Comparative genomics of the balanced lethal system in Triturus newts

France, J.M.

Introduction & Outline of Thesis

JAMES FRANCE[1,2]

[1] Institute of Biology Leiden, Leiden University, Leiden, The Netherlands

[2] Naturalis Biodiversity Center, Leiden, The Netherlands

General Background

Biological evolution can appear a simple, even obvious idea. Thomas Huxley recalls his initial reflection upon reading Darwin's 'On the Origin of Species' (1859) as "How extremely stupid of me not to have thought of that!" (Huxley 1888). Yet in nature this apparently simple concept plays out as a multitude of complex interactions between subtle processes, frequently producing results that seem counterintuitive, or even paradoxical. For example, Darwin initially found it difficult to explain the evolution of costly traits that seemed to reduce the chance of surviving or producing offspring, such as the sterility of many eusocial insects, or the cumbersome tail feathers of male peacocks (Burkhardt et al. 1993). Later researchers puzzled over questions such as how evolution might allow animals to aid unrelated individuals at cost to themselves? (Hamilton 1963) Or the 'C-value paradox' - why some species had evolved genomes many times larger and more expensive to replicate than other, similarly complex species (Thomas 1971). These paradoxical phenomena proved more than mere curiosities. Darwin's confusion gave way to insights that prompted the development of theories of kin selection and sexual selection (Darwin 1859, 1871). The later examples led to entire subfields studying reciprocal altruism (Trivers 1971) and selfish genetic elements (Orgel & Crick 1980). The potential for unexpected insight makes the investigation of evolution's most counterintuitive outcomes valuable.

Amongst the most important principles of evolutionary theory is natural selection, a concept so simple and self-evident that it has been described as a tautology (Waddington 1959) – organisms with traits that make them efficient at surviving and producing numerous offspring tend to survive and produce numerous offspring. It follows that, if those beneficial traits are inheritable, they will become more common, and opposingly, traits which inhibit reproduction or survival will become less common and eventually go extinct (Darwin 1859). Despite this incontrovertible logic, some organisms appear to defy natural selection. A notorious example of this are the newts of the genus *Triturus*. In these species 50% of all offspring spontaneously die during embryogenesis, cutting their reproductive potential in half. This trait is clearly massively disadvantageous, provides no known benefit, and is not shared by any other newt taxa – clearly it should be heavily selected against. However, it has persisted for over 20 million years and is fixed in every species in the genus.

Triturus – The Crested and Marbled Newts

Newts (subfamily Pleurodelinae) are salamanders in the family Salamandridae, which also includes the true salamanders. Newts are characterised by a semiaquatic lifestyle, where adults alternate between terrestrial and aquatic habitats, with breeding occurring in the water during spring and summer. All salamanders possess extremely large genomes and in newts the haploid genome consists of around 30 billion base pairs (30 Gbp). The newt genome is arranged into twelve pairs of chromosomes, except for the two new world genera, which have eleven pairs (Sessions 2008).

Triturus is a genus of newt distributed throughout Europe and western Asia. As of 2024 it is recognised to include three species of marbled newt and seven species of crested newt (Arntzen 2024). All *Triturus* species are capable of breeding together to produce viable offspring, however hybrids between the crested and marbled newt lineages have very low fertility (Arntzen et al. 2009, 2018). *Triturus* is part of a clade sometimes termed 'modern European newts', which also includes genera such as *Ichthyosaura* (the alpine newt) and *Ommatotriton* (banded newts) (Veith et al. 2018). The phylogenetic relationships within this lineage have been obscured by extensive ancient introgression, but recent publications place *Triturus* as the sister genus of *Lissotriton* (Rancilhac et al. 2021), which occupies a similar distribution. Europe is also home to 'primitive newts' of the genus *Pleurodeles*, which are much more distantly related, having diverged 60 mya (Marjanović & Laurin 2014). Compared to their close relatives, *Triturus* newts are notably larger and occupy different ecological niches. For example, the northern crested newt *Triturus cristatus* is approximately twice overall length and five times the total mass of the common newt *Lissotriton vulgaris*, with which it co-occurs across most of northern Europe and opportunistically predates upon (Cicort-Lucaciu et al. 2005; Sparreboom 2014).

The most striking difference between *Triturus* and its relatives concerns reproduction. Similar to other newt taxa, fertilisation occurs internally and the females lay clutches of 150-500 eggs, with approximately 200 being typical for most crested newt species (Sparreboom 2014). However, whereas in related genera close to 100% of fertilised eggs will hatch as viable larvae (Sessions et al. 1988), in *Triturus* only 50% will. The other 50% will develop normally until they reach the late tailbud stage, which occurs at day 6-7 in embryos developing at 20 °C, and then will spontaneously cease to develop and eventually die (Horner & Macgregor 1985).

The death of half of *Triturus* embryos had been noted over two centuries ago, by Italian naturalist Mauro Rusconi, in his treatise '*Amours des salamandres aquatiques*' - The love life of newts (Rusconi 1821). However, the mechanism behind this trait and its evolutionary implications remained uninvestigated until the second half of the 20th century. Superficially this phenomenon might appear similar to phenomena observed in other amphibians. Frogs of the genus *Oophaga* lay unfertilized eggs, which are eaten

by their tadpoles (Brust 1993). In fire salamanders, *Salamandra salamandra*, intrauterine cannibalism is observed in viviparous populations (Buckley et al. 2007). However, in these examples, the sacrifice of a mother's resources is balanced by a clear benefit to her surviving offspring. In contrast, *Triturus* larvae do not consume their unhatched brood mates (Grossen et al. 2012), and the result is *Triturus* losing half of their reproductive capacity, which must be considered strictly maladaptive.

Chromosome 1 in *Triturus*

The logic of natural selection suggests that trait as deleterious as the spontaneous loss of half of all offspring should be swiftly eliminated (Darwin 1859), but a peculiarity of the *Triturus* genome prevents this. The largest chromosome pair (chromosome 1) is unusual, while clearly a matched pair, the chromosomes are heteromorphic – the long arm of one chromosome is extended in comparison to the other (Callan et al. 1960). The larger of these forms is designated 1A and the smaller 1B. Under Giemsa staining, the long arms of chromosome 1 show an extreme amount of banding, in a pattern that differs between 1A and 1B (Sims et al. 1984). This implies that the long arms are a heterochromatic region that does not undergo homologous recombination, a suggestion supported by the lack of chiasmata observed in this region (Morgan 1978).

Initially chromosome 1A and 1B were proposed to be sex chromosomes, as the lack of recombination and accumulation of heterochromatin would indicate (Callan et al. 1960; Mancino & Nardi 1971). As many early investigations into the karyology of salamanders relied on lampbrush chromosomes prepared from female oocytes, and female heteromorphism (Z- and W-chromosomes) was already known in *Pleurodeles* (Lacroix 1970), this determination was accepted until it was realized that chiasmata were absent from the long arm in both males and females (Morgan 1978; Macgregor 1979) and chromosome 1 is heterozygous in both sexes. As all *Triturus* adults carry both chromosome 1A and 1B, Mendelian genetics would suggest that half of all offspring will be homozygous (1A,1A or 1B,1B). However, these genotypes are never observed in adults, because it is these homozygote embryos that undergo premature arrest (Macgregor & Horner 1980).

The requirement of both 1A and 1B for survival in *Triturus* is likely due to each form of the chromosome carrying genes that are essential for viability, that are either not functional or not present on the other form. This phenomenon, where heterozygosity is persevered in a population because homozygosity is invariably lethal, is termed a balanced lethal system (Muller 1918).

