# On the origin of 'bloopergenes': unraveling the evolution of the balanced lethal system in Triturus newts

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*Freshly laid* Triturus *egg in the wild*

# Chapter 2 - An evolutionary mystery: the deadly chromosome 1 syndrome in *Triturus* salamanders

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Originally, this popular science article is published as:

**De Visser, M.C.,** France, J.M., Meilink, W.R.M. & Wielstra, B.M. (2021). Een evolutionair raadsel: het dodelijke chromosoom 1 syndroom in *Triturus*-salamanders, ***RAVON*** 23(1): 9-12.

For this Chapter, the Dutch article has been freely translated to English. The lead text has been left out, and the English summary that was already in the article provided at the end is here provided as the abstract.

## Abstract

Crested and marbled newts (genus: *Triturus*) are stuck with a the deadly 'chromosome 1 syndrome', which is caused by lethal, genetic mutations. The syndrome is a result of a so-called balanced lethal system. Normally, natural selection will suppress lethal mutations. However, in a balanced lethal system unique, recessive lethal mutations exist on two different versions of a chromosome. These two versions compensate for one another: a functional gene copy on one chromosome type masks the damaged gene present on the other chromosome type, and vice versa. This way, the two different types of chromosome are both required to survive. Because all homozygotes die, this leads to a reproductive output that is cut in half, each generation. The *Triturus* newts are not the only organisms that suffer from such a wasteful system, as similar syndromes have been described in insects and plants. This is an evolutionary paradox: why would something so disadvantageous evolve time and again? Modern DNA-sequencing techniques allow us to compare *Triturus* DNA with that from newt species that do not suffer from chromosome 1 syndrome, to try and decipher the genetic basis of the syndrome. This will help us understand how balanced lethal systems repeatedly evolve in nature by the 'hijacking' of standard evolutionary processes.

## Introduction

According to evolutionary theory, natural selection reduces the likelihood that a lethal mutation that disrupts a crucial gene will be passed on to the next generation [1]. In this way, genetically determined diseases are suppressed in natural populations. However, newts of the genus *Triturus* are affected by the so-called 'chromosome 1 syndrome' [2] which causes 50% of the eggs to not hatch (Figure 1). All *Triturus* species, i.e. seven crested newt species and two marbled newt species, suffer from chromosome 1 syndrome [3].

The phenomenon is an evolutionary mystery that has puzzled scientists for two centuries. Mauro Rusconi was the first to realize that half of the eggs of crested newts stop growing halfway through their development. In his now two-hundred-year-old book *'Amours des Salamandres Aquatiques'* ('The Love Life of Newts'), he wrote the following regarding the relevant stages of embryonic development (freely translated into English): "Although this period is most entertaining for a naturalist to study, it appears that this stage is dangerous for the little embryos themselves, as almost half of them die at this moment - or shortly after" [4].

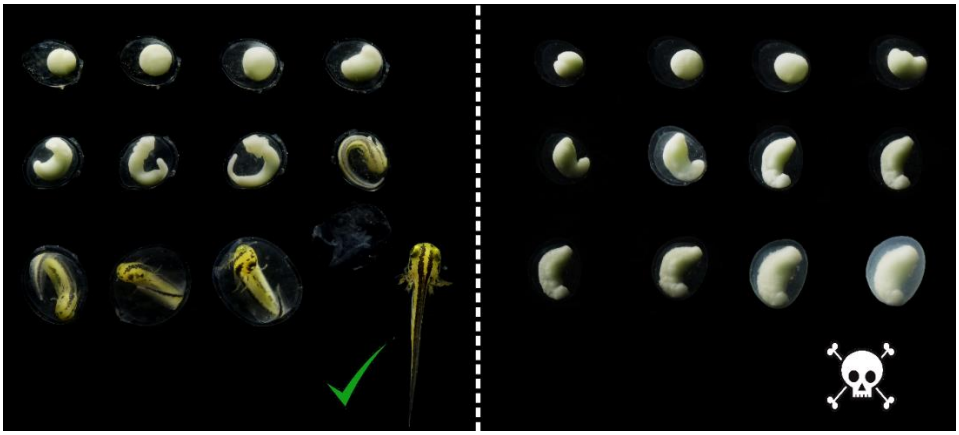

***Figure 1:*** *Half of the eggs laid by crested and marbled newts (genus:* Triturus*) do not hatch. Out of all eggs 50% go through embryonic development successfully (left), while the other 50% of eggs stop growing halfway through embryonic development and eventually succumb (right). (Photo's: Michael Fahrbach).*

