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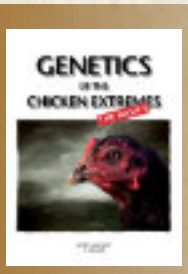
WHY Cp/Cp CREEPER EMBRYOS DIE IN THE EGG

The creeper gene is known for the very short legs of the, for example, Japanese Chabo. Breeders of breeds with the creeper gene (there are several breeds) know that a certain number of embryos die in the early stages of incubation.

Above and blelow examples of Japanese Chabo with the creeper gene. There is some variety in the leg length, they can be super short or hard to detect whether they are non-Cp or Cp/cp+. Non Cp legs can be very short too. This can be the explanation of much less dying embryos in creeper breeds. As you read in the article, there is no escape for Cp/Cp embryos, they will die. There is no other gene causing very short legs in standard ornamental chickens.



Basic info on the creeper gene you find in the book Genetics of the chicken Extremes.

The creeper gene (Cp) is an autosomal dominant gene (works the same in both sexes) and therefore visible when heterozygous (Cp/cp+).
It causes shortened legs, which is called chondrodystrophy, in heterozygous birds.

The homozygous creeper gene is lethal (Cp/Cp), the embryo dies. The creeper gene is located on chromosome 7 and is closely linked to MNR2, a protein responsible for rose comb.

The creeper gene causes this shortening of the legs by interfering with the **signalling proteine of bone growth**, this signalling pathway is called the Indian hedgehog IHH (not to be confused with Sonic the Hedgehog). Maybe you have heard about this before, that is possible, since polydactyly is messing with a Hedgehog too, the Sonic Hedgehog, you now know where the name of the game comes from.

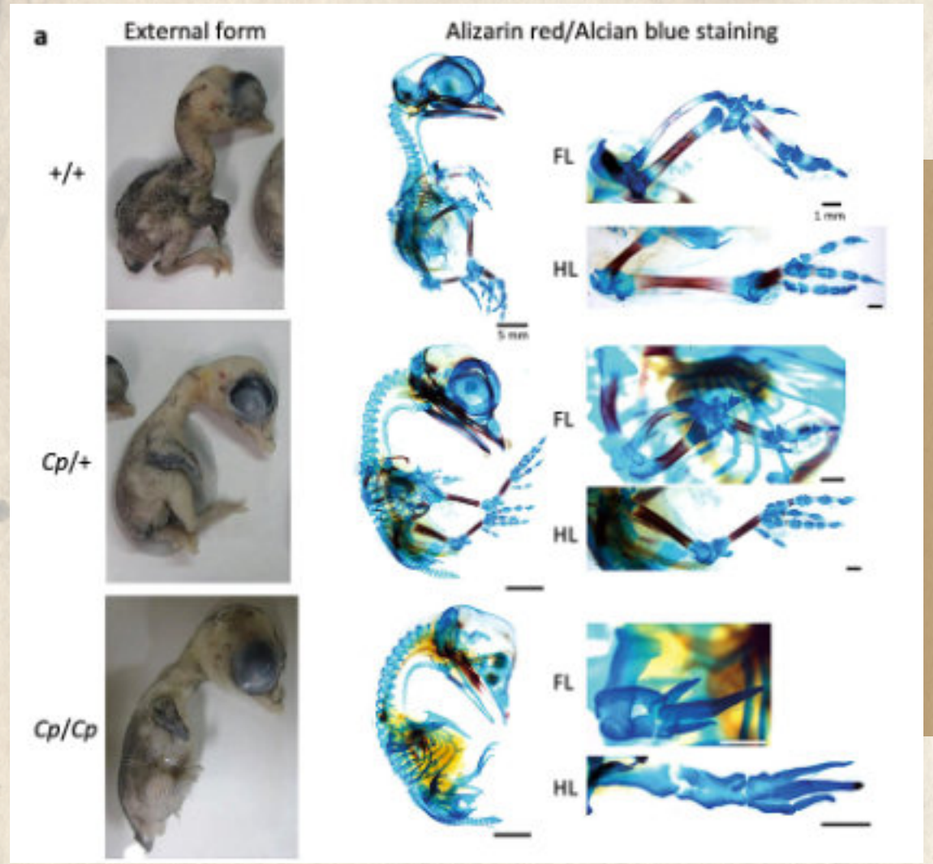


Figure 1a:
- **+/+ is cp+/cp+ is no-creeper**
- **Cp/+ is heterozygous creeper**
- **Cp/Cp is homozygous creeper**

Day 14 embryos from the Chabo and the Chabo/Fayoumi projects:
The Cp/cp+ embryo has shorter limbs than the +/+ embryo, the Cp/Cp embryo has phocomelia syndrom (in humans: caused by the drug Softenon end 1950s/early 60s).
The stained photos show the bones and cartilage.
FL is fore limb (wing) and HL is hind limb (leg).

