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A SECOND ROSE COMB ALLELE R2

THE JOYS WITHOUT THE PROBLEMS OF ROSE COMB R1

Reduced fertility rose comb

Regarding the reduced fertility of homozygous rose combed (R/R) cocks; the homozygous roosters (R/r+) pass on both rose comb and single comb to their offspring. The homozygous rose combed (R/R) hens do not suffer from reduced fertility.

R/R reduces sperm motility and longevity and the hens do not have this. Despite over 100 years of effort to breed rose combed Wyandottes, there are about 15% (per 100) single combs in each generation (Crawford, 1965).

It can be argued that the reduced fertility of homozygous rose combed Wyandottes is a pleiotropic effect, similar to the reduced amount of comb and lobe mass in the pea comb. In the recent study on rose comb and fertility, it was found that the mechanism in rose comb (reduced motility and sperm longevity) is similar to the mechanism in pea comb (reduced comb/lobe mass). So nothing can be done about it.

Cause of reduced fertility rose comb

The cause is a temporary (visible and measurable) but structural change in substances (due to a mutation in a gene that contributes to it) during comb formation in the embryo. In the developing embryo, a protein (MNR2) is activated, which in turn encodes a gene (CCDC108), and this gene causes a specific piece of DNA to be copied into RNA. So in the testes of homozygous rose combed cocks, this goes wrong in terms of sperm motility. This is a case of transcription (wiki: transcription biology). The case is that this reversal lasts for a short time and can be measured for a short time in the embryo, but the effects on the baby cockerel are lifelong (page 7 of the study).

While sequencing the DNA of a single combed Leghorn, a rose combed Chinese Silkie and a rose combed French Le Mans fowl (wiki: Poule le Mans, photo next page), the researchers noticed a sudden difference between the rose comb of the Silkie and the Le Mans. A difference could be seen that indicated the existence of a second rose comb allele. This second rose comb allele, called R2 (so the known rose comb allele is now called R1), is a recombination of a single wild-type comb and R1.

Bateson was the first to experiment with rose comb (1902) and he described rose comb as being incompletely dominant (i.e. you see it even when heterozygous).

Later, Bateson and Punnet discovered that the walnut comb was a hybrid of the rose comb and the pea comb.
The rose comb is an ancient mutation that occurred in both Europe and Asia, although there was a lot of time between them.

The rose comb has very different appearances in its surface and texture, angle of the comb thorn, etc.
This variation shows that there are many more genes involved in the appearance of the rose comb.

The rose comb is also known to make homozygous cocks less fertile than single combed cocks. This is also the reason for single combs. Many years have passed and there are some new findings. One of these is that there is a second rose comb allele that does not cause fertility problems and single combs in homozygous rose combed cocks.

The French breed of Le Mans with the R2/R2 rose comb, photo: www. picstopin.com.

R2 has two duplicated segments compared to R1, indicating an inversion. An inverted DNA fragment with a duplicated fragment, in simple terms. They then genotyped the entire Le Mans group on chromosome 7, which contains the rose comb, and of the cocks tested, four had R1, nine had R2 and three had r+.

They did some more tests and this too showed that there was a different fragment in R2, even when heterozygous (R1/r+ and R2/r+). Previous studies had already shown that the rose comb phenotype consists of an altered comb shape (compared to the r+ single comb), a dominant mode of inheritance and reduced fertility in homozygous cocks (R/R), which is inherited recessively.

The presence of two different rose comb alleles has demonstrated a causal relationship between what can be seen on chromosome 7 and these two different aspects of fertility in rose combed cocks. There are no visible (to the eye) differences between R1 and R2 (see photo above, page 6 of the study).