## Balanced lethal system

At the basis of chromosome 1 syndrome lies a so-called 'balanced lethal system' (Wielstra, 2020). Chromosome 1 is the longest pair of the 12 chromosome pairs that *Triturus* species possess. While a normal chromosome pair consists of two very similar

versions of a chromosome, there are to be two very different versions of chromosome 1 in *Triturus* newts [5]. An embryo needs both versions of chromosome 1, also known as chromosome 1A and 1B, in order to survive. They must thus be heterozygous for chromosome 1. Homozygotes, that have either two 1A or two 1B versions, die [2]. The genetic mechanism likely works as follows: both versions of chromosome 1 contain unique, harmful mutations in crucial genes that are not present on the other version. There are thus two so-called 'alleles' (gene variants) of these crucial genes: a lethal, non-functioning allele and a normal, unaffected allele. The lethal mutations on the 1A version of chromosome 1 can be compensated for by non-mutated, still functional genes on the 1B version, and *vice versa*. Hence, the defective genes and the genes that are still functional balance each other out. This is where the term 'balanced lethal system' comes from. In short: as long as an embryo possesses both versions of chromosome 1, so one 1A and one 1B (i.e., as long as an embryo is heterozygous), the lethal alleles are suppressed. However, if an embryo has two of the same versions, so either two times 1A or two times 1B (i.e., if an embryo is heterozygous), then things go wrong.

Because only heterozygotes survive, there is a consistent loss of 50% of the eggs. This follows directly from Mendel's laws of inheritance. Namely, when two heterozygous individuals with the genotype '1A1B' or '1B1A' mate, this results in 50% of the offspring being heterozygous. The remaining 50% of individuals are homozygous: 25% end up with the genotype '1A1A' and 25% with '1B1B' (Figure 2). The heterozygotes are lucky and get a chance to grow into adults and reproduce, while both types of homozygotes are doomed to die in the egg [2]. And every generation, this macabre cycle starts all over. What a waste!

## Originated millions of years ago

All *Triturus* species are affected by the chromosome 1 syndrome, while newts of the most related genus *Lissotriton* are not (for instance, the smooth newt *L. vulgaris* or the palmate newt *L. helveticus*, which are both found in the Netherlands). Therefore, it is likely that this anomaly originated in a common ancestor of *Triturus*. This means this system “got stuck” in the DNA of *Triturus* at least 24 million years ago [6, 7].

In scientific literature, chromosome 1 syndrome is *the* classic example of a balanced lethal system [8]. However, in addition to the newts there are examples of other organisms displaying a similar phenomenon, for instance some insects and plants [9]. In those cases, it is also the presence of two versions of a certain chromosome that plays a key role. The fact that balanced lethal systems occur in a broad range of taxonomic groups makes it plausible that; 1) their repeated emergence must be explainable on the basis of evolutionary principles, and 2) their occurrence may not be as rare as is currently believed. But then how could such a balanced lethal system actually arise?

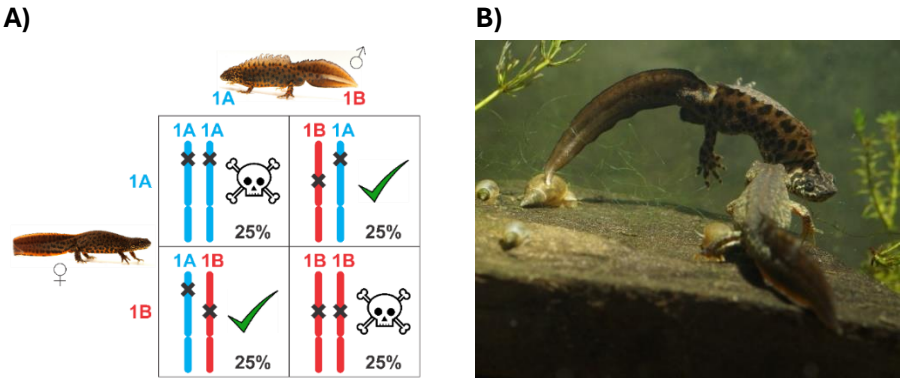


***Figure 2:*** *Crested and marbled newts suffer from a balanced lethal system, in which only heterozygotes survive.* ***A)*** *The two different versions of chromosome 1 possess unique, lethal mutations (crosses). Adult individuals always have a 1A and a 1B version and pass on one of the two to a sex cell with a 50% chance. After fertilization four combinations are possible, each with a chance of 50% x 50% = 25%. The heterozygotes survive because of their lethal mutations are compensated for, while both types of homozygotes are doomed to die.* ***B)*** *From the mating ritual (displayed on the photo) all the way through egg deposition:* Triturus' *chromosome 1 syndrome is considered an evolutionary mystery, because of the substantial amount of energy used in the reproductive process. (Photo: Michael Fahrbach)*

