On silkied, the simple molecular story

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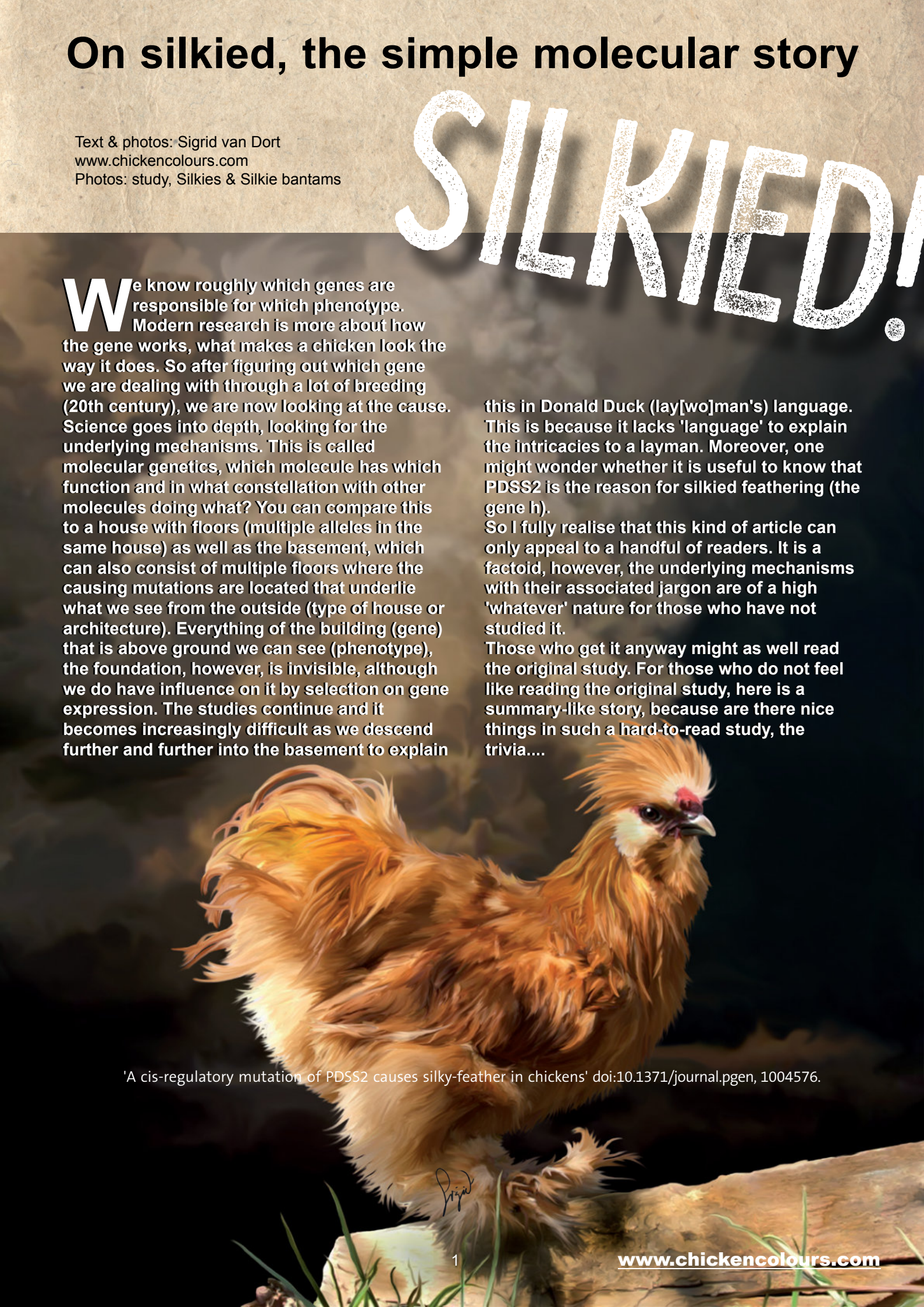
Photos: study, Silkies & Silkie bantams

SILKIED!

We know roughly which genes are responsible for which phenotype. Modern research is more about how the gene works, what makes a chicken look the way it does. So after figuring out which gene we are dealing with through a lot of breeding (20th century), we are now looking at the cause. Science goes into depth, looking for the underlying mechanisms. This is called molecular genetics, which molecule has which function and in what constellation with other molecules doing what? You can compare this to a house with floors (multiple alleles in the same house) as well as the basement, which can also consist of multiple floors where the causing mutations are located that underlie what we see from the outside (type of house or architecture). Everything of the building (gene) that is above ground we can see (phenotype), the foundation, however, is invisible, although we do have influence on it by selection on gene expression. The studies continue and it becomes increasingly difficult as we descend further and further into the basement to explain this in Donald Duck (lay[wo]man's) language. This is because it lacks 'language' to explain the intricacies to a layman. Moreover, one might wonder whether it is useful to know that PDSS2 is the reason for silkied feathering (the gene h).

