 1 8%, the weight of single support shall not be less than 400g.

## 2) Second class

The proportion is appropriate, full, the root is covered, the eyebrow method after processing is not exposed, the eyebrow branch is continuous, the texture is slightly old, the root bone pox is few, the processing is not peculiar smell, no insects, the blood holding purple orange or red color, the vertical section is white or pink, there is a little residual blood, the blood purple color is black brown, the section has sufficient blood content, the distribution is relatively uniform, the color is dark purple, the water content is not more than 18%, The weight of single support shall not be less

than 300g.

#### 3) Third class

Basically, it is trigeminal, no angle, uncoordinated eyebrow trunk, thin and sharp mouth, no separate mouth, no eyebrow, papilla at the root, old texture, more bones, no skin exposure, no smell, no insects, unclean blood flow, dark skin color, less blood content of blood antler, darker blood color, water content no more than 18%, and general weight no less than 200g.

#### 4) Fourth class

Pure dry, odorless, pest free, pollution-free, triple sawdust, the quality is below the fourth grade.

(4) Three pronged chopped antler

#### 1) Premium

The skull is white, no flesh, no damage, stable position, thick and firm velvet, symmetrical left and right, typical branches, balanced structure, full mouth, ingot shape, large and thick, regular root, young texture, no black skin, no black root, no bottom leakage, no broken skin, no fracture, the water content of the dried product is not more than 18%, and the whole dry weight is not less than 1750g.

#### 2) First class products

The skull is white, no residual meat, no serious damage, normal velvet branches, the proportion is appropriate, the left and right are basically symmetrical, the mouth is thin and long, the root is thin and thin, the root is irregular, the edge is large, the bone is many, the texture is old, the processing has no leakage, the skin has no antler exposure, no peculiar smell, the dry water content should not exceed 18%, and the total weight should exceed 1000g [12].

#### 3) Second class

The skull is white, no wound, the skin is elongated, the proportion is not harmonious, the base is large or moist, so the bone formation degree is large and the acne is clear, it will not cause serious damage to the skin, will not produce peculiar smell, will not cause worms, the whole water content is not more than 18%, and the weight is more than 850g.

## (5) Tricholoma rudimentary

Initial antler, commonly known as peach antler and vertebra antler, refers to the first stubble antler growing in male deer. It is characterized by low yield, general dry weight of 150g-300g, individual weight of more than 500g, thick and long hair, thick fat as the top grade, thin, dry and ossified as the bottom grade.

# (6) Regenerated antler

Regenerated antler, also known as second stubble antler, is the second harvested antler of adult male deer in the same year. The characteristics of regenerated antler are large degree of ossification, long hair, irregular shape of most regenerated antlers, abnormal number of branches and branches, ribs or nipples covering the trunk and roots, soft body shape, good antler shape, thin, aging and deformed ones are inferior.

## 3. Structure and Function of Androgen and AR

## 3.1. Structure and Function of Androgen

Androgen is a steroid with 19 carbon atoms. It is synthesized by cholesterol in vivo, mainly including testosterone (T) synthesized by leydig cells and dihydrotestosterone (DHT), the active metabolite in target cells. Testosterone is the main androgen in vivo. In some species, steroids play a very important role in gonadal development. Testosterone and white egg white in blood circulation and sex hormone binding globulin (SHBG) are bound by androgen receptor in surrounding tissues, which are utilized by 5  $\alpha$  - reductase and transformed into 5  $\alpha$  - dihydrotestosterone with higher activity. This transformation is irreversible. Under the action of

aromatase, testosterone can also be transformed into  $17~\beta$  - estradiol, and androgen is mainly inactivated in liver. At present, testosterone has become an important candidate hormone to regulate the reproductive balance of male vertebrates.