## Suppressed recombination

In regular chromosome pairs 'recombination' occurs during the production of sex cells (egg and sperm cells). Within a chromosome pair, this allows a part of one chromosome to be exchanged with the equivalent part of the other chromosome, and the other way around. This exchange is also called crossing-over and, in newts, it occurs on average twice on one chromosome per each cell division. Crossing-over is essential in evolution, as it ensures the constant creation of chromosomes that have a unique DNA code. Chromosome pairs are then split – a process called 'segregation' – and, one chromosome per pair is passed on to each sex cell. Thus, recombination ensures that unique combinations of chromosomes ultimately come together in the offspring during fertilization (Figure 3).

Crossing-over could theoretically swap the non-functional alleles on one of the two versions of chromosome 1 for the functional alleles from the other version. In this way, chromosome 1 syndrome could disappear in one go, as individuals with a 'corrected' chromosome 1 would suddenly be able to produce twice as many offspring: a huge selective advantage! However, crossing-over does not occur over a large part of chromosome 1 in *Triturus* [10, 11]. This suggests that the difference between chromosome 1A and 1B is probably so large that they no longer recognize each other as

equivalents, making crossing-over impossible. The result is that each sex cell possesses either chromosome 1A or 1B, but never a mix of both. Thus, if an egg and a sperm cell that carry the same version of chromosome 1 come together, this eventually results in a non-viable individual (Figure 3).

## From ‘supergene’ to ‘bloopergene’

Suppressed crossing-over is often observed in so-called 'supergenes.' Supergenes essentially comprise of a group of genes that lie on a piece of a chromosome and form alleles that are inherited together, i.e. as one overall stretch. In other words: these genes are 'linked' to each other. There are always at least two versions of a supergene, each with their own set of alleles for the genes involved. This allows the alleles on each supergene to evolve together, which makes it possible for complex adaptations to arise, hence the name: 'supergene.' Individuals heterozygous for a particular supergene may have a selective advantage over individuals that are homozygous. In that case, a so-called 'balancing selection’ exists, which leads to the retention of both the supergene versions across generations [12].

Supergenes are super, but they also have a downside. Because crossing-over between different supergene variants cannot occur within heterozygotes, it is difficult to eliminate any harmful mutations that arise. Thus, what follows is a slow accumulation of harmful mutations. In the field of evolutionary genetics, this phenomenon is referred to as 'Muller's Ratchet.' In heterozygotes, a still functioning allele on the other supergene variant can still compensate for such a new harmful mutation. But if both supergenes accumulate unique lethal alleles, no homozygote will be viable. In other words: a balanced lethal system, where all homozygotes are doomed to die, has originated.

It is thus likely that the supergenes initially provided an evolutionary advantage, but that the accumulation of harmful mutations spiraled out of control in such a way that this resulted in an irreversible, deadly syndrome. And once a balanced lethal system is fixed within a species, there is no escaping it. In this case, there must have been some sort of tipping point where 'supergenes' turned into what can be considered 'blooper genes.' In a way, it is reminiscent of the foolish greediness of King Midas from Greek mythology, who chose to have everything he touched turn to gold. This seemed like a good idea in the short term, but it turned out to be disastrous in the long term.