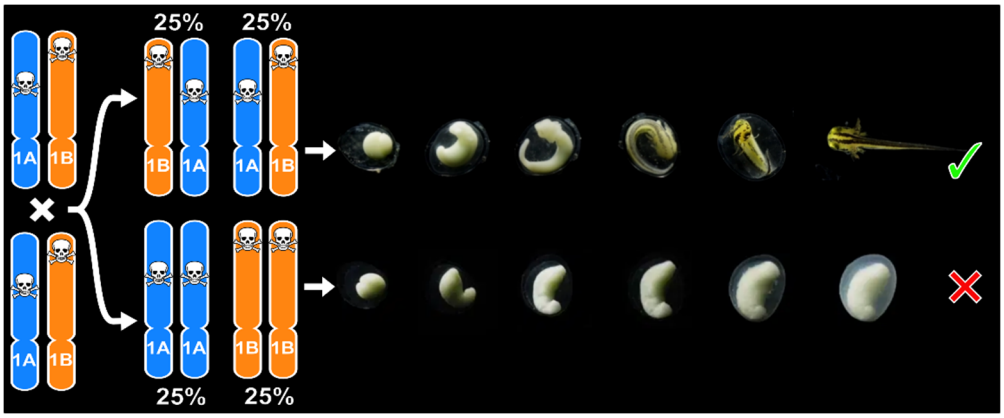

Figure 1: The balanced lethal system in *Triturus* chromosome 1. Each form of chromosome 1 has (recessively) lethal alleles at different locations. Consequently the 50% of offspring that inherit two copies of either version are unable to survive embryogenesis, and their development arrests, followed by eventual death. The 50% of offspring that inherit one copy of both versions of chromosome 1 survive and hatch as viable larvae. (Photographs courtesy of Micheal Fahrbach).

Balanced Lethal Systems

For over a century, artificial balanced lethal systems have been used to maintain stocks of organisms which carry alleles that are recessively lethal (Muller 1917). Most of these systems have been created in *Drosophila*, but they have also been engineered in nematodes (Herman et al. 1976) and mice (Zheng et al. 1999). Balanced lethal systems preserve recessively lethal alleles in a population by pairing them with a 'balancer chromosome' (Miller et al. 2019). This is a homologous chromosome which also carries a gene that is lethal when homozygous. This enforces heterozygosity, ensuring all that all individuals possess the allele of interest.

Genetic recombination is a problem for balanced lethal systems. If the balancer chromosome exchanges its lethal allele for a functional gene, it will become viable when homozygous and displace the allele of interest from the population. To prevent this, balancer chromosomes are developed with one or more genetic inversions (Miller et al. 2019). Recombination between the inverted and normal chromosome is inhibited within the inverted region both because the formation of chiasmata is often suppressed and because the chiasmata that do occur tend to result in inviable gametes (Coyne et al. 1993; Navarro & Ruiz 1997; del Priore & Pigozzi 2015).

The naturally occurring balanced lethal system in *Triturus* accounts for the inability of natural selection to purge what has been termed 'chromosome 1 syndrome' (Wallace 1994). However, it remains difficult to explain the origin of such a disastrously maladaptive trait. While extreme artificial selection has occasionally forced a balance lethal system into existence, as Dawson (1967) demonstrated with *Tribolium* beetles, it is improbable these conditions could occur in nature. However, the *Triturus* balanced

lethal system cannot be dismissed as an evolutionary fluke; similar systems have been described in widely divergent branches of the tree of life, including *Oenothera* (Steiner 1956), *Isotoma* (James et al. 1990) and *Drosophila Tropicalis* (Dobzhansky & Pavlovsky 1955).

Suppressed Recombination

For the balanced lethal system to remain stable, recombination between the two forms of chromosome 1 must be inhibited. This implies that the region of suppressed recombination we observe in *Triturus* chromosome 1 must have evolved before or concurrently with the balanced lethal system. The non-recombing region may have expanded afterwards, forming strata similar to those observed on sex chromosomes (Lahn & Page 1999).

Suppression of recombination is often primarily discussed in relation to genetic inversions, but other chromosomal rearrangements such as deletions and translocations can also inhibit homologous recombination, although these are much more likely to be immediately deleterious and hence are observed less frequently. Certain DNA sequences, for example the PRDM9 binding site (Paigen & Petkov 2018), also affect the local frequency of recombination and transposable elements have a bidirectional association with regions of suppressed recombination (Kent et al. 2017).

When initially describing the *Triturus* balanced lethal system Macgregor and Horner (1980) briefly posited that both chromosome 1A and 1B could have been created in a single event, during an unequal exchange between sister chromosomes. Sessions et al. (1988) elaborated upon this, suggesting that a pair of reciprocal deletions and duplications resulting from such an exchange would explain the non-viability of the homozygotes as well as suppressing recombination. However, this concept was dismissed by later authors as it provided no mechanism by which such deleterious mutations could achieve fixation (Grossen et al. 2012).

Recombination is a process of immense importance in evolution and so its suppression has significant consequences. Recombination is vital to the efficient purging of deleterious alleles, and its absence results in an accumulation of such alleles in an effect termed 'Muller's ratchet' (Felsenstein 1974), which may gradually reduce the fitness of the population. This effect is responsible for the accumulation of transposons and other repetitive elements in non-recombing regions resulting in the build-up of heterochromatin commonly observed (Charlesworth et al. 1994). As alleles for different loci within a non-recombining region will be inherited together for many generations the synergistic effects between them become more visible to selective pressures, which contributes to the evolution of phenomena such as sex chromosomes and supergenes (Charlesworth et al. 2005; Thompson & Jiggins 2014).

Supergenes

A supergene is a group of genes located on the same chromosome and consistently inherited together (Dobzhansky 1970). Supergenes can have profound effects on the evolution of a species (Thompson et al. 2014). By locking together a large number of genes a supergene can produce highly distinctive, complex phenotypes (Schwander et al. 2014). Among the most studied systems are the Müllerian mimicry supergenes in Heliconius butterflies (Joron et al. 2006) and the supergenes that control social organization in fire ants of the genus Solenopsis (Wang et al. 2013). Example in vertebrates include the supergenes that give rise to the spectacular mating morphs in the ruff (Küpper et al. 2016) and white throated sparrow (Tuttle et al. 2016).

Supergenes can also promote speciation, via several mechanisms. A lack of recombination can cause a supergene to rapidly accumulate mutations (Muller 1964), resulting in faster divergence between populations. Rigid genetic linkage also facilitates the evolution of meiotic drive systems, which selfishly destroy gametes that do not possess the supergene (Larracuente & Presgraves 2012). Drive systems typically impose a fertility penalty, favouring the evolution of genes which suppress them (Zanders & Unckless 2019). If a hybridization event introduces the drive system into a population that lacks the suppressor, then less fertile offspring will be produced (McDermott & Noor 2010). This will result in reproductive isolation and may explain some of the hybrid infertility often observed in nature (Patten 2018). Ironically, while supergenes can separate species by preventing hybridization, some supergenes are created by hybridization between two species (Tuttle et al. 2016; Jay et al. 2018). In addition, like any gene, supergenes can introgress between species, sometimes carrying over entire complex phenotypes with them (Corcoran et al. 2016).

As supergenes can only exist in regions of suppressed recombination, they often originate as a chromosomal inversion (Kirkpatrick 2010). If the inversion is not immediately deleterious, it may spread through the population and start to acquire mutations that cannot be passed back to the original chromosome, the beginnings of a supergene. With the inverted chromosome now in competition with the original, selection or genetic drift will eventually eliminate one of the variants. If the inversion is adaptive, it may become fixed in the population (Lande 1985). It must be noted that typically, an inverted chromosome will be capable of recombining with itself. Consequently, if the inversion becomes fully fixed, there will be no suppressed recombination, and the chromosome will cease to evolve as a supergene system.