The increase of testosterone content can promote the animal's courtship and sexual behavior, territorial invasion, secondary sexual sign and sperm production, and through its inhibitory properties, it can also reduce some expected behaviors, such as immune function and parental rearing. Male hormone is also a key factor affecting bone growth. In the adolescence of boys and girls, the level of testosterone also increases with the increase of bone mass It can also increase bone mineral density to a great extent. Androgen directly affects the function of osteoblasts. It cannot aromatize testosterone and androgen (dihydrotestosterone and fluoromethyltestosterone). It can increase the proliferation of osteoblast like cells in the initial cell culture of human osteoblasts. Meanwhile, androgen can Activate Src / SHC/ ERK signaling pathway can inhibit cell apoptosis, prolong the life of osteoblasts and promote bone formation. AR in prostate cancer has protein kinase pathway, which mediates extracellular signal regulated kinase mitogen activated ar transcription. M-RNA exists in bone cells, and AR protein can be expressed after androgen binds with its receptor, indicating that androgen directly acts on bone cells through its receptor.

#### 3.2. Structure and Function of AR

Androgen receptor (AR) is a type I sterol receptor in the nuclear receptor superfamily. It is a ligand dependent reverse transcription regulatory protein. It is located in the cell, classified as nr3c4, located in the stroma. The nerve nucleus also exists in the nervous system, kidney and adrenal gland, aorta, coronary artery, most in the atrium and ventricular muscle, and its gene code is located in x-staining In vivo, the molecular weight is 120000 Dalton, AR gene is located on xq11-12, AR contains 8 exons, encoding 919 amino acids, AR gene is mainly composed of 4 domains: exon 1 encodes N-terminal activation region with low preservation, there is a large difference between species, and the absence of this region does not affect the activity of LBD and DBD; DNA binding region consists of exon 2 and exon 3, encoding highly conserved, including two zinc finger structure consists of three  $\alpha$  helices; hinge region is encoded by the 5 'end of exon 4; ligand binding region is located at the C-end, highly conserved, and the coding of exon 4-8 mainly determines the specificity of receptor ligand binding.

According to whether ar participates in gene transcription, the mechanism of action is divided into genomic mechanism and non-genomic mechanism. Genomic mechanism depends on its nuclear translocation, and then combines with androgen response element to activate the transcription of target gene. Non genomic mechanism has been proved to have anti apoptosis effect in bone cells, bone cells, embryonic fibroblasts and HeLa cells, only in the presence of AR In addition, androgen can spread to target and non-target tissues. The intensity of androgen is closely related to the content of AR in cells, which is of great significance to the study of AR expression regulation. Androgen can be directly associated with AR, or regulate the expression of AR in the bones of male animals to regulate the bones in the developing parts of the bones of male animals Androgen, the metabolite of local enzymes, can also freely enter and exit the cell membrane nucleus, and combine with osteoblasts and osteoclasts to regulate the transcription of chromosomes; at the same time, androgen can also combine with related receptors on the surface of osteoblasts, regulate osteoblasts through a faster non chromosome mechanism, and AR activation of male animals can regulate the development of cancellous bone to a certain extent And cortical bone formation.

## 3.3. Regulation of Androgen and Its Receptor on Antler Production in Sika Deer

Antler is the second characteristic of the male deer. There is a certain relationship between antler growth and reproductive hormone cycle concentration. Relevant scientific research has found that the growth and ossification of the disk and antler are regulated by androgen. The antler tissue of the male deer can synthesize estradiol. Androgen is achieved by stimulating estrogen rather than directly stimulating bone formation. During the period of antler rapid growth, the level of estradiol and testosterone in the plasma decreased from the peak value, and then maintained at a lower stable level, indicating that the low level of testosterone and estradiol can promote the growth of antler to a large extent, while the high level of testosterone and estradiol can cause calcium and phosphorus deposition, promote the ossification of antler, and thus inhibit the growth of antler. By adjusting the concentrations and changes of testosterone and estradiol in the blood of sika deer, the growth of pilose antler can be promoted to a certain extent, and then the output of pilose antler can be increased. Testosterone is a major factor affecting the growth of pilose antler, but it does not play a role alone, but cooperates with estradiol. Estradiol may be more effective in promoting the formation of bone than in promoting the growth of pilose antler in the long run, it has a more prominent role. In the relevant production process, to control the growth of antler, consider the proportion of testosterone and estradiol, regulate the growth of antler through the combination of this hormone and the biological effect of antler, and the molecular biological changes of androgen receptor gene will directly affect the combination of androgen receptor and androgen.

## 4. Experiments and Discussion

## 4.1. Experiment

#### (1) Experimental sample

The Male Sika deer of 2-3 years old was used in the experiment. It was healthy, disease-free and in good condition.