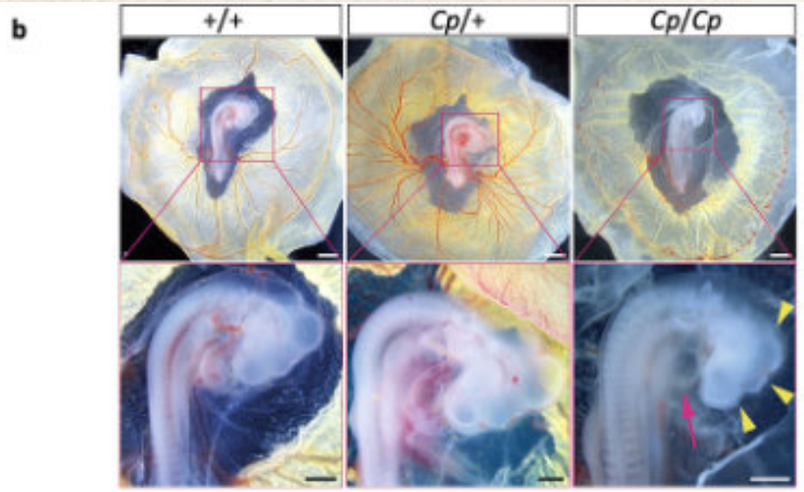


Figure 1b, above.
The embryos of the three phenotypes of the Chabo/Fayoumi group day 3. It can clearly be seen that the blood vessels of the yolk sac of the Cp/Cp embryo are underdeveloped. There is also malformation and slow growth of the brain (yellow arrows) and slow growth of the heart tube (red arrow). No blood cells circulate in the Cp/Cp embryo and the heart beats very slowly.

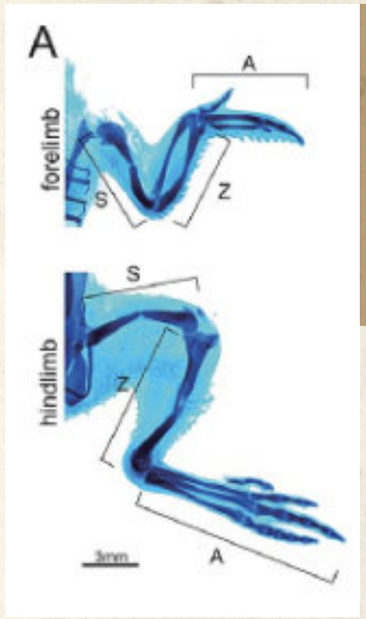
The creeper gene causes fewer signals to go to the Indian hedgehog to tell bone cells (chondrocytes) how to grow. Cp disrupts the signal to cartilage-forming cells, causing shortening of the bones in creeper heterozygotes. Homozygous Cp/Cp embryos completely lose the signal to bone cells to grow (IHH).

Cp/Cp causes damaged cells to die
The death of Cp/Cp embryos is caused by a so-called 'double-strand break' (DBS) in the DNA, caused by a by-product produced in the cell due to the homozygous creeper gene. Double-strand breaks are always fatal to the cell if they are not repaired.

The cells will always try to repair it, because cells are damaged all the time.
The damage can be caused by a lethal gene (Cp/Cp), but also by the cell's own (faulty) functioning. A double-strand break causes cell death. The cell can no longer divide and multiply. This is devastating for a growing embryo.

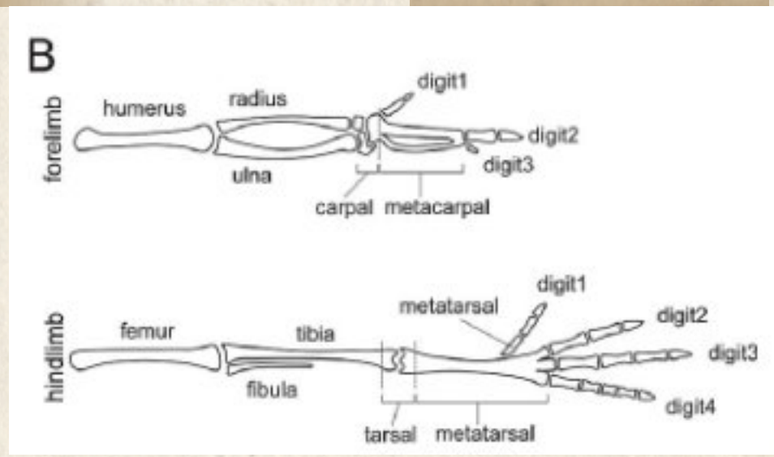
More on double-strand breaks
DSBs in DNA can be caused by environmental factors such as radiation, ultraviolet light (UV) or chemicals.
Organisms have the ability to repair themselves with special proteins. If this does not happen properly, it can lead to errors in cell division. This can look like skin cancer, for example, caused by UV radiation from the sun.