So I fully realise that this kind of article can only appeal to a handful of readers. It is a factoid, however, the underlying mechanisms with their associated jargon are of a high 'whatever' nature for those who have not studied it.

Those who get it anyway might as well read the original study. For those who do not feel like reading the original study, here is a summary-like story, because are there nice things in such a hard-to-read study, the trivia....



'A cis-regulatory mutation of PDSS2 causes silky-feather in chickens' doi:10.1371/journal.pgen, 1004576.

Silkied feathers

The feather is one of the most complex skin coverings available because of its enormous diversity in shape, size, arrangement and pigmentation. This makes the feather a great model for evolutionary and developmental biology because variations and differences (differentiations) are possible at every step of development. It starts with the embryo and continues after hatching and the entire feather development has thus been a challenge to unravel since the early 19th (!) century. Feathers appear in clusters on the chicken, to be precise: there are about 20 strips (clusters). Feathers of different strips can differ greatly from each other and this is due to where that part of the chicken originated from (mesenchyme - embryonic connective tissue arising from the mesoderm).

Feathers and their shapes

The three types of feathering on a chicken are contour feathers, down and filoplumes. Contour feathers can be divided into wing feathers (primaries and secondaries), tail feathers and the contour feathers on the body. A contour feather consists of a coil (calamus, which is in the skin), quill (rachis, in the centre of the feather, feather vane (barbs) and a mini feather (afterfeather) that sits behind the feather. The feather vane consists of feather barbs and attached to it are barbs that are hooked together with small hooks. What feathers all have in common is that they are branching structures. Through selective breeding, many different types of feather shapes have been created. Variations in structure, quantity, length, mode of arrangement and number of feathers are seen in chickens. The underlying factors of this variation have been studied for years by geneticists, biologists and embryologists, however, only a few of these feather-shape-changing factors are understood.

Some well-known factors include: why is a naked neck a naked neck (reason: BMP12, see Genetics of chicken extremes); why is a crest made of saddle feathers (HOXC8 gene, see Articles - chickencolours.com); why is a frizzled feather a frizzled feather (KRT75, not yet translated).

Variations in the structure of contour feathers usually start at the end of the feather vane and they disrupt the interlocking of the barbs/hooks. Variations in feather structure in chickens include: silkied feathering, frizzled feathering, henny feathering, hard and soft feather structure. Silkied is a typical breed characteristic of the Silkie named after this feather structure. The first to mention silkied was Mr M. Polo in his travelogues through Asia (1298), he wrote: 'chickens with hair like cats

Silkie-Serama F1 (Serama come in silkied too) and smooth feathered Serama chick.

Filoplumes (hairs) and new growing contour feathers (in sheeth) and old and new down feathers during moult (Brahma).

Left: Silkied chicks and smooth-feathered chicks have the same chick down. Only when the juvenile feathers emerge the gene 'h' strikes: as a result, the juvenile feathers of silkied chicks do not have hooks on the barbs.

laying the best eggs'. Darwin wrote that the offspring of Silkie x normal feathered chicken was not silkied. The recessive inheritance of silkied was confirmed by Dunn (1927).

All chicks have the same down feathers when they are born. The first moult starts a few weeks after birth and down is replaced by the first juvenile feathers. In silkied chicks, it can then be clearly seen that they have a different feather structure than smooth-feathered (wildtype) chicks.

The closed feather flag in wildtype feathers has hooks attached to the barbs (fig. 1C and E), silkied feathers do not have these hooks (fig. 1D and G). The additional mini feather (in the same coil) in both wild-type and silkied feathers has no hooks on the barbs (fig. 1F and H). The flight feathers and some feathers on the legs may have hooks on the barbs in Silkies, but much less than in wildtype feathers (Fig. 1B).Obviously, Silkies cannot fly well with those frayed flight feathers and they also do poorly in extreme temperatures in high winds or rain because they do not have a closed plumage.