- (2) Main solution and related buffer
- 1) Phosphate buffer solution (PBS): weigh 8g sodium chloride, 0.20g potassium chloride, 1.44g disodium hydrogen phosphate, 0.24g potassium dihydrogen phosphate, add water to dissolve, constant volume 1000ml, steam pressure 15min.
- 2) 1.0mol/l trisodium chloride: tris 30.29g, distilled water 200ml, after dissolution, adjust the pH value to the required point with concentrated hydrochloric acid, and finally make up 250ml with distilled water, sterilize at high temperature, and store at room temperature.
- 3) PMSF 1.74mg/ml (10mmol / L): PMSF 0.174g, isopropanol 100ml, dissolved in 1.5ml centrifuge tube, stored at 20  $^{\circ}$ C.
- 4) 10% sodium dodecyl sulfate: sds10g, add distilled water to 100ml, dissolve in a 50 °C water bath, store at room temperature, if there is precipitation in long-term storage, it can still be used after melting in the water bath.
- 5) 10% ammonium persulfate (AP): 0.1g ammonium persulfate, dissolve 1.0ml ultra pure water, and store at 4  $\,^{\circ}$ C for 1 week.
- 6) 1.5mol/l trisodium chloride (pH 8.8): tris 45.43g, dissolve 200ml of ultra pure water, adjust the pH value to 8.8 with concentrated hydrochloric acid, and finally adjust the volume to 250ml with ultra pure water, sterilize and store at room temperature and high temperature.
  - (3) DNA extraction and detection

Genomic DNA of sika deer was extracted by SDS method, dissolved in TE and stored at -20 C. the agarose gel electrophoresis and ultraviolet spectrophotometry were used to detect the purity and

concentration of the sika deer and dilute it to 30ng/ L.

# (4) Primer design

According to the relevant records of antler production, the DNA templates of two sika deer with high yield (saw blade yield > 500g) and two sika deer with low yield (saw blade yield < 250g) were selected for PCR amplification. After amplification, 1466bp target bands were sequenced and recovered for cloning. Then the homology of the four sequencing results was compared. A mutation site was found on the second intron and the third exon, respectively design primer.

## (5) PCR amplification

Reaction composition: 25  $\mu$  L system, including: 16.3  $\mu$  l sterile deionized water, 2.0  $\mu$  l30ng /  $\mu$  l DNA template, 10  $\times$  buffer 2.5  $\mu$  L, 2.5mmol/l dNTP 2.0  $\mu$  L, 10pmol /  $\mu$  L, upstream and downstream primers 1  $\mu$  L, 0.5u/ $\mu$  ltaq DNA, polymerase 0.2  $\mu$  L.

The PCR reaction cycle parameters of primer Z1: 94  $^{\circ}$ C pre denaturation for 7 min, then 35 cycles: 94  $^{\circ}$ C denaturation for 30 s, 55  $^{\circ}$ C denaturation for 40 s, 72.0  $^{\circ}$ C extension for 30 s, 72.0  $^{\circ}$ C extension for 10 min. The PCR reaction conditions of primer Z2 are the same as those of primer Z2, but the renaturation temperature is 57  $^{\circ}$ C.

## (6) Gene frequency

The calculation formula of gene frequency is as follows:

$$p = (2n_{AA} + n_{AB})/[2(n_{AA} + n_{AB} + n_{BB})]$$
(1)

$$q = (2n_{BB} + n_{AB})/[2(n_{AA} + n_{AB} + n_{BB})]$$
(2)

Among them, the gene frequency of p allele A, q allele B, and nAA,nAB,nBB are the number of individuals of genotype AA, AB and BB in the population respectively.

## (7) Statistical model and analysis

According to the characteristics of the experimental population in this study, the following linear regression model was constructed: observation value of antler yield  $q = (2n_{BB} + n_{AB})/[2(n_{AA} + n_{AB} + n_{BB})]_{character} = population average + fixed effect of genotype + random effect.$ 

## 4.2. Analysis of Experimental Results

#### (1) Analysis of DNA concentration and purity

The results of DNA concentration and purity determination are shown in Table 1.