Cp/Cp strand-break is fatal
Some DSBs cannot be repaired, such as the damage caused by the homozygous creeper gene. The creeper gene causes the loss of a repair protein, leading to a chain reaction in the vascular and nervous systems, which eventually leads to cell death in the (early) embryo.



2A: Skeletal patterns of the fore-and hind-limb in the chick embryo, as shown in one individual stained with Alcian blue for cartilage at E10.5. In this stage, most of the limb skeletal elements are cartilaginous tissue, which are then replaced by bony tissue as development proceeds. Both the fore-and hind-limb have three distinct segments along the proximo-distal axis; stylopod, zeugopod, and autopod. S, stylopod; Z, zeugopod; A, autopod. Scale bar = 3 mm.

2B: A schematic representation and terminology of the skeleton of the avian fore-and hind-limb. (Evolutionary and Developmental Aspects of Avian-Specific Traits in Limb Skeletal Pattern, 2012)



The embryos die mostly on the 3rd day of incubation, and they have many abnormalities of body shape. The embryos die before real bones are formed, they are still in the cartilage stage (embryonic cartilage later becomes bone and some of this continues after birth).

In this study, the researchers could not find out why the embryos died at the cartilage stage (day 3), because IHH blocks the proliferation and differentiation of bone cells, they died before osteogenesis, in other words before the chondrocytes (bone-forming cells) even started.

Why do Cp/Cp embryos die?
It is the reduced expression of the 'bone-building system' (Indian hedgehog) that causes shortened limbs and reduced body size in heterozygous creepers, and early death in Cp/Cp embryos due to the loss of the repair protein to repair DNA double-strand breaks.

Even the blood vessels outside the embryo, around the yolk sac, were abnormal (Figure 2). Since neither IHH nor the repair protein is present in the blood vessels outside the embryo, the reason for the underdevelopment of the brain and blood vessels, as well as bone formation, could be a secondary effect of the loss of IHH and the repair protein, accelerating death in the early stages of embryonic development.

Although the repair mechanism in the IHH is impaired in creepers, there are no major problems with their overall functioning repair system. No genetic diseases have been reported in creepers as a result of this in heterozygous chickens. Thus, the creeper gene has no effects (pleiotropic effects) other than limb shortening in heterozygotes.

Cp/Cp dying in later stages too
In the study, they crossed Chabo with a laboratory strain of Fayoumis. The homozygous Cp/Cp embryos from this project died at later stages of development and all had multiple abnormalities at stage 3. The abnormalities were underdeveloped brains, smaller hearts and loss of blood vessels. Some survived longer, but the later-stage death was caused by dominant modifiers previously identified by Landauer in his studies of the creeper gene in 1942, and later by Fujio and Shibuya in 1974.

Cp/cp+ development
In the heterozygous embryos, the limb bones were shorter at day 14, but the rest of the skeleton looked normal. This shows that only the ossification (bone formation) of the limbs is inhibited.
The Cp/Cp embryos were much smaller than the non-creeper and heterozygous creeper embryos. There was hardly any elongation of the forelimbs and hindlimbs and they were deformed, but the bones were still present. All parts of the limbs were deformed, which means that both the growth and the organisation of the bones were disturbed.

All in all, it is now known why embryos from Cp/Cp Chabo and other creeper breeds fail to hatch. A lot goes wrong in the development of the embryo because of the homozygous creeper gene.

More on the above in the paper: “Combined deletions in IHH and NHEJ1 cause chonrodystrophy and embryonic lethality in the creeper chicken”, 3-2020, Communications Biology, https://doi.org/10.1038/s42003-020-0870-z

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The papers by Landauer mentioned previously:
Landauer, W. & Dunn, L. C. Studies on the Creeper fowl. I. Genetics. J. Genet. 23, 397–413 (1930).
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