NEW RESEARCH 2021

CREST

Three alleles

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In crested chickens, the normally short feathers on the head are replaced by long feathers normally growing in the saddle region on the back of the chicken. Already in the third century crested chickens are described (Claudius Aelianus).
The Cr gene is autosomal incomplete (doesn't need to be homozygous) dominant with variable expression. In original Silkie as in Europe and hatchery Silkies in the US, the crest is small; in White crested Polish and bearded crested Padua, Houdan, Sultan, Houdan and others the crest is large. Davenport (1906) and Somes (1990) did extensive research.
A large crest goes mostly along with a vault (cerebral hernia) according to Fischer (1934), Frahm and Rehkämper (1998), Yoshimura (2012)...

1670-1690 Van Ruyven

Darwin wrote about Polish chickens (1868) and the vault. Vault is called 'cerebral hernia' in science lingo and wasseen in Houdan, Crèvecoeur and Sultan, all local derivatives of the crested chickens roaming the fields in Europe since... very long. Silkies have in origin no vault, the USA Silkies have it from Padua (bearded Polish) used to boost the crest and the beard came with it too. This is a rather recent happening somewhere in the last quarter of the 20th century.
Silkies in origin have a small crest compared to Polish and Houdan and they have no cerebral hernia. Krautwald (1910) saw that size of crest was directly proportional to the degree of the vault.
Crested chickens exist since the Roman times (Britain, Germany; search Gál et al 2010)) so must be older since they didn't suddenly pop up out of nothing.

In 2012 some of the crest was revealed by Wang et al, Cr was mapped in the (homeotic) HOXC8 cluster, abnormal expression happened in the skin on the head of embryos of crested breeds.
Hox genes in general (wild type) are expressed regional in the skin of the back of the chicken and they control the identity (shape) of the feathers.
HOXC8 can be found in the saddle region of chickens. Exactly these genes are found on the head of crested chickens. But that is not the whole story.
It was impossible to identify the mutation causing crest specifically and there is still no mutation found causing the cerebral hernia (Rizzi 2017, skull of Padovana).

Recent research identified the gene for crest

The gene for crest is located in an evolutionarily conserved region of HOXC10 or the mutation can found in a 197 bp duplication in the intron of HOXC10, for the nerds.
This mutation is found in ALL crested chickens (11 breeds, 68 chickens) tested in this research, which is all fancy breeds (Table 1 page 3) and not present in non-crested chickens.
The cluster of HOXC genes are on chromosome 33. The cluster of HOXC genes was investigated and it was found that actually three genes of five checked, are responsible for the variable crest expressions.
The scientists isolated mRNA from three tissues: skin of the head, skull and skin

TUIN Vee

of the back, from embryos in the stages E10, E13 and E17 and at hatch.
The expression of the HOXC genes (HOXC6, 8, 9, 10 and 11) differed at different stages of development but followed largely the same pattern.
A. they expressed in the skin of the back, there was not much different between crested and non-crested breeds;
B. they expressed highly in the skin on the head of Polish and moderately in Polish skull and the skin of the head of Silkie, but they didn't express in Silkie skull, Red Jungle Fowl skin on head.
C. Although in some tissues like head skin of Polish, although they all expressed in all four stages, the expression levels varied between the genes and during development.

In general the expression can be summarised in the following pattern:
1. expression of HOXC6, 8 and 9 is increasing from stage E10 to D1.
2. expression of HOXC10 and 11 first increase and then decrease from stage E10 to D1.

To find out whether HOXC genes expressed in epidermis or dermis, a piece of skin of the head from Polish and a white Leghorn was PCR'd at stage E13. (see for the embryonal developmental stages Genetics of Chicken Extremes)
First the Leghorn, the skin next to where the comb is (See next page, Fig. 1A) and feathers grow expressed HOXC6, 8, 9, 10 and 11 but very low level (Fig. 1B) in the feather bud epidermis, but not in the dermis or interbud epidermis (Fig. 1C).
In the Polish though, these genes expressed like crazy in the whole epidermis*), including feather bud and interbud epidermis (Fig. 1D) and also in the dermis. The Silkie though had less expression of HOXC where the Polish was massively upregulated, so much it affected the skull, compared to Silkies with smaller crest. In the previous research (Wang et al 212) they found that the expression of HOXC8 and not of HOXC12 or HOXC13 was found in the skin on the heads of Silkies, the two latter genes where therefore not included in the new study.