Sample ID	Abs 260nm	Abs 280nm	260nm 280nm	DNA μg/μL
LC110	0.0034	0.0020	2.0012	0.1627
LC197	0.0118	0.0114	1.0604	0.5818
LC251	0.0015	0.0013	1.2520	0.0537
LC303	0.0064	0.0074	0.8290	0.3008
LC313	0.0030	0.0036	0.6997	0.1341

Table 1. DNA concentration and purity determination results

As shown in Table 1, the template DNA concentration required for PCR amplification is 50-200ng/ 50-200ng/ L. According to the relevant concentration and the results of DNA template

electrophoresis, the amount of NDA template in the PCR reaction is adjusted. The initial DNA concentration is extracted from the blood yarn of the high yield group and the low yield group, and the results of purity determination are analyzed by agarose gel electrophoresis. All samples are concentrated in the polymer tenderness area. The condition of serious degradation shows that DNA quality can meet the requirements of downstream experiments.

## (2) Analysis of PCR amplification results

The results of PCR amplification are shown in Figure 1.

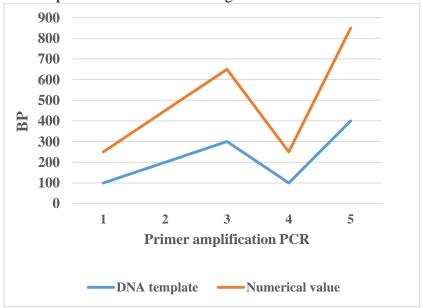


Figure 1. PCR results of primers

It can be seen from Figure 1 that PCR is amplified by designed primers with Sika Deer genomic DNA as template the PCR products were detected by 1% agarose gel. The results showed that the specific amplification effect was good. The fragment length was consistent with the expected fragment size. Compared with the sequences of 4 individuals, the DNAMAN (G to A) transition occurred in 490th place, and the primer was designed for this mutation site.

- (3) Analysis of allele frequency and AR gene mRNA expression by qPCR
- 1) Analysis of allele frequency

The frequency of 8 alleles in 40 antler deer populations is shown in Table 2.

Allele	Frequency	Allele	Frequency
A	0.6045	В	0.4252
A1	0.6705	D	0.2562
A2	0.6705	С	0.4630
A3	0.5763	C1	0.2747

*Table 2. frequency of 8 alleles in 40 antler deer populations* 

It can be seen from Table 2 that there are 8 alleles of A, A1, A2, A3, B, D, C and Cl in 40 male deer populations, among which the frequency of allele A is 0.6045, the frequency of allele A1 is 0.6705, the frequency of allele A2 is 0.6705, the frequency of allele A3 is 0.5763, the frequency of allele B is 0.4252, the frequency of allele D is 0.2562, and the frequency of allele C is 0.6705 the frequency of allele C1 was 0.2747.

2) Analysis of AR gene mRNA expression by qPCR The analysis of AR gene mRNA expression detected by qPCR is shown in Figure 2.

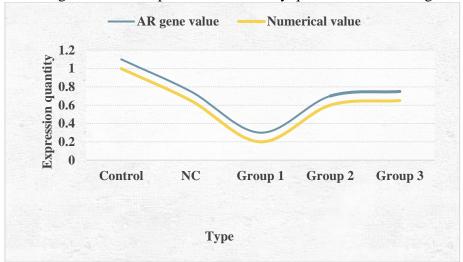


Figure 2. Detection of AR gene mRNA expression by qPCR

It can be seen from Figure 2 that after 48 hours of transfection, qPCR detects the expression of AR gene mRNA, extracts the total RNA of cells, and after reverse transcription of cDNA, uses 13actin as the internal control to detect the mRNA expression. Among them, the silencing efficiency of AR gene in the first group is 70%, the silencing rate of AR gene in the second group is 30%, and the silencing rate of AR gene in the third group is 28%. It can be seen that the relative expression of AR gene mRNA in the first group is relatively high expression has a significant impact, and the efficiency of silence is the highest.

- (3) Estimation of Antler Production in Sika deer and analysis of the relationship between AR gene and Antler Production
  - 1) Analysis on the estimated value of Antler Production in Sika Deer

See Figure 3 for the analysis of relevant estimated values of Antler Production Characteristics of sika deer.