For a supergene system to be stable in the long term, it must exist in a balanced polymorphism, where some selective mechanism actively maintains multiple alleles in a population (Charlesworth 2006). One possible mechanism is negative frequency dependent selection, where a trait is advantageous when rare, but disadvantageous when common (Ayala & Campbell 1974). This may be the case in grove snails *Cepaea*

nemoralis, which exist in several distinct morphs, coded for by a supergene (Gonzalez et al. 2019). It is suggested that the rarer a morph is, the less likely it is to be recognized by the snail's main predator, the song thrush (Clarke 1969).

Balanced polymorphism may also be sustained by heterozygote advantage. This occurs when possession of a single copy of an allele is more advantageous than having two (or zero) (Birchler et al. 2003). In severe cases an otherwise advantageous allele may be lethal when homozygous, which is the case in many supergene systems, such as those found in fire ants, ruffs and fruit flies (Kenvon 1972; Wang et al. 2013; Küpper et al. 2016). Recessive lethality may result from the accumulation of deleterious mutations, or from the disruption of an essential gene by the original inversion (Albornoz & Domínguez 1994).

The non-recombining region of *Triturus* chromosome 1, can be viewed as a supergene system exhibiting the most extreme form of heterozygote advantage possible. However, this prompts the question of how the original, un-inverted (or otherwise rearranged) form of the chromosome also loses viability when homozygous. A possible mechanism is that of a self-reinforcing heterozygote advantage: An increased proportion of a population heterozygous for a supergene leads to fewer opportunities for recombination within the affection section of the chromosome. With less recombination deleterious alleles are purged less efficiently, which in turn increases the relative heterozygote advantage. Berdan et al. (2021) model this scenario, showing that under strict conditions symmetric degeneration within a supergene locus can eventually result a balanced lethal system.

Alternatively, chromosomes 1A and 1B may be two incompatible descendants of the same supergene, with the original arrangement driven extinct. This would be the case if the balanced lethal system evolved from the most ubiquitous class of supergene: a sex chromosome.

Sex Chromosomes as Supergenes

The human Y-chromosome may be the most familiar example of a supergene (Charlesworth 2016). It displays all the typical characteristics of a supergene: it gives rise to a complex phenotype, it does not undergo homologous recombination over a portion of its length, and it is maintained in balanced polymorphism with the X-chromosome (Charlesworth 2017).

Sex chromosomes can also demonstrate some of the more diverse aspects of supergene biology. Haldane's rule summarizes how sex chromosomes decrease the fertility of hybrids and promote speciation (Haldane 1922). In some species highly divergent Y chromosomes can code for distinct morphs. This is the case in the guppy, *Poecilia reticulate*, where the polymorphism is maintained by females which preferentially mate with rare male morphs (Hughes et al. 2013). Interestingly it is

possible to breed viable YY guppies (by using temperature dependent sex reversal to produce an XY female), but only if the Y-chromosomes are heterozygous. This indicates that each Y chromosome linage possesses a unique set of recessively lethal alleles (Haskins et al. 1970).

In contrast to mammals and birds, where the XY and ZW systems have been relatively stable for hundreds of millions of years (Cortez et al. 2014; Zhou et al. 2014), amphibians exhibit a far more diverse array of sex determination systems (Nakamura 2009; Miura 2017). For example, the Iberian ribbed newt, *Pleurodeles waltl*, possesses a ZW system (Cayrol et al. 1983), but in *Triturus* chromosome 4 is identified an XY pair (Sims et al. 1984). In many Salamander species sex chromosomes are difficult to identify with a karyotype, as they are relatively homomorphic (Keinath et al. 2018), which is characteristic of evolutionarily young chromosomes (Stöck et al. 2011). This suggests the Salamanders experience rapid turnover of sex determination systems (Hillis & Green 1990).

Did Chromosome 1 Evolve from a Sex Chromosome System?

Wallace (1987) suggested that this rapid turnover explained the origin of the balanced lethal system - chromosome 1A and 1B were the remnants of an ancient sex determination system. This model relied upon the X-chromosome becoming self-incompatible but provided no mechanism by which this mutation might become fixed. A similar, but more developed hypothesis was later proposed by Grossen et al. (2012) where it was given the imaginative title "A Ghost of Sex Chromosomes Past?". This model is based on the following sequence of events:

1) Firstly, *Triturus* chromosome 1 was previously the Y-chromosome of a XY sex determination system.

2) Within this system the Y-chromosome diverged into two lineages, which were maintained in balanced polymorphism. The authors propose that, after divergence, each Y-chromosome lost several (different) essential genes which were maintained on the X-chromosome, creating a unique set of recessively lethal alleles on each lineage of the Y-chromosome. This created a system much like that observed in guppies.

3) The climate became colder. The sex determination mechanisms of salamanders are affected by temperature, with lower temperatures having a feminizing effect (Wallace & Wallace 2000). The environmental shift resulted in a proportion of XY *Triturus* developing as female.

4) When XY females bred with XY males, YY offspring resulted. These behaved similarly to sex reversed guppies: they were viable, but only when heterozygous for the two Y-chromosome linages. To preserve these linages, they must have been so divergent that they were incapable of homologous recombination with each other.

5) The climate became even colder resulting in all XY individuals developing as female. However, the YY individuals had two copies of the masculinizing chromosome, so a proportion of them remained male.

6) The temperature induced sex reversal resulted in a large excess of females over males. In this situation the X-chromosome (which was always in females) was at a disadvantage and was eventually lost from the gene pool. This resulted in a population that was purely YY, with temperature dependent sex determination.

7) The balanced lethal system was now fully established. All individuals carry two copies of the chromosome, but only those which had each of the two lineages were viable. This resulted in the 50% embryonic mortality rate now characteristic of *Triturus*.

8) Finally, a masculinizing mutation evolved on chromosome 4. As the sex ratio was still biased in favour of females, this mutation spread rapidly and eventually evolved into the XY sex determination system observed today on chromosome 4.

As each step in this sequence is plausible under the conditions proposed it presents a viable theoretical route to the fixation balanced lethal system – which is supported by the simulations performed by the authors. However, the pre-requisites are exacting. The Y-chromosome lineages must have diverged early enough that they share no common lethal factors and then been maintained in polymorphism, likely requiring some degree of balancing selection. They must also have evolved separate chromosomal rearrangements to prevent recombination between them in the YY individuals. The mechanism relies on specific patterns of climate alteration and depends on the final masculinizing mutation being delayed until after the balanced lethal system has been fixed, despite a strong selection pressure in its favour from the beginning of the scenario.

A major virtue of the "Ghost of Sex Chromosomes Past" model is that it is not simply an evolutionary "Just So Story" (Smith 2016), but a falsifiable hypothesis that implies testable predictions. If *Triturus* chromosome 1 is a former Y-chromosome, the most parsimonious scenario is that it descended from (and hence is homologous with) the same common ancestor as the current XY systems found in related European newt genera such as *Lissotriton*. Furthermore, because the model involves sex-chromosome turnover specific to the *Triturus* lineage, it implies that the current *Triturus* Y-chromosome is different to that of any common ancestor, and so is extremely unlikely to be homologous to those found its relatives.