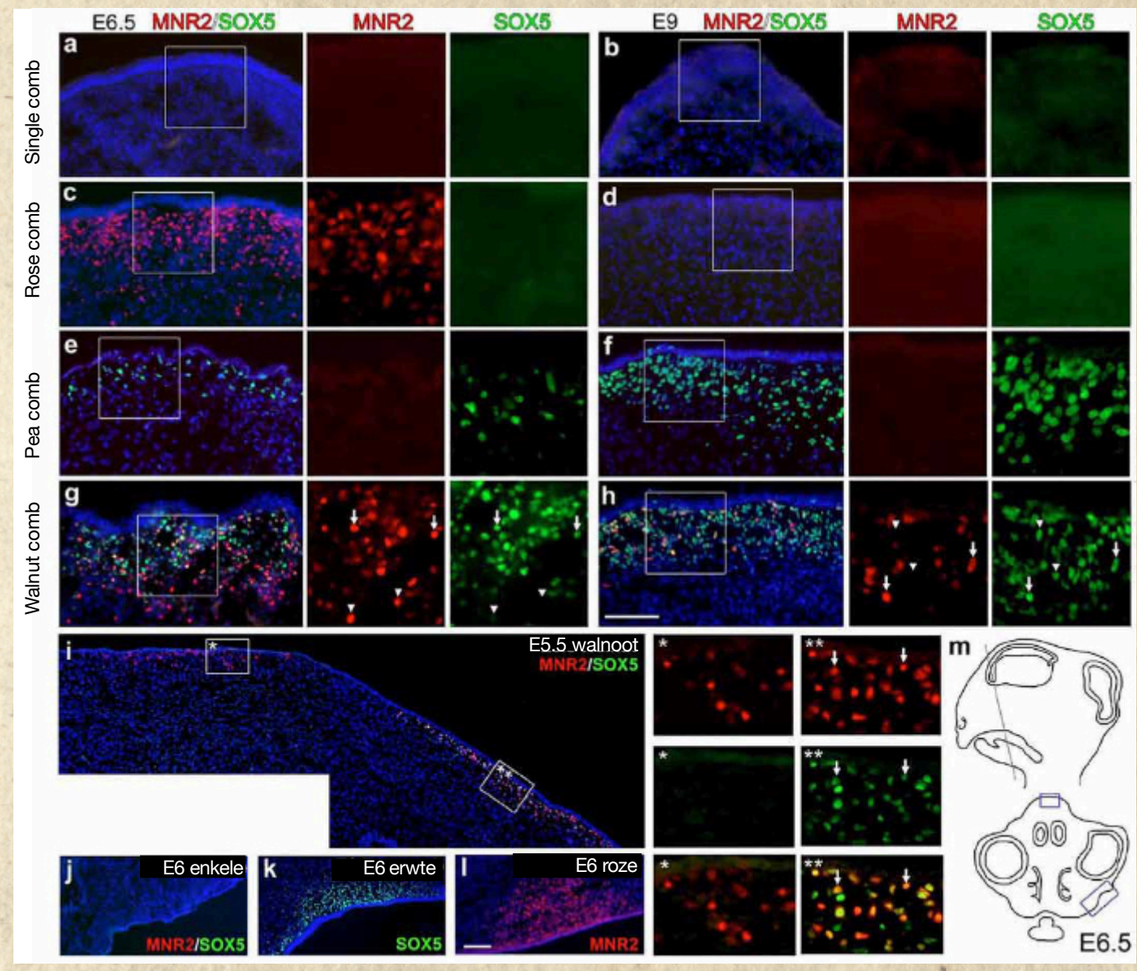
Similar to the pea comb study (see also my article 'What wattle'), it was the signalling substance SOX5 and now together with a partner signalling substance MNR2, both present in the single comb, that appeared to be responsible for changing the (embryonic - mesenchymal) comb tissue in the single comb to produce a rose comb (MNR2) or a pea comb SOX5. MNR2 does not have the same effect on wattle tissue as pea comb SOX5. The wattles of a rose comb are normal wild-type (as in the single comb, page 8 fig 5 of the study). The walnut comb (rose + pea) shows both substances.

What the researchers also wanted to know was whether the side effect of the R1 rose comb, reduced motility and longevity of R/R sperm, would also apply to the R2 rose comb, given the variable experience of this reduced fertility.