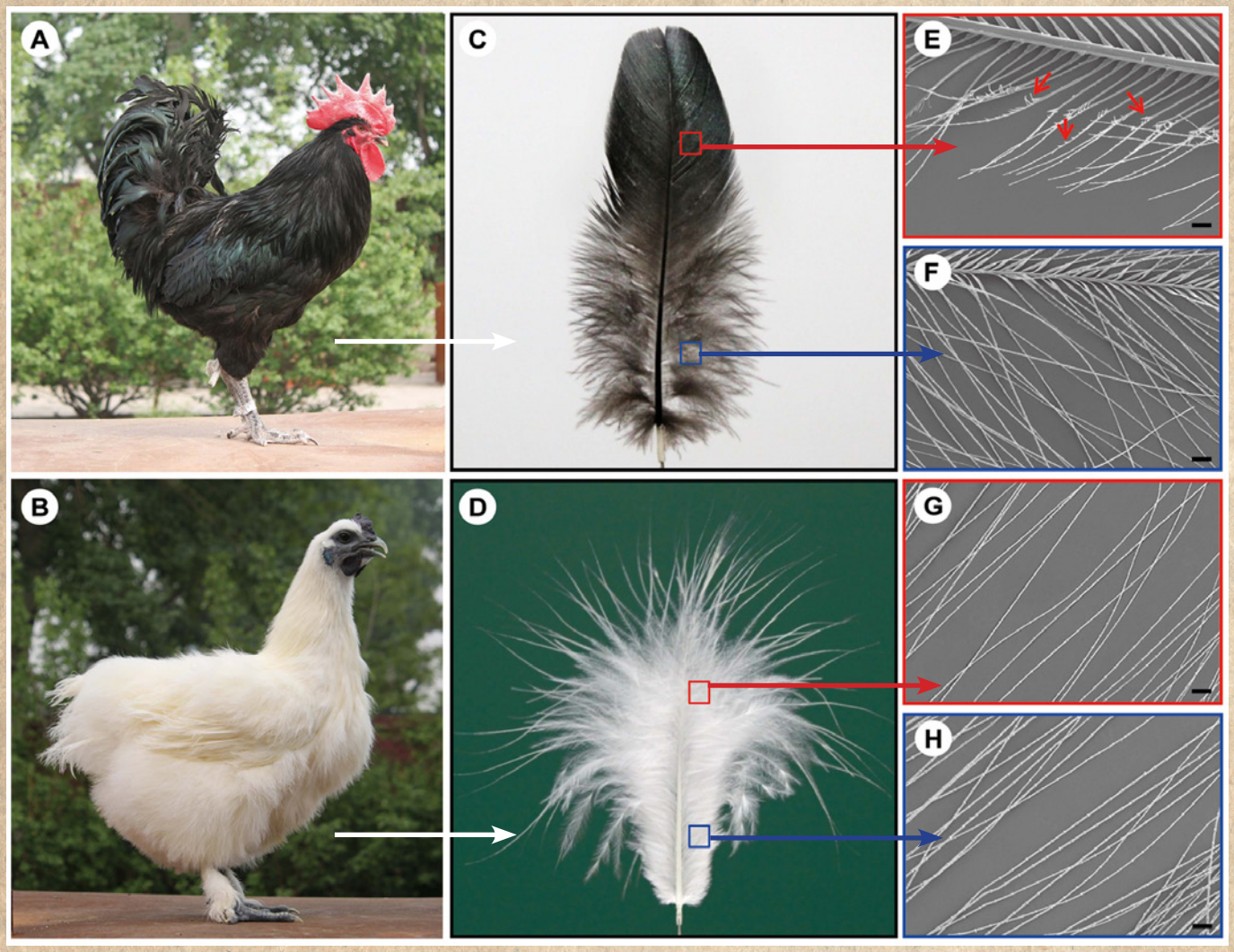
It is normal for flight and tail feathers in Silkies not to be frayed, these feathers do have hooks on the barbs. In the photo above of a lavender cuckoo Silkie cock, though, a lot of the tail feathers have hooks so they are hardly silkied feathers. This is a matter of gene expression.

There are a few feather structure variations in birds related to the hooks. One is frizzled feathers in the chicken where the curl goes backwards and mainly the feather quill is changed. The adult curly feather structure also shows other changes like a thickening of the barbs and also barbules, a change of the hooks and other abnormalities in the structure, yet the frizzled feather remains a feather as we know it. Frizzled inherits autosomal incomplete dominant.