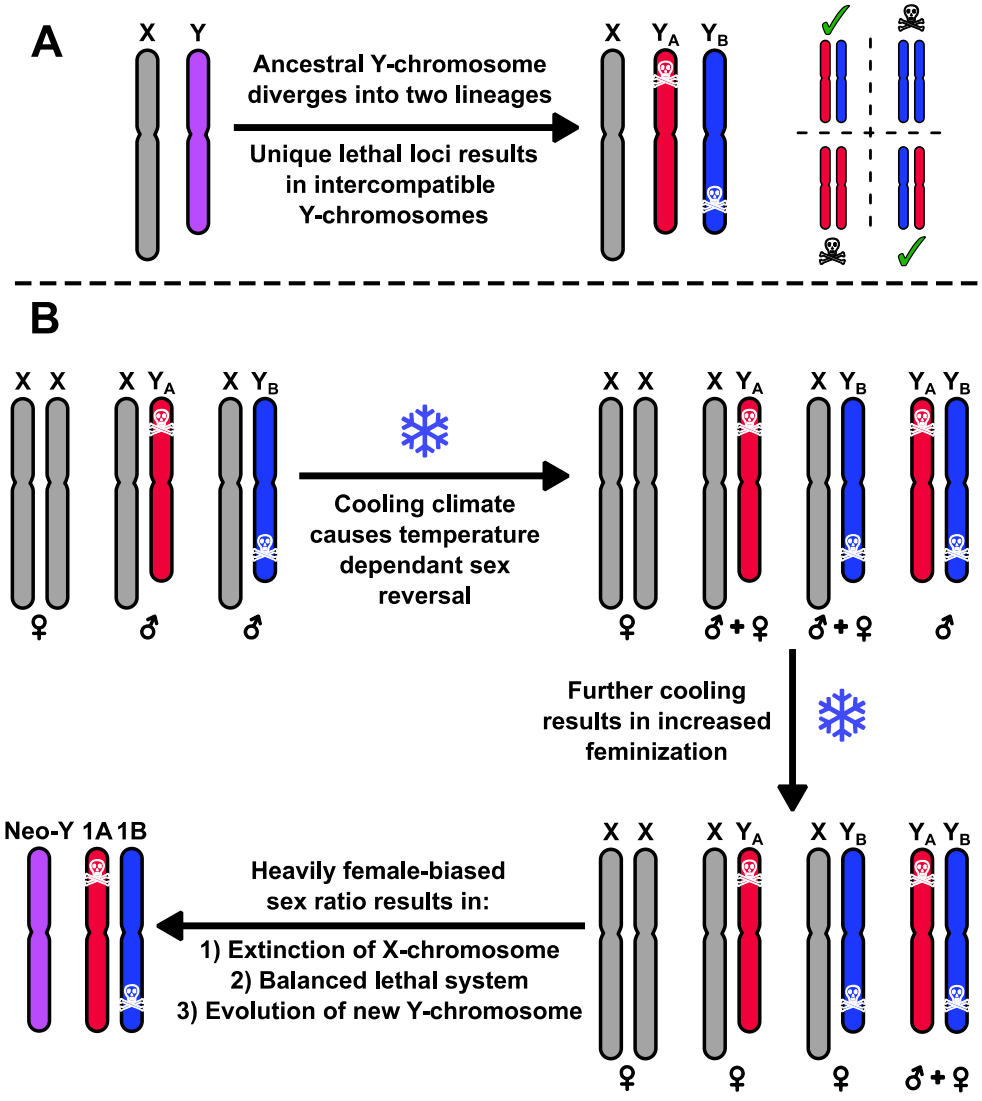

Figure 2: The hypothesized evolution of the *Triturus* balanced lethal system from a Y-chromosome (Grossen et al. 2012). A) The ancestral Y-chromosome diverges into two lineages, each other which possesses its own lethal alleles. Normally the Y-chromosomes cannot meet, but if they did only individuals with one copy of each of the two different lineages would be viable. **B)** As the climate cooled, temperature dependent sex reversal would result in XY females, producing the possibility of YY individuals. With drastic climate change even a YY genotype may not always be sufficient for masculinization, resulting in a sufficiently female biased sex-ratio that the X-chromosome is driven extinct, even though this results in a balanced lethal system.

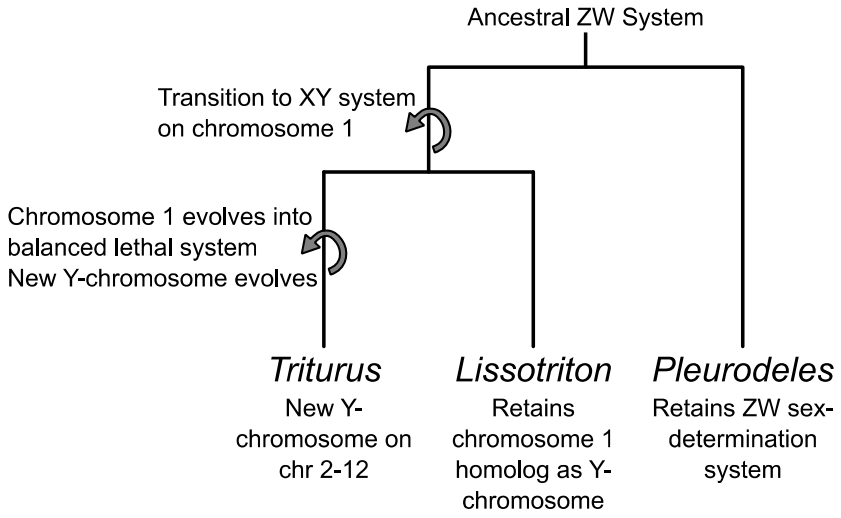

Figure 3: Sex chromosomes turnover events in newts required by the Y-chromosome origin hypothesis. A transition between an ancestral male-determining region on chromosome 1 to a new Y-chromosome is an essential feature of this mechanism. This implies that the Y-chromosome of *Lissotriton* cannot be homologous with that of *Triturus* and will instead likely be homologous with the *Triturus* balanced lethal system. Also indicated is transition from the ancestral ZW system of 'primitive newts' to the XY system in 'modern' newts.

A Degenerate Supergene?

While Grossen et al. (2012) presented the first detailed simulation of the evolution of a balanced lethal system the alternative hypothesis of a runaway heterozygote advantage has recently been modelled by Berdan et al. (2022) These simulations concern different arrangements of a supergene that are initially mildly overdominant with respect to each other (i.e. the supergene locus drives heterozygote advantage) which can be either inherent to the arrangement, or a consequence of genetic linkage to other overdominant alleles (associative overdominance). As individuals carrying two different arrangements of the supergene have a fitness advantage in this scenario, they will experience disproportionate reproductive success and may constitute the majority of the breeding population.

The supergene arrangements can only undergo recombination in homozygous individuals, and so if these homozygotes reproduce less often, there will be less frequent recombination within the supergenes. This will reduce the efficiency of purifying selection within these regions and therefore cause an accumulation of deleterious mutations within each supergene arrangement. As most deleterious mutations are recessive, and the mutations will be different in each supergene arrangement, they will have little impact on individuals that are heterozygote for the supergene. However, homozygotes will suffer reduced fitness due to the additional mutation burden and so will make up even less of the breeding population. This forms the basis of a vicious cycle where there is ever less recombination and purifying selection within the supergene

arrangements, resulting in an ever-increasing mutation burden which eventually drives the fitness of the homozygotes to zero, producing a balanced lethal system.

Berdan et al. (2022) show that this is a plausible mechanism for the formation of a balanced lethal system, but in common with the degenerate sex-chromosome scenario, its viability depends on specific conditions, especially a small effective population size and low rates of gene conversion. An interesting aspect of this mechanism is that the degeneration must be symmetric. If the mutation burden in one of the supergene arrangements results in significantly lower fitness of its homozygotes than the other arrangement, then its frequency within the population will decrease. Any imbalance in frequency will reduce purifying selection within the less common supergene arrangement (and enhance its efficiency in the more common arrangement) resulting in this arrangement experiencing an even greater mutation burden and exacerbating the original imbalance. This cycle would continue until the less common arrangement was either driven extinct, or, if the heterozygote advantage was very high, evolved into a 'half-lethal' supergene – like those observed in ruffs or fire ants (Kenvon 1972; Wang et al. 2013; Küpper et al. 2016).

