CASE STUDY

Solving Ohno's puzzle — resolving an ancient sex chromosome system

In the 1960s, prominent cytogeneticist Susumu Ohno described an atypical sex chromosome system in the creeping vole (*Microtus Oregoni*). He proposed that females had an XO and males had an XY karyotype; with males contributing either the Y chromosome or no sex chromosome during gamete development. Although sparking much debate at the time, the mechanisms behind this puzzle remained unresolved for almost 60 years, until the recent work of Couger *et al.* (2021)[1,2].

An initial dive into this fascinating system saw the use of short-read sequencing of male and female vole genomic DNA and pointed to a male-specific X chromosome. Intriguingly, amplicon sequencing of conserved Y chromosome genes revealed their presence in both male and female voles. No such Y chromosome genes were detected in females of closely related *Microtus* species, indicating that this system had been evolving independently for approximately 150 million years.

The placement and order of these Y genes was, however, still unclear, and so the team performed genome assembly with long reads. Despite the initial long-read assembly being '*quite excellent*', sex chromosome contigs were shorter than autosomal contigs, likely due to the highly repetitive nature of the sex chromosomes[1].

> ***"[With nanopore ultra-long reads] we were able to bridge the regions we were really interested in, which we had not with any of the other technologies we tried[1]"***

To resolve this, the team turned to ultra-long nanopore sequencing reads. Using the recently released Ultra-Long DNA Sequencing Kit, they got an '*amazing turnaround time for data*' from two PromethION Flow Cells, with an N50 of 91 kb, and a significant proportion of their reads being ultra-long (>50 kb)[1].

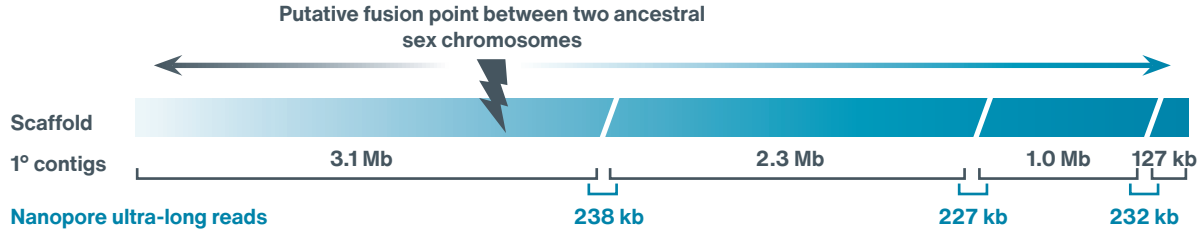

Figure 1
Ultra-long nanopore reads closed the gaps in the initial assembly of the repetitive vole sex chromosome regions of interest. Figure courtesy of Matthew Brian Couger, Brigham and Women's Hospital, USA[1].

The high-quality, ultra-long nanopore data aligned '*extremely well*' to the genome, and further supported accurate SNP calling and phasing[1]. Highlighting a 410 kb read that aligned to a region of the sex chromosome, lead researcher Matthew Brian Couger commented: '*that's a really solid contig for most peoples' assemblies, not their actual read generation*'. With these long reads, unassembled regions of the repeat-rich sex chromosomes were connected (**Figure 1**). The position of genes relative to each other, both within a chromosome, and between the paternal X and maternal X chromosomes, was revealed.

And so Ohno's puzzle was resolved, revealing a unique sex chromosome system whereby male gametes contain either a paternal X chromosome, generating male offspring, or no X chromosome, giving rise to female offspring (**Figure 2**).

Products used

Kit	Ultra-Long DNA Sequencing Kit
Device	PromethION
Tools	Winnowmap2 (alignment)

Find out more: nanoporetech.com/products

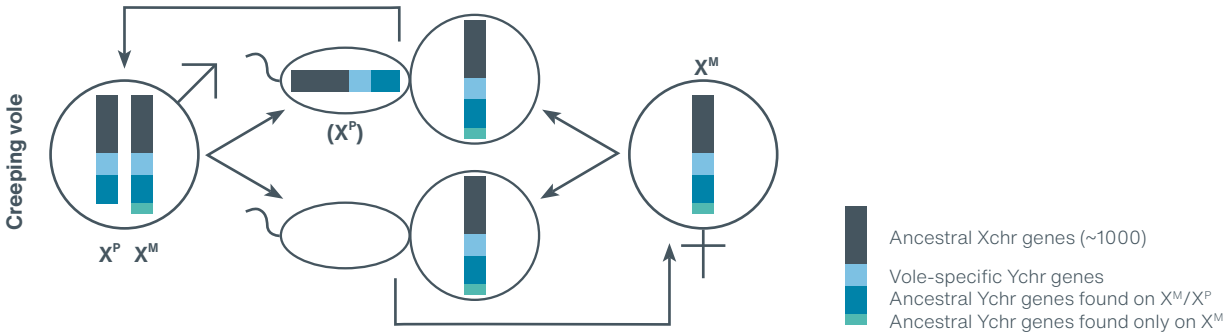

Figure 2
Accurate mapping of the maternal and paternal X chromosomes supported a new model of sex determination in the creeping vole. Males have both maternal (X^M) and paternal (X^P) X chromosomes, with Xist-based silencing of the paternal copy, while females inherit just one X chromosome (X^M) from the mother. Figure adapted from Couger *et al.* (2021)[2].

>70 Gb
Ultra-long read data

91 kb
Read N50

2.48 Mb
Max read length

NANOPORE SEQUENCING

- enabled assembly of large, highly repetitive genomic regions using ultra-long reads
- delivered fast access to results through rapid workflows and real-time basecalling

> ***"Ultra-long read data generated superior alignments even in repetitive regions[1]"***

Find out more about using nanopore sequencing for animal genomics: nanoporetech.com/applications/animal-genomics

References

1. Couger, M.B. Presentation. Available at: https://nanoporetech.com/resource-centre/video/lc21/ultra-long-nanopore-sequencing-for-assembly-and-scaffolding-of-sex-chromosomes [Accessed 20 January 2022]
2. Couger, M.B. et al. Science.372(6542):592-600 (2021).