A number of molecules have been investigated that are involved in the shape of the feather and the branching that takes place. Among others, the expression of sonic hedgehog SHH (Not the game! See book Genetics of Chicken Extremes for more on this), and the bone morphogenetic protein BMP (plays role in bone and cartilage and interacts with SHH), Noggin (a signalling molecule in embryo) etc play a role in this. SHH plays a mediating role in the interaction between the epithelium and mesenchyme (look up at wiki, you probably learned this at highschool) during feather development. On the other hand, BMPs actually inhibit feather development. It was found that an activator-'counterholder' (antagonist) model is going on between SHH and BMP2 signalling which may explain the development of feather barbs and also the shape of the feather and its branching structure. SHH and BMP2 and their mutual interaction thus determine how the feather will look like. The variation in the interaction between these two molecules together with an antagonistic interaction between Noggin and BMP4 are necessary for a feather to branch where SHH causes barbs to appear. Noggin takes care of the branching itself and the BMPs take care of the quill formation and stop the barbs from being made. The balance between BMPs and SHH ensures the number and size of feather barbs. The interaction of everything together determines the number and fate of the cells involved in feather formation.

Adult frizzled feathers have not only a backwards curved quills, they also have thickened barbs, therefore the hooks no longer interlock, causing the feather to fray. Left a smooth feather, on the right the same feather (wing) of a frizzled.

Reading this like this, you can see that there are many variations possible and the whole thing is a perfectly balanced exercise to grow a feather on a biochemical level. Nice to know and that's about it. While one now knows how the feather is created (sort of) and why it has a certain shape, one does not know how the formation of the hooks on the feather barbs comes about and what molecules are responsible for it and what mechanisms ensure the shape of the hooks.
The study discussed here deals with exactly this, and they have discovered that a gene is responsible for it.



Searching for the silkied gene

Silkied is on chromosome 3 in the chicken, that was already known. Now they went looking for which other genes were nearby. They found three in the first population of chickens, so they joined a second population and found an overlap which they combined. Looking for a causative yet unknown gene is done by making the fragment to be examined smaller and smaller. Thus, the spot on chromosome 3 could be made even smaller than before by excluding all kinds of genes by comparing heterozygous and homozygous animals. Data from two homozygous groups were also combined. There are various research methods, each of which has its advantages and disadvantages. These can be read in the research and are dead boring although they did ensure they came to know what they know now. With one research method, the researchers were not getting there, they had to do several because not everything could be made visible in each method.

A. Smooth feathered (wildtype +)
B. Silkied
C. Wildtype contour feather
D. Silkied contour spring
E. Barbs with hooks wildtype
F. Down of smooth feathered contour feather
G. Barbs without hooks silkied feather
H. Down of silkied contour feather.
E to H magnification of 100 um (human hair is 90 um).
From: the study.

Testing and downsizing the location further

The parents were a white Plymouth Rock with wildtype feathering and a Silkie who was homozygous for silkied (obviously). The white Plymouth Rock was not suspected of possibly being heterozygous for silkied as they had never been crossed to Silkie. From the parents to the F2, bits of gene were examined. In the F2, some birds were homozygous for one piece but not homozygous for another which should fall within the silkied piece (haplotype). The piece that matched the Silkie parent and gave a silkied in the F2 was a different piece from the piece that matched the Plymouth Rock parent and the F2 with smooth feathers. Thus, within the piece on chromosome 3, the spot where the causative gene for silkied was located could be further reduced.

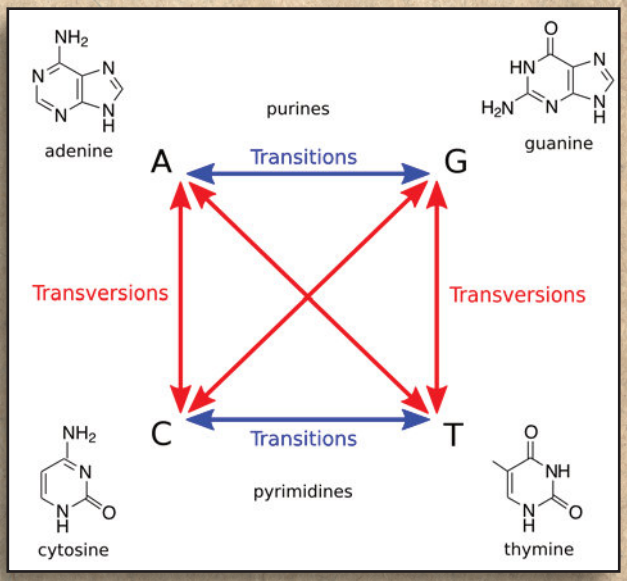
Then they started comparing that piece (which consists of 34 SNP markers,