The genomic signature that this mechanism would leave in modern *Triturus* requirement is less obvious than evolution from a 'ghost' sex chromosome. However, the requirement for steady, symmetric degeneration of both supergene arrangements suggests that we would not expect to find evidence of macro-mutations (such as multi-gene deletions) that would have abruptly incurred major fitness penalties. This is a stark contrast to the first mechanism proposed for the evolution of the *Triturus* balanced lethal system, the unequal exchange described by Sessions et al. (1988) which likely would leave clear evidence of large-scale deletions.

Exploring the Balanced Lethal System and the Giant *Triturus* Genome

There has been considerable investigation of the *Triturus* balanced lethal system at the embryonic and cytological level. Multiple mechanisms have been proposed, and in some cases simulated, to explain the evolution of naturally occurring balanced lethal systems in general (Berdan et al. 2021) and the *Triturus* system in particular (Wallace 1987; Sessions 2008; Grossen et al. 2012). Despite this interest, essentially nothing is known about the system at the genetic or genomic level. The specific mechanism of lethality remains a mystery. Not a single gene has been shown to be associated with the non-recombing regions of either chromosome 1A or 1B. No structural rearrangements have been definitively identified with the *Triturus* genome. This is unfortunate, as understanding the evolution of the balanced lethal system may be almost impossible without knowledge of the structure and content of this part of the genome and so far, any predictions implied by the proposed hypotheses have remained untested.

The relative lack of progress has been typical of salamander genetics/genomics in the first two decades of the 21st century (for example no male-linked markers have been identified on the Y-chromosome of any salamander) and may be due to the intimidating size of the genomes involved. Other than lungfish (Metcalfe et al. 2012) (Meyer et al. 2021), salamanders have the largest genome of any vertebrate. In *Triturus*, estimates of the haploid length varies between 27 GB and 32 GB depending on species (Litvinchuk et al. 2007) and this does not account for the heteromorphy observed in the largest chromosome. This size complicates sequencing and genome assembly. Chromosome scale assemblies have been published for the axolotl and Iberian ribbed newt (Brown et al. 2025; Nowoshilow et al. 2018; Smith et al. 2019), but while more are sure to follow, these remain very resource intensive projects.

An alternative to whole genome sequencing is reduced representation sequencing (Hirsch et al. 2014). This is diverse category of techniques that aim to interrogate subset of loci. Importantly, if the techniques are applied consistently, the same loci are sequenced across multiple samples. Compared to whole genome sequencing, these approaches have several advantages. Sequencing a single sample is much cheaper, large sample sizes become viable, very high coverage is possible (making it easier to identify rare polymorphisms) and useful data can be quickly generated even from the largest of genomes.

Two techniques of particular interest are RADseq and target capture sequencing. RADseq sequences sections of DNA flanked by the binding sites of selected restricted enzymes (Davey & Blaxter 2010). Though restriction sites will be scattered randomly throughout the genome, their position will be conserved between samples of the same species. RADseq may yield hundreds of thousands of loci with little preliminary work, but these are rarely coding sequences, and often poorly conserved between species. Target capture relies on the hybridization of synthetic complementary RNA probes to preselected target sequences (Mertes et al. 2011). The targets chosen are generally coding genes, and often highly conserved between species. However, one limitation is that the general sequences of the targeted loci must be known in advance. Often transcriptome data is used to design the probes, but this is not helpful for non-transcribed sequences, making investigation of gene-poor regions, such as sex chromosomes, difficult. For *Triturus* a probe set targeting ca. 7000 genes has already been designed and used to construct a phylogeny of the genus (Wielstra et al. 2019).

Unlike a whole genome assembly, reduced representation sequencing does not directly give information about the position of any marker within a genome. However, if a family of related individuals is sequenced, the relative position of any two markers can be inferred by calculating the frequency of recombination between them. With enough markers a linkage map covering all chromosomes within the genome can be constructed (Kai et al. 2014).

Aims and Structure of the Thesis

The primary objectives of this PhD project are to map *Triturus* genome, to compare the structure of the *Triturus* genome to that of related genera and identify regions of shared synteny, to identify any chromosomal rearrangements which may have been involved in the evolution of the non-recombing region of *Triturus* chromosome 1, and to test the "A Ghost of Sex Chromosomes Past" hypothesis by determining whether or not chromosome 1 ever has acted as a sex chromosome.

Chapter 2

To verify whether *Triturus* chromosome 1 has ever acted as a sex chromosome it will be necessary to identify the sex-linked regions of the genome of its close relatives (on the assumption that these still retain the sex determination system of their common ancestor). To this end, a RADseq based linkage map will be constructed for the common newt *Lissotriton vulgaris*, which, like *Triturus*, also possess a small heteromorphic Y-linked region close to the telomere of one of its middle-ranked chromosomes.

Other researchers working in amphibians have shown that a sex-linked region may be identified directly from such a map without any previously known sex-associated sequence and without knowledge of the sex any samples other than the parents of a family (Brelsford et al. 2016). If this is also possible in newts it would negate the need for a separate study searching for sex-associated markers. However, the large genomes and apparently small sex-linked regions observed in the karyotypes of both *Triturus* and *L. vulgaris* make it questionable whether this approach is reliable. Consequently, for *L. vulgaris*, an association based RADseq approach is also employed, and the markers identified used to benchmark the suitability of a purely linkage mapping based study. The sex-specific performance of any Y-linked markers identified in *L. vulgaris* will also be verified across the genus *Lissotriton*.

Chapter 3

With the Y-linked region of the *Lissotriton* genome identified, the same must be achieved for *Triturus*. The same RADseq methodology used in chapter 2 will be employed for the Balkan crested newt *T. ivanbureschi*. Sex association will be used to identify male-linked RAD markers in sequences from samples of adults of known sex. These markers will then be located a on RADseq-based linkage map. Bioinformatic approaches will be used to determine whether the *Lissotriton* and *Triturus* Y-chromosomes are homologous. Additionally, the Y-linked regions of both genera will be placed on target capture-based linkage which also include genes involved in the *Triturus* balanced lethal system. Finally, the performance of Y-linked markers developed in *T.*

ivanbureschi will be assessed throughout the genus *Triturus*, as multispecies sex-specific markers may be valuable for many future research projects.

Chapter 4

This chapter will focus directly on the genes involved in the *Triturus* balanced lethal system and the identification of structural rearrangements. Target capture sequencing of F2 hybrid families will be used to construct linkage maps for both *Triturus* and *Lissotriton*. As the *Triturus* dataset will include both viable and arrested embryos it will become possible to distinguish alleles associated with each of the chromosome 1 heteromorphs. Via the linkage maps any structural rearrangements between *Triturus* and *Lissotriton* involving these loci will become apparent. Additionally, allele ratio analysis will be used to investigate any change in the copy number of the genes. Finally, the viability of the various hypotheses for the evolution of the *Triturus* balanced lethal system will be discussed in light of the results, and an evolutionary scenario will be modelled.

Chapter 5

In the final chapter of this thesis will summarize and synthesize the findings of the previous three chapters and place them in the context of the surrounding literature. Topics of discussion will include: Which, if any, the different proposed models for the evolution of balanced lethal system best fit the observations made in *Triturus*? Whether the evolutionary processes experienced by *Triturus* are relevant to other naturally occurring balanced lethal systems? What are the broader relationships between the origin of the balanced lethal system and other evolutionary processes, such as genetic surfing and speciation? And, what is the outlook for future research?

References

Albornoz J, Domínguez A (1994) Inversion polymorphism and accumulation of lethals in selected lines of *Drosophila melanogaster*. *Heredity*. 73: 92–97. DOI: 10.1038/hdy.1994.103.