There were breeding groups made with R1/R1, R2/R2, R1/r+ and test crosses done with both rose comb types. The results showed that the reduced fertility of the R1/R1 cocks was consistent with previous data. However, there was no evidence of reduced fertility in the R2/R2 cocks. This proved that lazy sperm only occurred in R1/R1 cocks.

The researchers also knew where it was on the gene and what caused it (CCDC108 damaged therefore lazy sperm, which also happens in humans). The conclusion of this study is therefore quite surprising, because two mutations have occurred in the rose comb. The way the mutation occurred: recombination single x rose 'but different' is also interesting because this can basically happen anywhere, in any place/gene and in any animal (explained on page 9 of the study).

There is a huge variety of comb phenotypes in rose combs, with the comb surface also contributing, such as traditionally smooth (Wyandottes, Vogtländer) and rough (Redcap, Watermael) or classically rough (Hamburg). These standard combs are just some of the many possibilities. Additional variation near MNR2 or other genes will also contribute to the many shapes and sizes. On the next page you will find an overview of well-known and lesser-known breeds and which rose comb allele they have.



Above: images of comb tissue from embryos showing the compounds MNR2 (single and rose comb) and SOX5 (single and pea comb).

a. and b. single comb; c. and d. rose comb; e. and f. pea comb; g. and h. are walnut comb. The E means embryo and 5.5 means 5.5 days old, the same applies to 6 days old or 6.5 days old.

In i. the wattle region in single comb, the squares are enlarged below it in j. , k. and l. for single, pea and rose respectively as comparison. Magnification: 100 um.

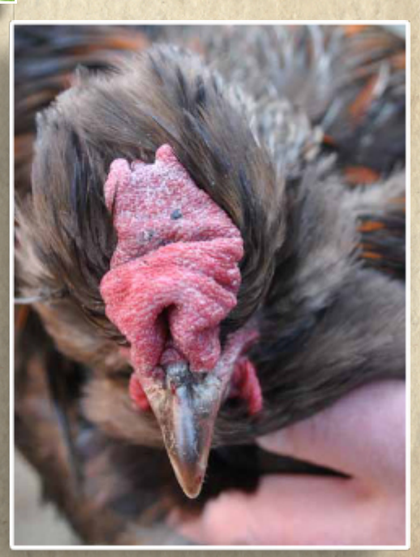
m. is schematically the head of the embryo and the squares indicate the regions on day 6.5 where a. to h. and the wattles j. to l. are seen.