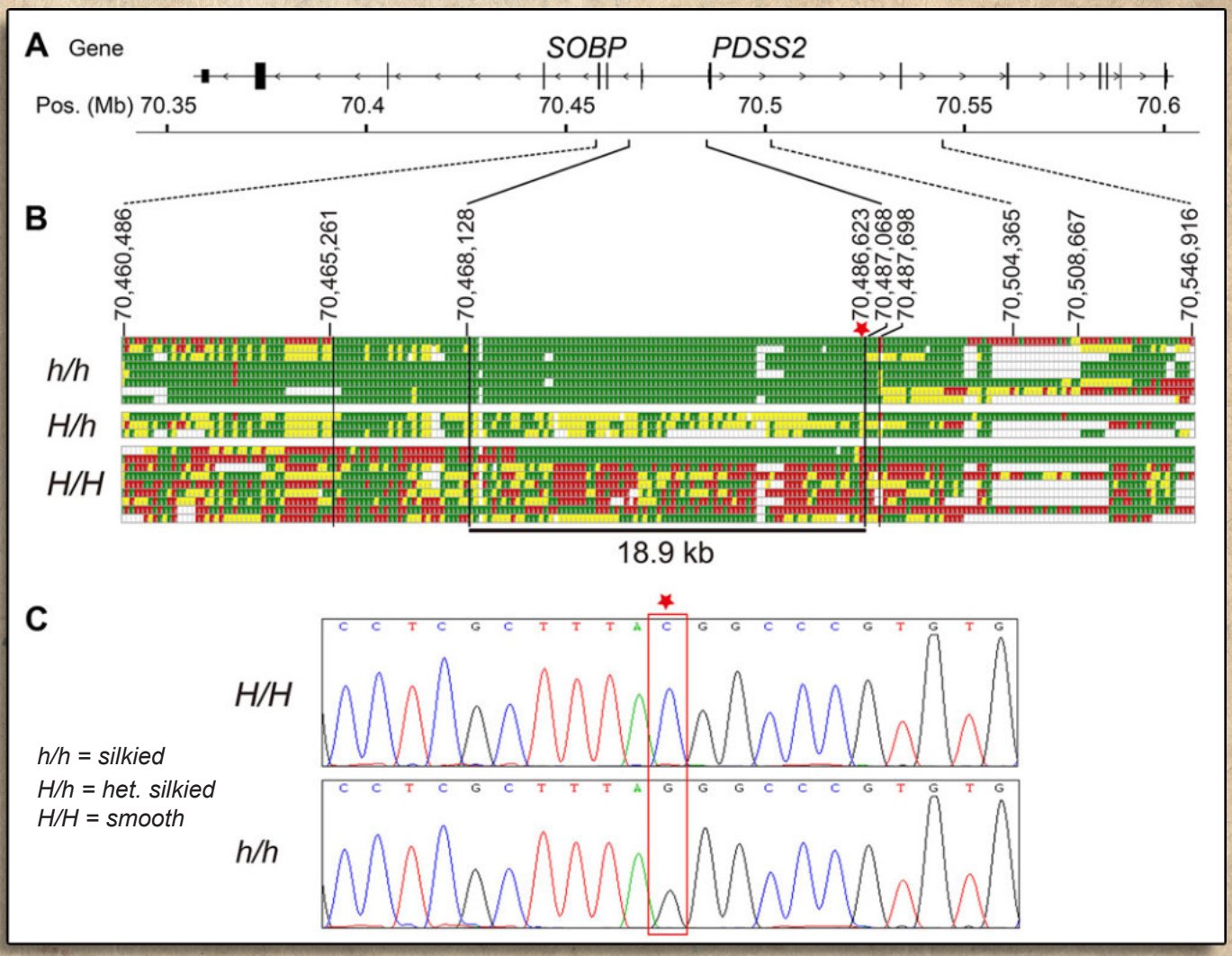
wiki: SNP genotyping for explanation) with 12 other breeds of which 76 samples were taken from three types of Silkies and 95 samples from nine other breeds with smooth feathers. This process was repeated a few more times when they found a piece in a White Leghorn and a White Plymouth Rock that was also on the silkied haplotype. The Silkies examined were of Chinese origin so 15 more American Silkies were also examined and compared.
After a lot of fuss, the silkied causing mutation could be located in the 18.9 kb* region. And this mutation involved a transversion, a way of mutating that has major consequences for the amino acids formed.

*) look up wiki: base pair > length measurements

At the locus found where silkied was located, the C and G were interchanged (see image wiki and fig 2 research).
All 718 hens from 33 populations were re-genotyped and all 337 silkieds had G/G, the purebred 341 gladveds had C/C and the 40 known heterozygous gladveds had G/C. These results led them to find the locus where the silkied causing gene (ss666793747) was located. It was decided to call this gene PDSS2(-103C-G). Well, we can easily remember that (not!), so quickly forget it.