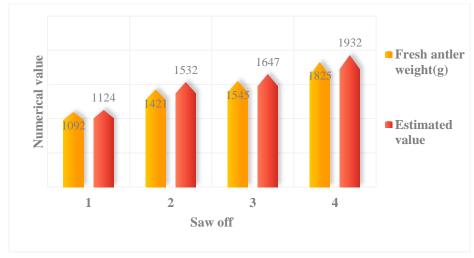


Figure 3. Estimated value of antler production in sika deer

It can be seen from Figure 3 that when the saw type is 1, the fresh antler weight of sika deer is 1092g, with an estimated value of 1124g; when the saw type is 2, the fresh antler weight of sika

deer is 1421g, with an estimated value of 1532g; when the saw type is 3, the fresh antler weight of sika deer is 1545g, with an estimated value of 1647g; when the saw type is 4, the fresh antler weight of sika deer is 1825g, with an estimated value of 1932g.

2) Analysis of the relationship between AR gene and the estimated value of Antler Production in Sika Deer

The analysis of the relationship between AR gene and estimated Antler Production of sika deer is shown in Figure 4.

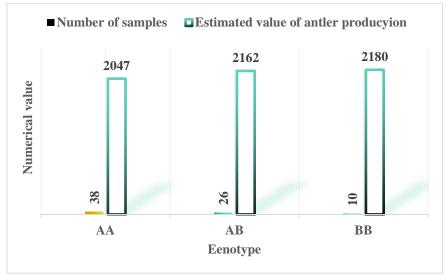


Figure 4. Analysis of the relationship between AR gene and estimated antler production of sika deer

It can be seen from Figure 4 that the estimated value of Antler Production in Sika deer is 115g more in genotype AB than in genotype AA, with significant difference (P < 0.05), 18G more in genotype BB than in genotype AB, with no significant difference (P > 0.05), 133g more in genotype AA, with significant difference (P < 0.05).

## 5. Conclusion

Antler is a kind of immature horn growing on the forehead of male deer, and it is also the main product obtained by artificial breeding of deer. In deer animals, except for male and female reindeer, all other deer animals are under natural conditions, belonging to male individuals who produce antler, while female individuals do not produce antler. Therefore, academic circles claim that antler is the second characteristic of male deer, pilose antler is a derivative between periosteum and scalp. It falls off regularly in a certain period of time and regenerates once a year. Pilose antler is one of the main products in artificial breeding of sika deer. The growth of pilose antler is regulated by endocrine hormone, especially the role of androgen and the growth of pilose antler are more closely related.

Antler is a derivative of deer bone and scalp, and its products have high medicinal value and economic value. In this study, androgen receptor gene is used as a candidate gene to affect antler yield traits of sika deer. Molecular biological methods are mainly used to study and analyze the molecular markers that affect antler yield and the correlation between them and deer production traits. This study shows that in 40 There are 8 alleles a, A1, A2, A3, B, D, C, CL in the population of male deer, among which the frequency of allele A is 0.6045, the frequency of allele A1 is 0.6705, the frequency of allele A2 is 0.6705, and the frequency of allele A3 is 0.5763; for the expression of mRNA detected by qPCR, the silencing efficiency of AR gene in the first group is 70%, and that of

AR gene in the second group is 30%, In the third group, the silencing efficiency of AR gene was 28%; when the sawage was 1, the fresh antler weight of sika deer was 1092g, the estimated value was 1124g; when the sawage was 2, the fresh antler weight of sika deer was 1421g, the estimated value was 1532g; when the sawage was 3, the fresh antler weight of sika deer was 1545g, the estimated value was 1647g; the estimated value of AB genotype was 115g more than AA genotype, the difference was significant (P = 0.05).

Breeding is one of the most important tasks in breeding work, antler breeding is also an important work in deer industry. Traditional antler breeding methods include pure breed and hybrid breeding, but the current antler breeding methods are mainly pure breed breeding, antler high-yield is one of the important economic characteristics of high-quality bucks. Therefore, the breeding of male deer mainly focuses on how to steadily improve antler yield. At present, transmission the traditional breeding method of sika deer has the disadvantages of slow speed, low efficiency and large breeding base. These disadvantages will have a negative impact on the further development of sika deer breeding to a certain extent, and then affect the production, sales and economic benefits of the entire sika deer industry.