Arntzen JW (2024) Morphological and genetic diversification of pygmy and marbled newts, with the description of a new species from the wider Lisbon Peninsula (*Triturus*, Salamandridae). *Contributions to Zoology*. 93: 178–200. DOI: 10.1163/18759866-bja10057.

Arntzen JW, Jehle R, Bardakci F, Burke T, Wallis GP (2009) Asymmetric viability of reciprocal-cross hybrids between crested and marbled newts (*Triturus cristatus* and *T. marmoratus*). *Evolution*. 63: 1191–1202. DOI: 10.1111/j.1558-5646.2009.00611.x.

Arntzen JW, Üzüm N, Ajduković MD, Ivanović A, Wielstra B (2018) Absence of heterosis in hybrid crested newts. *PeerJ*. 6: e5317. DOI: 10.7717/peerj.5317.

Ayala FJ, Campbell CA (1974) Frequency-dependent selection. *Annual Review of Ecology, Evolution, and Systematics*. 5: 115–138. DOI: 10.1146/annurev.es.05.110174.000555.

Berdan EL, Blanckaert A, Butlin RK, Bank C (2021) Deleterious mutation accumulation and the long-term fate of chromosomal inversions. *PLOS Genetics*. 17: e1009411. DOI: 10.1371/journal.pgen.1009411.

Berdan EL, Blanckaert A, Butlin RK, Flatt T, Slotte T, Wielstra B (2022) Mutation accumulation opposes polymorphism: supergenes and the curious case of balanced lethals. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 377: Article 20210199. DOI: 10.1098/rstb.2021.0199.

Birchler JA, Auger DL, Riddle NC (2003) In search of the molecular basis of heterosis. *The Plant Cell*. 15: 2236–2240. DOI: 10.1105/tpc.151030.

Brelsford A, Dufresnes C, Perrin N (2016) High-density sex-specific linkage maps of a European tree frog (*Hyla arborea*) identify the sex chromosome without information on offspring sex. *Heredity*. 116: 177–181. DOI: 10.1038/hdy.2015.83.

Brown T, Mishra K, Elewa A, Iarovenko S, Subramanian E, Araus AJ, Petzold A, ... Simon A (2025) Chromosome-scale genome assembly reveals how repeat elements shape non-coding RNA landscapes active during newt limb regeneration. *Cell Genomics*. 100761. DOI: 10.1016/j.xgen.2025.100761.

Brust DG (1993) Maternal brood care by *Dendrobates pumilio*: a frog that feeds its young. *Journal of Herpetology*. 27: 96–98. DOI: 10.2307/1564914.

Buckley D, Alcobendas M, García-París M, Wake MH (2007) Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. *Evolution & Development*. 9: 105–115. DOI: 10.1111/j.1525-142X.2006.00141.x.

Burkhardt F, Browne J, Porter DM, Richmond M, eds. (1993) *The Correspondence of Charles Darwin*. Vol. 8 Cambridge University Press ISBN: 978-0-521-44241-1.

Callan HG, Lloyd L, Waddington CH (1960) Lampbrush chromosomes of crested newts *Triturus cristatus* (Laurenti). *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 243: 135–219. DOI: 10.1098/rstb.1960.0007.

Cayrol C, Jaylet A, Ferrier V, Gasser F (1983) A genetic study of various enzyme polymorphisms in *Pleurodeles waltl* (Urodele, Amphibian). III. The relationship between sex-linked peptidase-1 expression and gene-dose effects. *Biochemical Genetics*. 21: 551–559. DOI: 10.1007/BF00484446.

Charlesworth B, Sniegowski P, Stephan W (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature*. 371: 215–220. DOI: 10.1038/371215a0.

Charlesworth D (2006) Balancing selection and its effects on sequences in nearby genome regions. *PLOS Genetics*. 2: e64. DOI: 10.1371/journal.pgen.0020064.

Charlesworth D (2017) Evolution of recombination rates between sex chromosomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372: 20160456. DOI: 10.1098/rstb.2016.0456.

Charlesworth D (2016) The status of supergenes in the 21st century: recombination suppression in Batesian mimicry and sex chromosomes and other complex adaptations. *Evolutionary Applications*. 9: 74–90. DOI: 10.1111/eva.12291.

Charlesworth D, Charlesworth B, Marais G (2005) Steps in the evolution of heteromorphic sex chromosomes. *Heredity*. 95: 118–128. DOI: 10.1038/sj.hdy.6800697.

Cicort-Lucaciu A Ş., Ardeleanu A, Cupsa D, Naghi N, Dalea A (2005) The trophic spectrum of a *Triturus cristatus* (Laurentus 1768) population from Plopiș Mountains area (Bihor County, Romania). *North-Western Journal of Zoology*.

Clarke B (1969) The evidence for apostatic selection. *Heredity*. 24: 347–352. DOI: 10.1038/hdy.1969.52.

Corcoran P, Anderson JL, Jacobson DJ, Sun Y, Ni P, Lascoux M, Johannesson H (2016) Introgression maintains the genetic integrity of the mating-type determining chromosome of the fungus *Neurospora tetrasperma*. *Genome Research*. 26: 486–498. DOI: 10.1101/gr.197244.115.

Cortez D, Marin R, Toledo-Flores D, Froidevaux L, Liechti A, Waters PD, Grützner F, Kaessmann H (2014) Origins and functional evolution of Y chromosomes across mammals. *Nature*. 508: 488–493. DOI: 10.1038/nature13151.

Coyne JA, Meyers W, Crittenden AP, Sniegowski P (1993) The fertility effects of pericentric inversions in *Drosophila melanogaster*. *Genetics*. 134: 487–496. DOI: 10.1093/genetics/134.2.487.

Darwin C (1859) *On the origin of species by means of natural selection, or, The preservation of favoured races in the struggle for life*. John Murray London, England.

Darwin C (1871) *The descent of man, and, Selection in relation to sex.* John Murray London, England.

Davey JW, Blaxter ML (2010) RADSeq: next-generation population genetics. *Briefings in Functional Genomics*. 9: 416–423. DOI: 10.1093/bfgp/elq031.

Dawson PS (1967) A balanced lethal system in the flour beetle, *Tribolium castaneum*. *Heredity*. 22: 435–438. DOI: 10.1038/hdy.1967.52.

Dobzhansky T (1970) *Genetics of the evolutionary process*. Columbia University Press ISBN: 978-0-231-08306-5.

Dobzhansky T, Pavlovsky O (1955) An extreme case of heterosis in a Central American population of *Drosophila tropicalis*. *Proceedings of the National Academy of Sciences of the United States of America*. 41: 289–295.

Felsenstein J (1974) The evolutionary advantage of recombination. *Genetics*. 78: 737–756. DOI: 10.1093/genetics/78.2.737.

Gonzalez DR, Aramendia AC, Davison A (2019) Recombination within the *Cepaea nemoralis* supergene is confounded by incomplete penetrance and epistasis. *Heredity*. 123: 153–161. DOI: 10.1038/s41437-019-0190-6.

Grossen C, Neuenschwander S, Perrin N (2012) The balanced lethal system of crested newts: a ghost of sex chromosomes past? *The American Naturalist*. 180: E174-183. DOI: 10.1086/668076.

Haldane JBS (1922) Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics*. 12: 101–109. DOI: 10.1007/BF02983075.

Hamilton WD (1963) The evolution of altruistic behaviour. *The American Naturalist*. 97: 354–356. DOI: 10.1086/497114.

Haskins CP, Young P, Hewitt RE, Haskins EF (1970) Stabilised heterozygosis of supergenes mediating certain Y-linked colour patterns in populations of *Lebistes Reticulatus*. *Heredity*. 25: 575–589. DOI: 10.1038/hdy.1970.64.