Transversion and transition, two of the five mutation mechanisms. The others are: insertion, deletion and substitution. (woki)





B: the gene 'h', green and red, two alternative homozygous genotypes. Yellow: heterozygous genotype. White: missing genotype. The thin black vertical lines are the boundaries of the h/h birds. The thick black vertical lines are the boundaries where h shares the haplotype with het. genotypes. The minimum shared haplotype of silkied is 18.9 kb. Asterisk indicates where the mutation is located. C Electropherogram of DNA sequence where the (putative) mutation is located, see asterisk at C and G.From: the study.

During normal activity of PDSS2, hooks form on the beards, creating a smooth feather. In the above Silkie, PDSS2 seems to be unevenly active or inactive throughout. However, it could also be due to this hen's thyroid gland. Off topic: this hen clearly has a pea comb: hardly any skin formation and a dewlap.

The mutation PDSS2(-103C-G) is a mutation which causes the expression of PDSS2 to be altered causing feathers to become silkied because the hooks are not formed properly. There is a possibility that this mutation may affect the degree of translation and affect mRNA stability and possibly other genes at some point in skin/feather development. In normal feather formation in the embryo, there is a competition between two regulatory signal molecules (SHH) that are activators and on the other hand, antagonists (BMP) which also result in a certain phenotype of the feather because they affect the very earliest cells that later divide into specialised feather follicles (also consisting of different cells with their own specific tasks).

The gene PDSS2 is present in the chick down of both smooth-feathered and silkied chicks. Slowly the expression of PDSS2 increases simultaneously with feather development in the chick. So PDSS2 plays a role in feather development regardless of smooth- or silkied.

Different types of keratin - the simple story

The hooks on the feathers are formed in the final stage during feather development. The feather pulp is filled with other material and the barbs are formed by differentiated cell death (Wiki: cell death). The different parts of the feather are made of different types of keratin proteins whose presence or absence can be seen in the many different types of feathers, including mutated ones.

Hook formation occurs due to an increased number of feather barb cells that start growing in hooks after a change has taken place in the keratin so that the barbs interlock when the feather is ready.

There is a difference in feather making cells that make regular feathers or down feathers. So you also have these differences in cells when making a contour feather that consists of feather vane and down at the bottom of the feather. The cells that make the vane die and then keratin cells start producing down which consists of a different kind of keratin made by the

keratin cells (feather pulp). Experiments have been done with this and it is the substance BLSK1 (barbule specific keratin 1) that is made specifically in feather follicles that need to produce down. This also indicates that the expression of BLSK1 is also involved in the formation of hard feathers with barbs, barbules and hooks.The feather follicle is formed at the embryonic stage and the contour feathers are formed only after the first moult (chick down gone, hello youth or juvenile feathers). During the development of the contour feathers after the first moult (chick down gone), no hooks are formed on the feathers in silkieds. In the study, it has become clear that the expression of PDSS2 is greatly reduced at that time, causing the cells responsible for making the feather barbs to change the spatial organisation of the feather in the feather follicle, resulting in the absence of hooks in the silkied feather.

Feather growth goes through a number of successive steps according to a certain structure in which successively barbs, quills, barbules and hooks are formed leading to the feather vane. Special purpose feathers are formed by independent varying parts in this process. The earliest cells to make the barbs would mature earlier than the cells that make the quill for transporting nutrients/materials. To date in 2014 (maybe they are further along now in 2022, I haven't figured out), all studies on the formation of feathers have focused on the formation of the cells that produce the barbs. What happens to the keratin cells at the stage between barb > barbule, hooklet in not known and has yet to be investigated.

Unlike frizzled where mainly the quill and the formation of the barbs themselves were changed, this study on silkied looked for the first time at what happens to the hooks. Because the hooks are totally absent in the contour feathers of silkieds, it is difficult to determine what the exact role of the PDSS2 gene is when looking at the development of the entire (silkied) feather. This needs to be investigated.

It does happen that Silkies to a lesser extent show silkied. In the end, it all comes down to gene expression. And gene expression is thus also selecting for signalling molecules under the gene. The breeder does this without knowing the bottom line....

What PDSS2 does

The PDSS2 gene plays a role in the formation of coenzyme Q (a molecule), something that may sound familiar. This coenzyme Q is required for cell metabolism related to oxygen. In humans, an error in this gene causes Leigh syndrome (inability to absorb CoQ10) result: kidney disease. Mice with a mutation in PDSS2 and thus impaired CoQ metabolism developed thickened skin, hair loss and damage was done in kidneys, brain, liver and muscle tissue. Mutation in the gene PDSS2 and hence disruption of CoQ metabolism and thus a lack of oxygen in cells is responsible for kidney problems which in turn leads to numerous other problems in humans and mice.
Despite the fact that PDSS2 plays a major role in CoQ biosynthesis and abnormal expression of PDSS2 leads to kidney problems and also nervous system damage in humans and mice, nothing is known about how mutated PDSS2 affects the skin. Lower expression of PDSS2 in chicken skin leads to silkied feathering, that's all.