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## **Data Availability**

Data sharing is not applicable to this article as no new data were created or analysed in this study.

## **Conflict of Interest**

The author states that this article has no conflict of interest.

#### References

- [1] Dómhnall J. Jennings, & Gammell, M. P. (2017). "Lateralization During Lateral Display and Its Relationship with Antler Size and Symmetry in Fallow Deer ( Dama Dama)", Laterality, 23(1),pp. 1-19.
- [2] Noguchi, J.(2016). "Overabundance of Sika Deer and Immunocontraception", Journal of Reproduction and Development, 63(1), pp.13-16. https://doi.org/10.1262/jrd.2016-132
- [3] Zhang, H. L., Yue, Z. P., Zhang, L., Yang, Z. Q., & Guo, B.. (2017). "Expression and Regulation of Angiopoietins and Their Receptor Tie-2 in Sika Deer Antler", Animal cells and systems the official publication of the Zoological Society of Korea, 21(3),pp. 1-8.
- [4] He, Y. Hasan, I. Keilig, L. Fischer, D. Ziegler, L. & Abboud, M. et al. (2018). "Numerical Investigation of Bone Remodelling around Immediately Loaded Dental Implants Using Sika Deer (Cervus Nippon) Antlers as Implant Bed", Computer Methods in Biomechanics & Biomedical Engineering, 21(1-4), pp.359-369.
- [5] Tang, Y. Jeon, B. T. Wang, Y. Choi, E. J. & Kim, E. K. (2016). "Effect of Sika Seer (Cervus Nippon) Velvet Antler on Prostate-Specific Antigen Levels and Migration of Lncap Human Prostate Cancer Cells", Asian Journal of Animal and Veterinary Advances, 11(11), pp.665-674. https://doi.org/10.3923/ajava.2016.665.674
- [6] Takeshita, K. Ikeda, T. Takahashi, H. Yoshida, T. & Kaji, K. (2016). "Comparison of Drive Counts and Mark-Resight as Methods of Population Size Estimation of Highly Dense Sika Deer

- (Cervus Nippon) Populations", PLoS ONE, 11(10), pp.e0164345. https://doi.org/10.1371/journal.pone.0164345
- [7] Terada, C. & Saitoh, T. (2018). "Phenotypic and Genetic Divergence Among Island Populations of Sika Deer (Cervus Nippon) in Southern Japan: a Test of The Local Adaptation Hypothesis", Population Ecology,60(3), pp.211-221. https://doi.org/10.1007/s10144-018-0607-8
- [8] Ohashi, H. Kominami, Y. Higa, M. Koide, D. Nakao, K. & Tsuyama, I. et al. (2016). "Land Abandonment and Changes in Snow Cover Period Accelerate Range Expansions of Sika Deer", Ecology & Evolution, 6(21), pp.7763-7775. https://doi.org/10.1002/ece3.2514
- [9] Cheng, Z. B. Bai, J. D. & Zhong, Z. Y. (2016). "Antler Cycle and Its Influencing Factors in Pére David's Deer (Elaphurus Davidianus)", Acta Ecologica Sinica, 36(1), pp.59-68.
- [10] Diaz, A. Walls, S. Whitmarsh, D. Smith, M. & Green, I. (2018). "Habitat Selection of Invasive Sika Deer Cervus Nippon Living in a Uk Lowland Heathland-Woodland-Grassland Mosaic: Implications for Habitat Conservation Management", Journal of Scientific Research and Reports, 17(3), pp.1-15. https://doi.org/10.9734/JSRR/2017/38579
- [11] Jan Dvo &, & Lenka Palyzov & (2016). "Analysis of the Development and Spatial Distribution of Sika Deer (Cervus Nippon) Populations on the Territory of the Czech Republic", Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 64(5), pp.1507-1515.
- [12] Kozlovskiy, E. E. Kisleiko, A. A. Fukuda, T. Kawai, K. & Ohdachi, S. D. (2020). "Records of Sika Deer Cervus Nippon from the Southern Kuril Islands in 1986–2019, with Special Reference to a Continuous Record of Living Deer on Kunashir Island since 2017", Mammal Study, 45(2),pp. 1. https://doi.org/10.3106/ms2020-0008