Herman RK, Albertson DG, Brenner S (1976) Chromosome rearrangements in *Caenorhabditis elegans*. *Genetics*. 83: 91–105.

Hillis DM, Green DM (1990) Evolutionary changes of heterogametic sex in the phylogenetic history of amphibians. *Journal of Evolutionary Biology*. 3: 49–64. DOI: 10.1046/j.1420-9101.1990.3010049.x.

Hirsch CD, Evans J, Buell CR, Hirsch CN (2014) Reduced representation approaches to interrogate genome diversity in large repetitive plant genomes. *Briefings in Functional Genomics*. 13: 257–267. DOI: 10.1093/bfgp/elt051.

Horner HA, Macgregor HC (1985) Normal development in newts (*Triturus*) and its arrest as a consequence of an unusual chromosomal situation. *Journal of Herpetology*. 19: 261–270. DOI: 10.2307/1564180.

Hughes KA, Houde AE, Price AC, Rodd FH (2013) Mating advantage for rare males in wild guppy populations. *Nature*. 503: 108–110. DOI: 10.1038/nature12717.

Huxley TH (1888) *On the reception of the 'Origin of Species'*. Vol. 2 pp. 179–204 John Murray. DOI: 10.1037/13919-005.

James SH, Sampson JF, Playford J (1990) Complex hybridity in *Isotoma petraea*. VII. Assembly of the genetic system in the O6 Pigeon Rock population. *Heredity*. 64: 289–295. DOI: 10.1038/hdy.1990.36.

Jay P, Whibley A, Frézal L, Rodríguez de Cara MÁ, Nowell RW, Mallet J, Dasmahapatra KK, Joron M (2018) Supergene evolution triggered by the introgression of a chromosomal inversion. *Current Biology*. 28: 1839-1845.e3. DOI: 10.1016/j.cub.2018.04.072.

Joron M, Papa R, Beltrán M, Chamberlain N, Mavárez J, Baxter S, Abanto M, Bermingham E, Humphray SJ, Rogers J, Beasley H, Barlow K, ffrench-Constant RH, Mallet J, McMillan WO, Jiggins CD (2006) A conserved supergene locus controls colour pattern diversity in *Heliconius* butterflies. *PLOS Biology*. 4: e303. DOI: 10.1371/journal.pbio.0040303.

Kai W, Nomura K, Fujiwara A, Nakamura Y, Yasuike M, Ojima N, Masaoka T, Ozaki A, Kazeto Y, Gen K, Nagao J, Tanaka H, Kobayashi T, Ototake M (2014) A ddRAD-based genetic map and its integration with the genome assembly of Japanese eel (*Anguilla japonica*) provides insights into genome evolution after the teleost-specific genome duplication. *BMC Genomics*. 15: 233. DOI: 10.1186/1471-2164-15-233.

Keinath MC, Timoshevskaya N, Timoshevskiy VA, Voss SR, Smith JJ (2018) Miniscule differences between sex chromosomes in the giant genome of a salamander. *Scientific Reports*. 8: 17882. DOI: 10.1038/s41598-018-36209-2.

Kent TV, Uzunović J, Wright SI (2017) Coevolution between transposable elements and recombination. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372: Article 20160458. DOI: 10.1098/rstb.2016.0458.

Kenvon A (1972) Heterozygous effects on viability of *Drosophila* supergenes that are lethal when homozygous. *Genetica*. 43: 536–551. DOI: 10.1007/BF00115597.

Kirkpatrick M (2010) How and why chromosome inversions evolve. *PLOS Biology*. 8: e1000501. DOI: 10.1371/journal.pbio.1000501.

Küpper C, Stocks M, Risse JE, dos Remedios N, Farrell LL, McRae SB, Morgan TC, Karlionova N, Pinchuk P, Verkuil YI, Kitaysky AS, Wingfield JC, Piersma T, Zeng K, Slate J, Blaxter M, Lank DB, Burke T (2016) A supergene determines highly divergent male reproductive morphs in the ruff. *Nature Genetics*. 48: 79–83. DOI: 10.1038/ng.3443.

Lacroix JC (1970) Demonstration on lampbrush chromosomes of *Pleurodeles poireti Gervais*, urodele amphibia, of a structure linked with sex, identifying the sexual bivalent and labelling the chromosome

W. *Comptes Rendus Hebdomadaires Des Seances De l'Academie Des Sciences. Serie D: Sciences Naturelles*. 271: 102–104.

Lahn BT, Page DC (1999) Four evolutionary strata on the human X chromosome. *Science*. 286: 964–967. DOI: 10.1126/science.286.5441.964.

Lande R (1985) The fixation of chromosomal rearrangements in a subdivided population with local extinction and colonization. *Heredity*. 54: 323–332. DOI: 10.1038/hdy.1985.43.

Larracuente AM, Presgraves DC (2012) The selfish segregation distorter gene complex of *Drosophila melanogaster*. *Genetics*. 192: 33–53. DOI: 10.1534/genetics.112.141390.

Litvinchuk SN, Rosanov JM, Borkin LJ (2007) Correlations of geographic distribution and temperature of embryonic development with the nuclear DNA content in the Salamandridae (Urodela, Amphibia). *Genome*. 50: 333–342. DOI: 10.1139/G07-010.

Macgregor HC (1979) In situ hybridization of highly repetitive DNA to chromosomes of *Triturus cristatus*. *Chromosoma*. 71: 57–64. DOI: 10.1007/BF00426366.

Macgregor HC, Horner H (1980) Heteromorphism for chromosome 1, a requirement for normal development in crested newts. *Chromosoma*. 76: 111–122. DOI: 10.1007/BF00293412.

Mancino G, Nardi I (1971) Chromosomal heteromorphism and female heterogamety in the marbled newt *Triturus marmoratus* (Latreille, 1800). *Experientia*. 27: 821–822. DOI: 10.1007/BF02136887.

Marjanović D, Laurin M (2014) An updated paleontological timetree of lissamphibians, with comments on the anatomy of Jurassic crown-group salamanders (Urodela). *Historical Biology*. 26: 535–550. DOI: 10.1080/08912963.2013.797972.

McDermott SR, Noor MAF (2010) The role of meiotic drive in hybrid male sterility. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 365: 1265–1272. DOI: 10.1098/rstb.2009.0264.

Mertes F, El Sharawy A, Sauer S, van Helvoort JMLM, van der Zaag PJ, Franke A, Nilsson M, Lehrach H, Brookes AJ (2011) Targeted enrichment of genomic DNA regions for next-generation sequencing. *Briefings in Functional Genomics*. 10: 374–386. DOI: 10.1093/bfgp/elr033.

Metcalfe CJ, Filée J, Germon I, Joss J, Casane D (2012) Evolution of the Australian lungfish (*Neoceratodus forsteri*) genome: a major role for CR1 and L2 LINE Elements. *Molecular Biology and Evolution*. 29: 3529–3539. DOI: 10.1093/molbev/mss159.

Meyer A, Schloissnig S, Franchini P, Du K, Woltering JM, Irisarri I, Wong WY, ... Schartl M (2021) Giant lungfish genome elucidates the conquest of land by vertebrates. *Nature*. 590: 284–289. DOI: 10.1038/s41586-021-03198-8.

Miller DE, Cook KR, Hawley RS (2019) The joy of balancers. *PLoS Genetics*. 15: Article e1008421. DOI: 10.1371/journal.pgen.1008421.

Miura I (2017) Sex determination and sex chromosomes in Amphibia. *Sexual Development*. 11: 298–306. DOI: 10.1159/000485270.

Morgan GT (1978) Absence of chiasmata from the heteromorphic region of chromosome I during spermatogenesis in *Triturus cristatus carnifex*. *Chromosoma*. 66: 269–280. DOI: 10.1007/BF00330555.