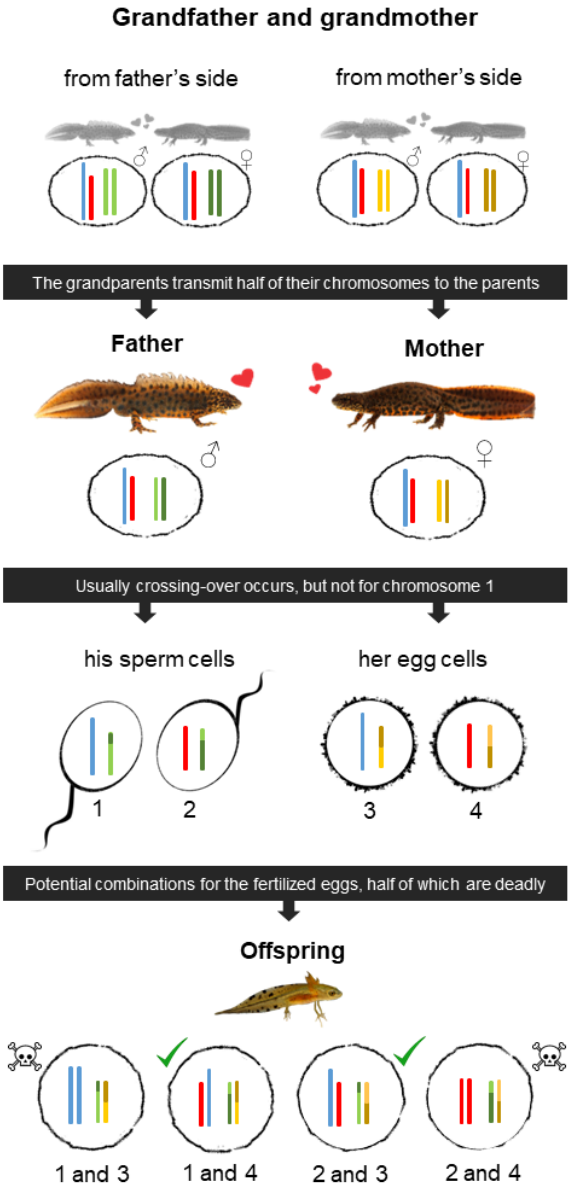


***Figure 3:*** *Chromosome pairs usually shuffle and exchange DNA, however chromosome 1 in the genus* Triturus *poses an exception. When fathers and mothers produce sperm and egg cells, it is generally the case that crossing-over happens in the chromosomes of the grandparents (shown for one chromosome pair, displayed with two shades of green or yellow for the grandparents of father's and mother's side, respectively). However, between the two versions of chromosome 1, 1A (in red) and 1B (in blue) there is no crossing-over, meaning that an egg cell or a sperm cell can receive 1A or 1B, but never a mix of both. (Photo's: Michael Fahrbach).*

## DNA research will solve the puzzle

So far, scientists have only been able to look at the overall development of *Triturus* embryos in the egg (Figure 4) and at the 'karyotype' (the general shape of the chromosomes) of these newts. Investigating it more in depth by means of DNA research used to be virtually impossible – until now. Modern DNA sequencing techniques should make it possible to identify the genes responsible for the catastrophe that befalls half the *Triturus* eggs.

By comparing the genome of *Triturus* species with that of other salamander species that do not suffer from the chromosome 1 syndrome, such as *Lissotriton* species, the lethal mutations hidden in the DNA of *Triturus* can be discovered. Furthermore, by following the line of evolutionary descent it is possible to "reconstruct" what chromosome 1 must have looked like in the ancestor of all the crested and the marbled newts, so before the syndrome originated.

Figuring out how chromosome 1 syndrome evolved hopefully leads to an understanding of how a balanced lethal system can be created by standard evolutionary processes, despite it *seeming* evolutionarily impossible.

***Figure 4**: A viable* Triturus *embryo inside the egg. (Photo: Michael Fahrbach).*

## References


1. Darwin, C., *On the origin of species by means of natural selection, or preservation of favoured races in the struggle of life*. 1859.
2. Macgregor, H.C. and H. Horner, *Heteromorphism for chromosome 1, a requirement for normal development in crested newts.* Chromosoma, 1980. **76**: p. 111-122.
3. Fahrbach, M. and U. Gerlach, *The genus Triturus: History, Biology, Systematics, Captive Breeding.* 2018.
4. Rusconi, M., *Amours des salamandres aquatiques: et developpement du tetard de ces salamandres depuis l'oeuf jusqu'a l'animal parfait.* 1821.
5. Callan, H.G. and L. Lloyd, *Lampbrush Chromosomes of Crested Newts Triturus cristatus (Laurenti).* Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 1960. **243**: p. 135-219.
6. Steinfartz, S., et al., *A Bayesian approach on molecules and behavior: reconsidering phylogenetic and evolutionary atterns of the Salamandridae with emphasis on Triturus newts.* Journal of experimental zoology. Part B, Molecular and developmental evolution, 2007. **308B**: p. 139-162.
7. Rancilhac, L., et al., *Phylotranscriptomic evidence for pervasive ancient hybridization among Old World salamanders.* Molecular Phylogenetics and Evolution, 2021. **155**.
8. Wielstra, B., *Balanced lethal systems.* Current Biology, 2020. **30**: p. R742-R743.
9. Grossen, C., S. Neuenschwander, and N. Perrin, *The balanced lethal system of crested newts: A ghost of sex chromosomes past?* American Naturalist, 2012. **180**: p. E174-E183.
10. Wickbom, T., *Cytological Studies on Dipnoi, Urodela, Anura and Emys.* Hereditas, 1945. **31**: p. 241-346.
11. White, M.J.D., *The spermatogenesis of hybrids between Triturus cristatus and T. marmoratus (Urodela).* The Journal of Experimental Zoology, 1946. **102**: p. 179-207.
12. Schwander, T., R. Libbrecht, and L. Keller, *Supergenes and complex phenotypes.* Current Biology, 2014. **24**: p. R288-R294.