Silkied without genetic basis

Skin transplants of Silkies have shown that the formation of hooks on the feather barbs happens in the feather follicle. This indicates that silkied has nothing to do with what is going on inside the chicken in terms of substances. However, silkied feathers are also known to have no genetic background, so no gene ‘h'. Approximately similar-looking silkied feathers are known from a brown Leghorn (1927) whose thyroid gland had been removed. Later, silkied was also found in white Leghorns that had an under-functioning thyroid gland (thyroid hormone deficiency). Chickens who had this were small, bulky, had more body fat and a small dry comb, they were definitely not silkied (genetic) Leghorns. Silkied can also occur in smooth-feathered chickens if far too much thyroid hormone is produced. Silkies can be made smooth feathered locally by pulling out the feathers and giving them a subcutaneous injection of thyroid hormone (thyroxine) at that spot. Thyroid hormone along with other hormones is known to affect hair growth.

Some more useless knowledge

Thyroid hormone is known from experiments to play a role in feather development with respect to shape and also the rate of regeration of cells in the feather papilla. Thyroid hormone has been much studied with regard to its effect on oxygen metabolism where it altered the expression of genes related to this by the hormone altering the structure of cells involved (via gene expression, that is). Everything is connected as can be read.... Assuming that too little thyroid hormone in smooth-feathered birds leads to the loss of feather hooks (result: silkied) and that with the supplemental injection of the hormone Silkies produce smooth-feathered feathers with hooks, it may be that the formation of hooks depends on normal oxygen metabolism in cells in the presence of thyroid hormone. In Silkies' skin, the lesser expression of PDSS2 may lead to reduced CoQ10 synthesis, reducing oxygen metabolism in the feather follicle.

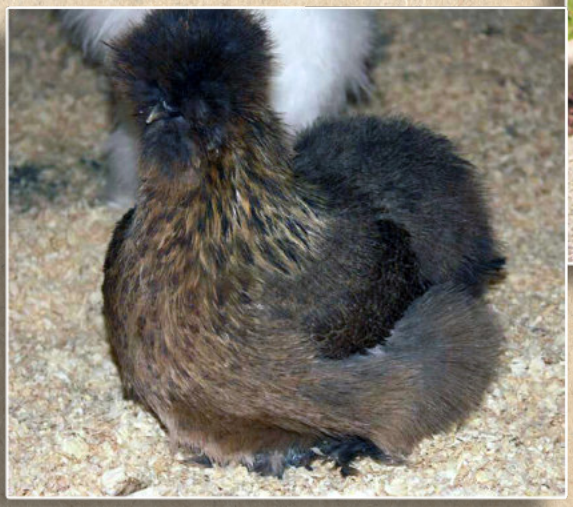
Chickens are dinosaurs, what you already knew

There is debate about the origin of feathers anyway and this has been going on for almost 150 years (in 2014). Many types of feather structures have been found in dinosaur fossils which does improve the concept of 'feather' and also the evolutionary origin of these 'highly branched structures' growing on the skin. Feathers found on dinosaurs and what they looked like and where they were in terms of location on the animal have provided insight into the hypothesis of whether chickens are dinosaurs.