Muller HJ (1917) An *Oenothera*-like case in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*. 3: 619–626.

Muller HJ (1918) Genetic variability, twin hybrids and constant hybrids, in a case of balanced lethal factors. *Genetics*. 3: 422–499.

Muller HJ (1964) The relation of recombination to mutational advance. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 1: 2–9. DOI: 10.1016/0027-5107(64)90047-8.

Nakamura M (2009) Sex determination in amphibians. *Seminars in Cell & Developmental Biology*. 20: 271–282. DOI: 10.1016/j.semcdb.2008.10.003.

Navarro A, Ruiz A (1997) On the fertility effects of pericentric inversions. *Genetics*. 147: 931–933. DOI: 10.1093/genetics/147.2.931.

Nowoshilow S, Schloissnig S, Fei J-F, Dahl A, Pang AWC, Pippel M, Winkler S, ... Myers EW (2018) The axolotl genome and the evolution of key tissue formation regulators. *Nature*. 554: 50–55. DOI: 10.1038/nature25458.

Orgel LE, Crick FHC (1980) Selfish DNA: the ultimate parasite. *Nature*. 284: 604–607. DOI: 10.1038/284604a0.

Paigen K, Petkov PM (2018) PRDM9 and its role in genetic recombination. *Trends in Genetics*. 34: 291–300. DOI: 10.1016/j.tig.2017.12.017.

Patten MM (2018) Selfish X chromosomes and speciation. *Molecular Ecology*. 27: 3772–3782. DOI: 10.1111/mec.14471.

del Priore L, Pigozzi MI (2015) Heterologous synapsis and crossover suppression in heterozygotes for a pericentric inversion in the zebra finch. *Cytogenetic and Genome Research*. 147: 154–160. DOI: 10.1159/000442656.

Rancilhac L, Irisarri I, Angelini C, Arntzen JW, Babik W, Bossuyt F, Künzel S, Lüddecke T, Pasmans F, Sanchez E, Weisrock D, Veith M, Wielstra B, Steinfartz S, Hofreiter M, Philippe H, Vences M (2021) Phylotranscriptomic evidence for pervasive ancient hybridization among Old World salamanders. *Molecular Phylogenetics and Evolution*. 155: Article 106967. DOI: 10.1016/j.ympev.2020.106967.

Rusconi M (1821) *Amours des salamandres aquatiques: et developpement du tetard de ces salamandres depuis l'oeuf jusqu'a l'animal parfait*. Chez Paolo Emilio Giusti DOI: 10.5962/bhl.title.59626.

Schwander T, Libbrecht R, Keller L (2014) Supergenes and complex phenotypes. *Current Biology*. 24: R288–R294. DOI: 10.1016/j.cub.2014.01.056.

Sessions SK (2008) Evolutionary cytogenetics in salamanders. *Chromosome Research*. 16: 183–201. DOI: 10.1007/s10577-007-1205-3.

Sessions SK, Macgregor HC, Schmid M, Haaf T (1988) Cytology, embryology, and evolution of the developmental arrest syndrome in newts of the genus *Triturus* (Caudata: Salamandridae). *Journal of Experimental Zoology*. 248: 321–334. DOI: 10.1002/jez.1402480311.

Sims SH, Macgregor HC, Pellatt PS, Horner HA (1984) Chromosome 1 in crested and marbled newts (*Triturus*). *Chromosoma*. 89: 169–185. DOI: 10.1007/BF00294996.

Smith JJ, Timoshevskaya N, Timoshevskiy VA, Keinath MC, Hardy D, Voss SR (2019) A chromosome-scale assembly of the axolotl genome. *Genome Research*. 29: 317–324. DOI: 10.1101/gr.241901.118.

Smith RJ (2016) Explanations for adaptations, just-so stories, and limitations on evidence in evolutionary biology. *Evolutionary Anthropology: Issues, News, and Reviews*. 25: 276–287. DOI: 10.1002/evan.21495.

Sparreboom M (2014) *Salamanders of the old world: The salamanders of Europe, Asia and northern Africa*. KNNV Publishing DOI: 10.1163/9789004285620.

Steiner E (1956) New aspects of the balanced lethal mechanism in *Oenothera*. *Genetics*. 41: 486–500.

Stöck M, Horn A, Grossen C, Lindtke D, Sermier R, Betto-Colliard C, Dufresnes C, Bonjour E, Dumas Z, Luquet E, Maddalena T, Sousa HC, Martinez-Solano I, Perrin N (2011) Ever-young sex chromosomes in European tree frogs. *PLOS Biology*. 9: e1001062. DOI: 10.1371/journal.pbio.1001062.

Thomas CA (1971) The genetic organization of chromosomes. *Annual Review of Genetics*. 5: 237–256. DOI: 10.1146/annurev.ge.05.120171.001321.

Thompson MJ, Jiggins CD (2014) Supergenes and their role in evolution. *Heredity*. 113: 1–8. DOI: 10.1038/hdy.2014.20.

Trivers RL (1971) The evolution of reciprocal altruism. *The Quarterly Review of Biology*. 46: 35–57.

Tuttle EM, Bergland AO, Korody ML, Brewer MS, Newhouse DJ, Minx P, Stager M, Betuel A, Cheviron ZA, Warren WC, Gonser RA, Balakrishnan CN (2016) Divergence and functional degradation of a sex chromosome-like supergene. *Current Biology*. 26: 344–350. DOI: 10.1016/j.cub.2015.11.069.

Veith M, Bogaerts S, Pasmans F, Kieren S (2018) The changing views on the evolutionary relationships of extant Salamandridae (Amphibia: Urodela). *PLOS ONE*. 13: e0198237. DOI: 10.1371/journal.pone.0198237.

Waddington CH (1959) Evolutionary adaptation. *Perspectives in Biology and Medicine*. 2: 379–401. DOI: 10.1353/pbm.1959.0027.

Wallace H (1987) Abortive development in the crested newt *Triturus cristatus*. *Development*. 100: 65–72. DOI: 10.1242/dev.100.1.65.

Wallace H (1994) The balanced lethal system of crested newts. *Heredity*. 73: 41–46. DOI: 10.1038/hdy.1994.96.

Wallace H, Wallace BM (2000) Sex reversal of the newt *Triturus cristatus* reared at extreme temperatures. *The International Journal of Developmental Biology*. 44: 807–810.

Wang J, Wurm Y, Nipitwattanaphon M, Riba-Grognuz O, Huang Y-C, Shoemaker D, Keller L (2013) A Y-like social chromosome causes alternative colony organization in fire ants. *Nature*. 493: 664–668. DOI: 10.1038/nature11832.

Wielstra B, McCartney-Melstad E, Arntzen JW, Butlin RK, Shaffer HB (2019) Phylogenomics of the adaptive radiation of *Triturus* newts supports gradual ecological niche expansion towards an incrementally aquatic lifestyle. *Molecular Phylogenetics and Evolution*. 133: 120–127. DOI: 10.1016/j.ympev.2018.12.032.

Zanders SE, Unckless RL (2019) Fertility costs of meiotic drivers. *Current Biology*. 29: R512–R520. DOI: 10.1016/j.cub.2019.03.046.

Zheng B, Sage M, Cai W-W, Thompson DM, Tavsanli BC, Cheah Y-C, Bradley A (1999) Engineering a mouse balancer chromosome. *Nature Genetics*. 22: 375–378. DOI: 10.1038/11949.

Zhou Q, Zhang J, Bachtrog D, An N, Huang Q, Jarvis ED, Gilbert MTP, Zhang G (2014) Complex evolutionary trajectories of sex chromosomes across bird taxa. *Science*. 346: 1246338. DOI: 10.1126/science.1246338.