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Comparative genomics of the balanced lethal system in *Triturus newts*

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Summary

The balanced lethal system found in the crested and marbled newts that make up the genus *Triturus* is an especially puzzling phenomenon. In these newts half of all fertilised eggs undergo developmental arrest and die before hatching. This phenomenon results in a catastrophic loss of reproductive output and is not seen in any other species of newt or salamander. While we understand evolution to be a stochastic process with no ability to recognise or progress towards any ‘objective’, it is still surprising that a lethal mutation has become fixed in an entire genus. This thesis aims to reveal the nature of this mutation and the evolutionary mechanisms that produced it.

In [Chapter 1](#) I give a broad overview of the research background. I introduce the organisms of interest (*Triturus* newts) and place them in a phylogenetic context, including their sister genus *Lissotriton* and more distant relatives inside and outside of the family Salamandridae. I explain the general mechanics of balanced lethal systems and discuss the literature regarding the system found in *Triturus*, which comprises the two distinct, non-recombining forms of chromosome 1. I describe several possible hypotheses which seek to explain the evolution of the balanced lethal system, including evolution from sex chromosomes, and degeneration of a supergene system. I then set out the objectives of the PhD project: to examine these hypotheses by mapping the genomes of *Triturus* and its relatives, and identifying structural changes associated with the evolution of the balanced lethal system.

In [Chapter 2](#) we begin the process of testing the sex chromosome origin hypothesis by identifying the Y-chromosome of *Lissotriton*, which in this hypothesis would be expected to be homologous to the non-recombining region of *Triturus* chromosome 1. This is challenging because newts have extremely large genomes (20-30 Gbp) and small, homomorphic, sex-linked regions. Two RADseq based approaches are attempted, identifying male specific markers in adults of known sex, and identifying a paternal-linked, non-recombining region via linkage mapping. The sex association approach proves to be effective, identifying several Y-linked markers which are validated in multiple *Lissotriton* species. However, despite the construction of a high-density linkage map, a Y-linked non-recombining region is not immediately apparent until the markers discovered in the adult males are highlighted.

In [Chapter 3](#) we apply the techniques used in [Chapter 2](#) to *Triturus* itself. A high-density RADseq linkage map is constructed, and Y-linked markers identified via sex association in adult males. The Y-linked markers are then incorporated into the linkage map to highlight the Y-chromosome. By comparing the *Triturus* and *Lissotriton* linkage

maps, we observe that the two genera have two different Y-chromosomes. This indicates that at least one of the lineages evolved a new Y-chromosome after they split from their last common ancestor. This is important because the sex chromosome origin model requires this kind of Y-chromosome turnover event to occur in this time frame. However, we also observe that the *Lissotriton* Y-chromosome is not homologous to *Triturus* chromosome 1. Consequently, we conclude that, while there is clear evidence of recent Y-chromosome turnover within newts, this is likely incidental to the evolution of the *Triturus* balanced lethal system.

In [Chapter 4](#) we seek to explore other models of the evolution of the balanced lethal system by directly identifying any genomic rearrangements that have occurred since *Triturus* and *Lissotriton* diverged from their last common ancestor. We use target capture sequencing to construct linkage maps for both genera, each including the position of over 3,500 coding genes. We also compare these maps to the genome assemblies of the Iberian Ribbed Newt (*Pleurodeles waltl*) and Axolotl (*Ambystoma mexicanum*). Surprisingly, at the whole genome level, there is very little structural variation between the three newt genera, even though their last common ancestor lived over 60 million years ago. However, in *Triturus* chromosome 1 specifically, there are rearrangements associated with the balanced lethal system. We observe that each of the two forms of chromosome 1 is missing a single large section of DNA, approximately 200 Mbp in length. This explains the lethality of the system: the 50% of embryos unlucky enough to inherit two copies of the same version of chromosome 1 are completely missing a significant portion of their genome. Curiously, it also appears that each deletion is compensated for by duplication of the homologous region of the opposite version of the chromosome, so the 50% of embryos that inherit one copy of each version of chromosome 1 still have two copies of all genes. This pattern of deletion and duplication is the predicted result of an unequal exchange between sister chromosomes, which is a mechanism that has been hypothesised to create a balanced lethal system in a single step, in a single individual. We model this scenario and find that, counterintuitively, the balanced lethal system can become fixed in a sub-population that then becomes resistant to invasion by the ancestral, pre-rearrangement form of chromosome 1. This resistance is due to the fact the duplications in the balanced lethal system fully compensate for the deletions, which is not the case for ‘hybrids’ between the balanced lethal system and ancestral chromosome.

In [Chapter 5](#) I synthesise the findings of the preceding three chapters and provide directions for future research. The identification of two completely different Y-chromosomes in two sister genera is somewhat surprising and suggests a need for further investigation of sex determination systems across newts, which I suggest could be accomplished using the same methodology as I describe in [Chapters 2 and 3](#). A consistent finding across the chapters is a tight conservation of genome scale synteny within newts, and it would be interesting to know whether this is restricted to *Triturus*,

Lissotriton and *Pleurodeles*, or is characteristic of the entire family Salamandridae. I also discuss the implications of the reproductive isolation modelled in Chapter 4 and the unexpected link this suggests between the balanced lethal system and speciation. I conclude that the *Triturus* balanced lethal system offers an excellent example of how applying modern genomic tools to previously intractable problems can offer fascinating and surprising new insights