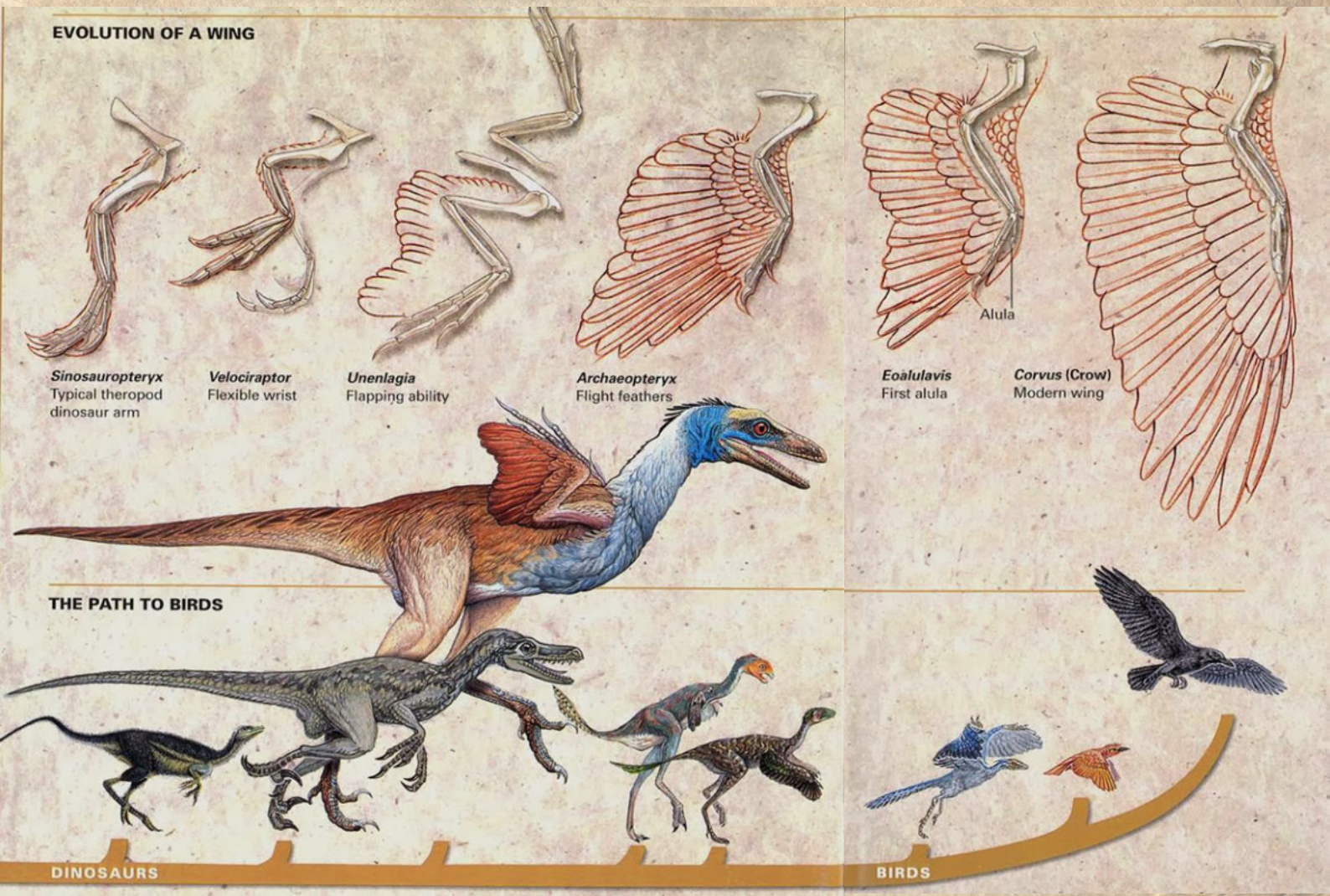
The tail and wing feathers found from the Protoarcheopteryx show that they were there before the two-legged dinosaurs took to the air. The later Anchiornis showed that there were different feather sizes and there may have been colour in them as well. These are all Things that give a better understanding about the evolution of the feather and its use. The research on silkied feathering has revealed another tip of the veil on feather structure and certainly how a substance can lead to loss of carrying capacity when flying/flapping. Follow-up studies on how exactly barbs, barbules and hooks are involved should bring further understanding about the developmental history of the feather.

The PDSS2(-103G-C) mutation alters the interaction of one or more transcription factors (from RNA > DNA) has been proposed in this study. Linking mutations that alter gene expression to a watertight 'cause and effect' situation from genotype to phenotype is always a challenge. However, quite a few of these mutations have already been found to be responsible for changing the appearance of chickens by acting on cell-type expression of genes. What has been found include: BCD02 which causes yellow skin (w), SOX5 which gives pea comb (P) plus side effects, SOX10 which gives the gene Db its action (not by me donaldduckerised), BMP12 which causes naked neck, EDN3 which causes black skin (fibromelanosis, Fm), MNR2 which gives rose comb (R) and SLCO1B3 which is responsible for blue eggshell colour.

This research along with quite a few previous studies over the past years show that there is still quite a bit for us genetic freaks to sniff out. As stated earlier, it is no longer about the above-ground gene, as here h (hookless) for silkied and how it inherits which we obviously do know - we are in the basement on which chicken colours are built, i.e. the causative mutations that lead to the expression we know as silkied. The story told above is the Donald Duck version of the research: A cis-regulatory mutation of PDSS2 causes Silkie-Feather in chickens (28 August 2014). Those who want to know the details can read this research on the website PloSOne.org.



Two hens from the same (American) breeding line. On a less silkied one, the multi-laced partridge pattern is much more visible. From: Silkies and Silkie bantams.



Stolen from internet in 2014.
Do you know the origin?