Looking at Table 1, mentioned are Cr1, Cr2 and Cr3, these are three alleles of the gene carrying a slightly different duplication. Cr2 carries an additional single base change in the 3rd copy of the duplication, while Cr3 has an expansion of the short TG dinucleotide repeat in the 5th copy. Yeah, yeah... Cr1 is the allele of the Asian breeds: Silkie and Beijing You.
European crested like Houdan and Polish have allele Cr2 and Cr3 or both.

Three alleles: Cr1 Asia, Cr2 & Cr3 Europe

**A scenario why there are three Cr alleles can be: Cr1 happened in Asian chickens and it accumulated additional changes in its sequence after the introduction to Europe which happened about 2000 years ago.
It is not possible to judge whether any of the two additional sequences to the alleles (Cr2 & Cr3) are functional important or possibly contribute to the development of a larger crest in the European crested breeds. Appenzeller has an upright slightly forwards, tapered mohawk. All four Appenzellers from three different populations have the 197 bp duplication at HOXC10 and are homozygous for Cr2/Cr2, the difference between an upright crest and a normal large crest must therefore be controlled by (an)other genetic factor(s). Crossing Appenzeller x Polish would give an answer what determines the shape of the Appenzeller crest.**

**) The interpretation is that the 197 bp duplication includes a HOXC enhancer element that becomes substantially stronger by the duplication. Wang et al. (2012) proposed that ectopic expression of HOXC8 in cranial skin changes body region identity and that the crest feathers resemble feathers generated from dorsal skin where the HOXC genes are expressed in wild-type chickens. The present study fully supports the interpretation of an altered body region identity but shows that the mechanism may involve ectopic expression of a whole cluster of HOXC genes.*

Streamers reflect the long feathers on the saddle

Short feathers reflect the feathers of the cushion

Breed	Phenotype	Genotype				
		Cr1/Cr1	*Cr2/Cr2*	*Cr2/Cr3*	*Cr3/Cr3*	cr^{WT}/cr^{WT}
Beijing You	Small crest	1	0	0	0	0
Silkie	Small crest	22	0	0	0	0
Crested Dutch	Large crest	0	1	0	0	0
Crevecoeur	Large crest	0	1	0	0	0
Dutch-Polish	Large crest	0	3	1	4	0
Houdan	Large crest	0	8	0	0	0
Polish	Large crest	0	11	2	1	0
Sultan	Large crest	0	5	0	2	0
Appenzeller	Upward crest	0	4	0	0	0
Schijndelaar	Upward crest	0	1	1	0	0
Other breeds	Non-crested	0	0	0	0	433

Table 1. Summary of Cr genotyping results across breeds with or without crest.
Data based on whole genome sequencing or diagnostic tests.