		Genotype					
Breed	**Phenotype**	*rr*	*R1R1*	*R2R2*	*R1R2*	*R1r*	*R2r*
Aijiao Yellow	Single	10	-	-	-	-	-
Alsacienne	Rose	-	1	4	1	-	-
Ameraucana	Pea	1	-	-	-	-	-
Anka	Single	10	-	-	-	-	-
Araucana	Pea	4	-	-	-	-	-
Ayam Cemani	Single	4	-	-	-	-	-
	Pea	3	-	-	-	-	-
Baier	Single	10	-	-	-	-	-
Beijing Fatty	Single	10	-	-	-	-	-
Bian	Rose	-	-	-	-	7	-
	Single	18	-	-	-	-	-
Brahma	Pea	4	-	-	-	-	-
Chahua	Single	10	-	-	-	-	-
Charollaise	Rose	-	5	-	-	1	-
Chongren Ma	Single	10	-	-	-	-	-
Cobb	Single	10	-	-	-	-	-
Cochin	Single	3	-	-	-	-	-
Crossbred Layer	Single	4	-	-	-	-	-
	Pea	7	-	-	-	-	-
Dagu	Single	10	-	-	-	-	-
Dongxiang Green Eggshell	Single	10	-	-	-	-	-
Dorking	Single	2	-	-	-	-	-
Faverolle	Single	2	-	-	-	-	-
Geline de Touraine	Single	4	-	-	-	-	-
Gushi	Single	10	-	-	-	-	-
Guyuan	Rose	-	10	-	-	55	-
	Single	5	-	-	-	-	-
Hamburg	Rose	-	1	-	-	3	-
Henan Game	Walnut	-	7	-	-	61	-
	Pea	113	-	-	-	-	-
Huiyang Beard	Single	10	-	-	-	-	-
Icelandic	Rose	-	3	-	-	9	-
	Single	28	-	-	-	-	-
	Undetermined	-	2	-	-	9	-
INRA F_0s	Single	16	-	-	-	-	-
	Rose	-	-	-	-	2	-
INRA F_1s	Rose	-	-	-	-	16	-
	Single	15	-	-	-	-	-
INRA resource	Rose	-	-	6	3	28	5
	Single	72	-	-	-	-	-
	Abnormal	-	2*	-	-	37*	-
Jinhu Wu	Rose	-	33	-	-	111	-
	Single	36	-	-	-	-	-
Kuaida Wu	Rose	-	38	-	-	105	-
	Single	36	-	-	-	-	-
Langshan	Single	10	-	-	-	-	-
Le Mans	Rose	-	1	2	2	-	3
Luyuan	Single	10	-	-	-	-	-
New Hampshire	Single	1	-	-	-	-	-
Oravka	Rose	-	6	1	7	2	4
Orlov	Variable	7	-	1	3	4	5
Orpington	Single	1	-	-	-	-	-
Plymouth Rock	Single	3	-	-	-	-	-
Poltava Clay	Variable	1	-	7	4	4	4
Qingyuan Ma	Single	10	-	-	-	-	-
Red Junglefowl	Single	13	-	-	-	-	-
Sebright	Rose	-	4	-	-	-	-
Shiqiza	Single	10	-	-	-	-	-
Shouguang	Single	10	-	-	-	-	-
Silkie	Walnut	-	5	-	-	2	-
	Rose	-	136	-	-	183	-
	Single or Pea	42	-	-	-	-	-
Sussex	Single	2	-	-	-	-	-
Tibetan	Single	10	-	-	-	-	-
Wahui	Single	10	-	-	-	-	-
Wenchang	Single	10	-	-	-	-	-
Westfälischer Totleger	Rose	-	13	-	3	4	-
White Leghorn	Single	10	-	-	-	-	-
White Rock	Single	10	-	-	-	-	-
Wyandotte	Rose	-	2	-	-	2	-
Xianju	Single	10	-	-	-	-	-
Xiaoshan	Single	10	-	-	-	-	-
Youxi Ma	Single	10	-	-	-	-	-
Yurlov	Rose	-	-	12	-	-	8
Total		687	269	33	23	645	29

For the breeds marked with an *, it was initially assumed that they had a single comb.
However, genotyping showed that they had R1, further inspection of the comb showed that the apparent single comb was not really single because the shape deviated, see photos next page.

Paper: The Rose-comb mutation in chickens constitutes a structural rearrangement causing both altered comb morphology and defective sperm motility (6/2012) doi: 10.1371/ journal. pgen.1002775. Full download: www.plosgenetics.org.