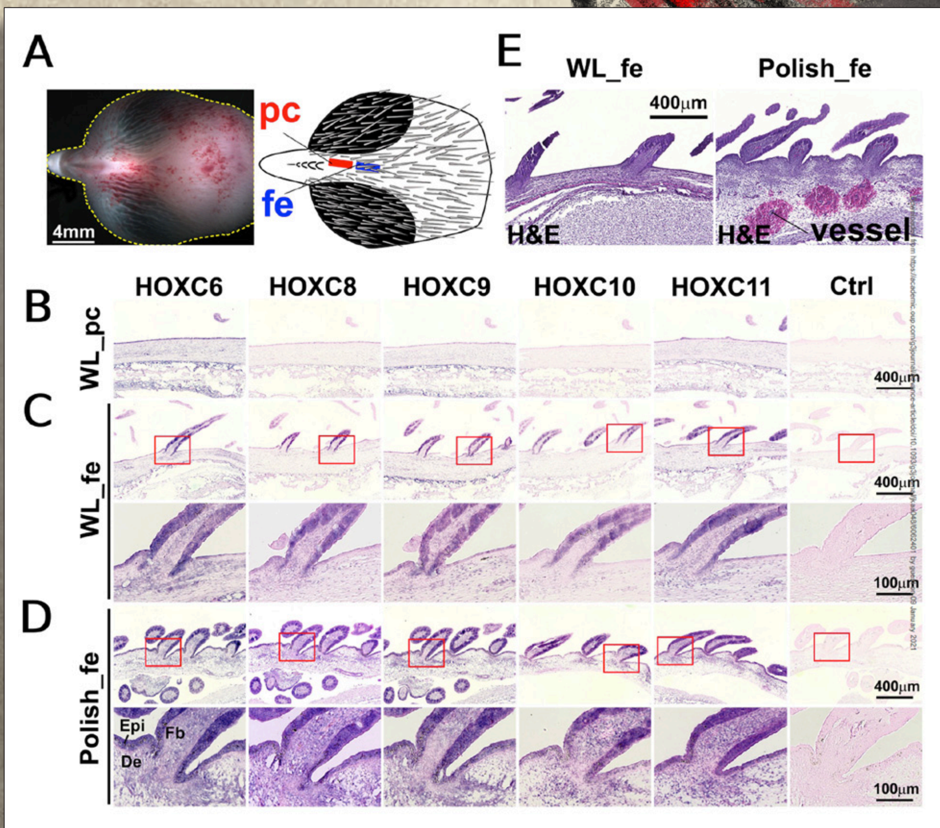

Fig 1. HOXC genes on the scalp skin of E13 chicken embryos
(A) E13 White Leghorn (WL) head showing cranial skin. White dots mark the contour of the head. The cranial skin is composed of the putative comb region (pc) and adjacent feather growing regions (fe) (indicated in red and blue, respectively). (B-D), Section ISH using HOXC6, HOXC8, HOXC9, HOXC10, HOXC11 probes, respectively; the most right column is the control with no added RNA probe.
(B) Sagittal sections of the putative comb region in WL chicken show no signal.
(C) Sagittal sections of the feather growing region in WL chicken show staining on bud epidermis but absence in dermis.
(D) Polish chicken. High level and diffuse Hox C staining are seen in both epidermis and dermis.
(E) H&E staining of the feather growing cranial skin region in WL (left) and in Polish chicken (right). Polish chicken skin shows longer feathers, dense upper dermis and abnormally enlarged blood vessels.
De: dermis, **Epi:** epidermis, **Fb:** feather bud, **fe:** feather-growing region, **pc:** putative comb region, **WL:** White Leghorn.

Also the **structure** of the skin on the head of Leghorn and Polish was investigated. It appeared the upper dermis of the Polish skin was thicker and had a higher dermal cell density compared to the Leghorn skin. In the lower dermis are abnormally enlarged blood vessels (Fig. 1E). These blood vessels in lower dermis and thickened upper dermis of the Polish, suggest that effects of HOXC gene expression expands deep into the dermis, and thus likely affects even deeper tissues, and may cause the cerebral hernia in chickens with a large crest. The 197 bp duplication in HOXC10 is required but not sufficient for causing a vault, because the vault is only found in the Cr2 and Cr3 alleles (large crest), but not in breeds with a small crest like Silkie (Cr1 allele).
It is therefore still not known whether a large crest and the associated vault are caused by mutation(s) affecting HOXC expression or other mutation(s) affecting the growth of the crest feathers, resulting in a deformed skull.

Previously (Yoshimura et al 2012) was suggested, after a cross breeding experiment, the vault was caused by a pleiotropic (by-) effect of the Cr mutation or a closely linked recessive mutation. In this research there was a clear difference in HOXC expression between Silkie and Polish. The Polish had HOXC expression not only in the skin but also in skull tissue.
In Polish embryos the peak of the expression of HOXC10 and 11 happens during the early stage E13. It is possible that the skull development, which is more critical in the early stages of development is more affected by the high expression of HOXC10 and 11. Later, when the down feathers start to grow, the other three genes (HOXC6, 8 and 9) may contribute to the big crest. This is only an idea, based on a mouse model (Yu et al. 2018).

For Cr2 allele, the mutation is this one might cause a large crest plus susceptibility to a vault because this mutation results in several actions affecting hair and skull development in mammals. The Cr3 allele's specific mutation is likely less functionally important.
Breeds having both alleles (Cr2 and Cr3) all have large crests and Cr1 breeds of Asian origin, have a small crest, the breeds differ genetically a lot more than only crest type. Therefore the alleles only might not provide evidence for a possible causal relationship between crest and vault. An experiment crossing breeds with Cr1 and Cr2 or Cr3 might reveal why the last two and not Cr1 have a predisposition for a vault. All in all, the HOXC genes are responsible for unusual shaped and sized feathers in birds (peacock, birds of paradise, hoopoe and chicken.

The crest phenotype in domestic chicken is caused by a 197 bp duplication in the intron of HOXC10, Leif Andersson, Uppsala University 2021