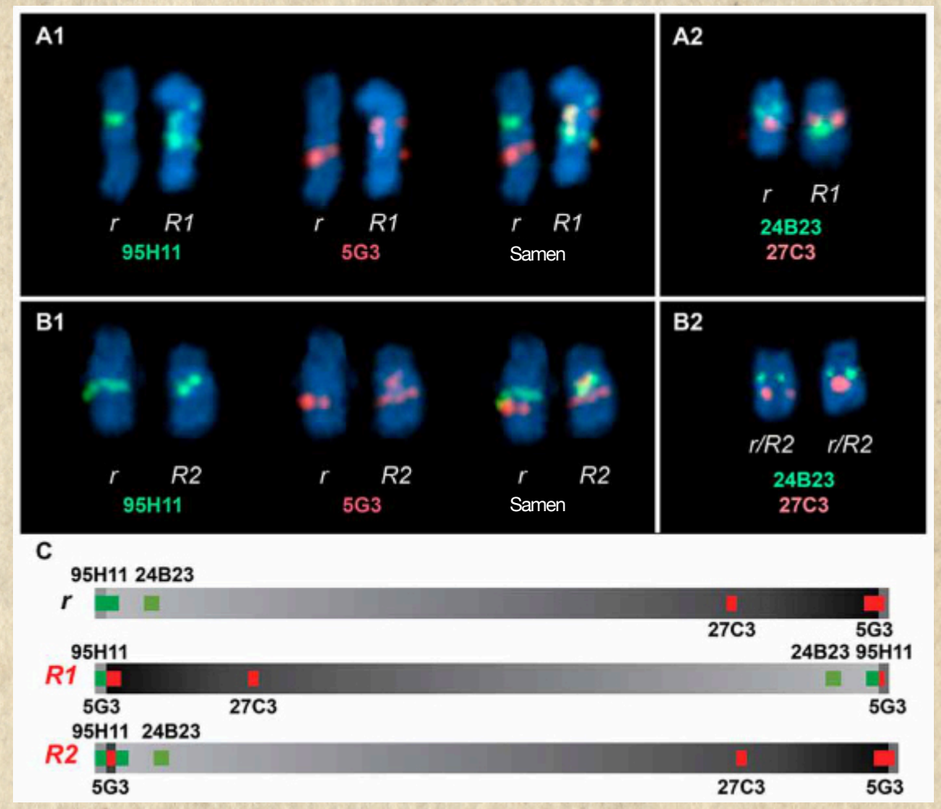
Several R1/r1+ combs of a cross of single x rose comb, this shows how many different appearances there are of the heterozygous rose comb , R is incompletely dominant.



Photos: A-W Freyja Imsland, X Michèle Tixier-Boicard, Y, Z, a, b David Gourichon.

Many phenotypic differences in rose combs in Icelandic chickens (A to W), Alsacienne (X) and INRA lab chickens (Y,Z, a, b). D, H, L, P and T are homozygous R1/R1 combs and X is a homozygous R2/R2 and all others are homozygous R1/r+ combs. All Icelandic chickens whose appearance was noted as single comb were indeed r+. From this it may be inferred that there is no duplex and pea comb in the birds and if so, it is rare. So only rose comb and crest determine the comb shapes of Icelandic chickens which can have many different shapes. All INRA lab chickens were assumed to have a single comb. On closer inspection, some were found to be positive for R1 see images Y, Z, a and b. That a split at the front of the single comb can be caused by R1 is new, usually duplex comb is assumed, especially if you know that duplex comb is present in a line because a Padua was once used. On the previous page some more R1/r+ phenotypes that can occur, some of which I have called out for duplex rose comb. However a heterozygous duplex crest splits at the back and not the front (I think?) see Crested of Lasne, my photo on the right.

For enthusiasts: chromosome 7 and the spots where single comb, R1 and R2 are located...



Two-colour test by fluorescence technique (FISH: fluorescent molecule is added to the piece of DNA examined on chromosome 7). The chromosome was cloned into a bacterium (BAC), which started dividing and at some point in that division these recordings were made. Image A1 is a heterozygous R1/r+ and shows two different placements for CH261-95H11 and for CH261-5G3 when comparing r+ to R1.

Figure A2 shows the inversion of BW27C3 and TAM32-24B23 between r+ and R1, indicating a very large inversion (reversal of a piece of DNA).

B1, TAM32-24B23 and BW27C3 (B2: both r+ and R2) together with CH261-5G3 show additional localisation at R2 (B1), this is consistent with the translocation duplication of a segment in the 23.88 MB region to the 16.50 MB region. A small spatial separation for CH261-95H11 is consistent with the insertion (insertion) of the translocation duplication, this can be seen at R2 (B1).

Shown here is a schematic representation of the types of types of mutations may be involved. R2 involves inversion (reversal), duplication (x2) and translocation (the chromosome breaks and a piece of it attaches itself to another chromosome), in short it is a DNA hotchpotch.