Improving genomic tools to investigate ivermectin resistance in *Teladorsagia circumcincta*

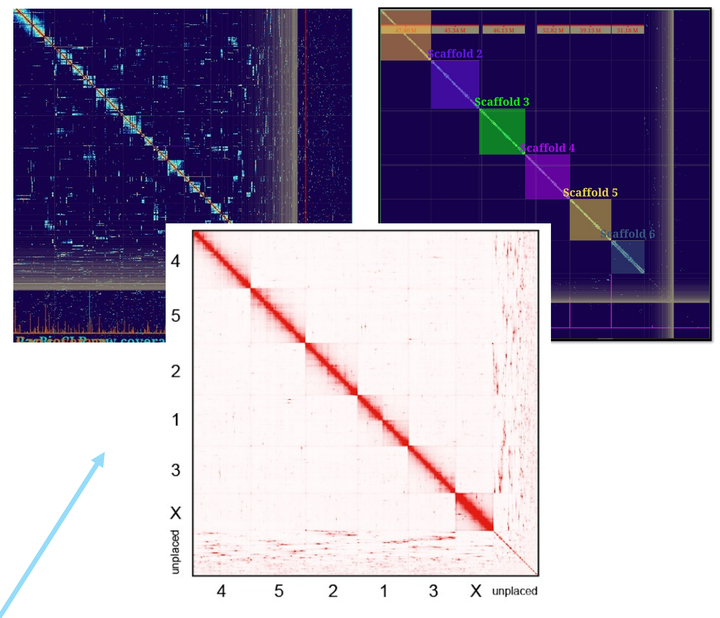

Jenni McIntyre [1], Kirsty Maitland [1], Duncan Berger [2], Alison Morrison [3] David Bartley [3], Katie Bull [4], Hannah Vineer [5], Eric Morgan [6], Neil Sargison [7], Nancy Holroyd [2], Alan Tracey [2], Matt Berriman [1,2], Sam Dougan [2], Dio Grigoriadis [2], James Cotton [1,2], Dan Price [3], Al Nisbet [3], Eileen Devaney [1], Roz Laing [1], **Stephen R. Doyle [2]**

Contact: jennifer.mcintyre@glasgow.ac.uk, stephen.doyle@sanger.ac.uk

[1] University of Glasgow, UK, [2] Wellcome Sanger Institute, UK, [3] Moredun Research Institute, UK, [4] University of Bristol, UK, [5] University of Liverpool, UK, [6] Queen's University Belfast, UK, [7] Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

Why *Teladorsagia circumcincta* and ivermectin?

- Primary gastrointestinal nematode pathogen of small ruminants in temperate climates
- Causes severe welfare and production issues – concern for food sustainability if unable to control infections
- Ivermectin is an endectocide, used to treat both animals and humans (MDA programs); resistance is widespread and increasing
- The genetic mechanisms driving ivermectin resistance (in any parasitic nematode) are unknown



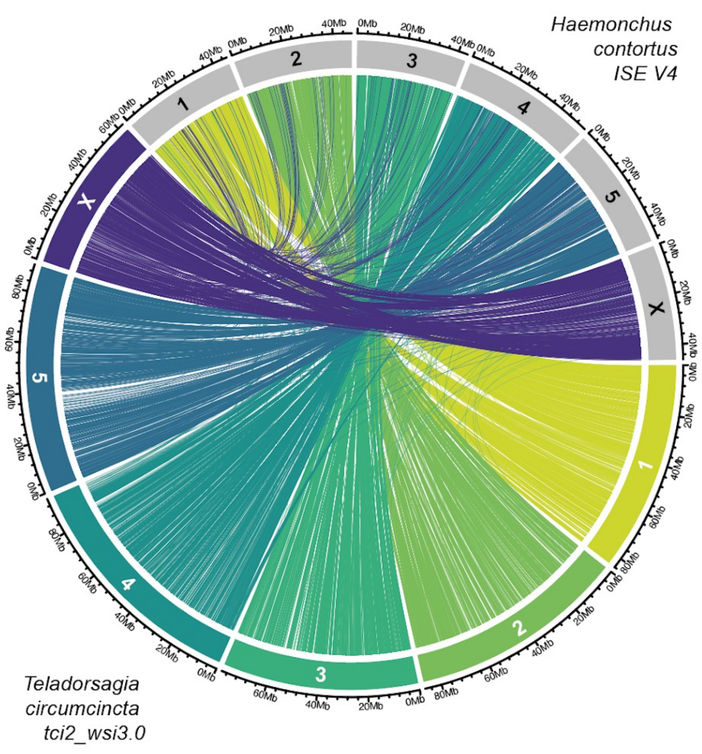
1. We used PacBio long-read sequencing and Illumina Hi-C to assemble chromosome-scale scaffolds

2. We further improved the genome, in particular the coding regions;
   a) Corrected tiny indels using RNA-Seq data
   b) Removed haplotypic duplicates (incorporation errors) and recovered missing genes (on contigs incorrectly discarded by software), facilitated by BUSCO tools
   c) Removed contaminating sequences (not *T. circumcincta*) with BlobTools

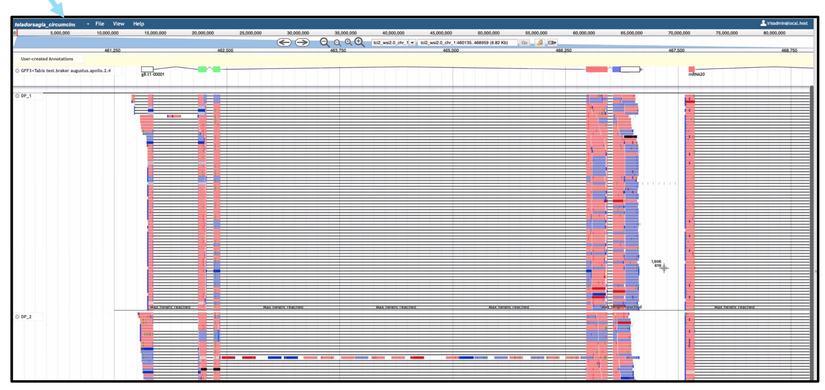
Genome assembly	Assembly size (Mb)	Scaffolds (n)	N50 (Mb)	Largest (Mb)	Genes (n)	Complete BUSCOs (%) Genome [a]	Complete BUSCOs (%) Protein [a]
T. circumcincta tci2_wsi3.0	573.0	1,286	84.0	94.8	22,948	85.2	96.3
T. circumcincta WASHU	700.6	81,734	0.047	1.5	25,572	67.4	40.2
T. circumcincta DNAZOO [b]	593.2	52,507	57.1	66.6	28,082	65.8	na
H. contortus ISE V4	283.4	7	47.4	51.8	19,778	83.5	96.2
C. elegans [c]	100.3	7	17.5	20.9	18,178	98.8	100.0

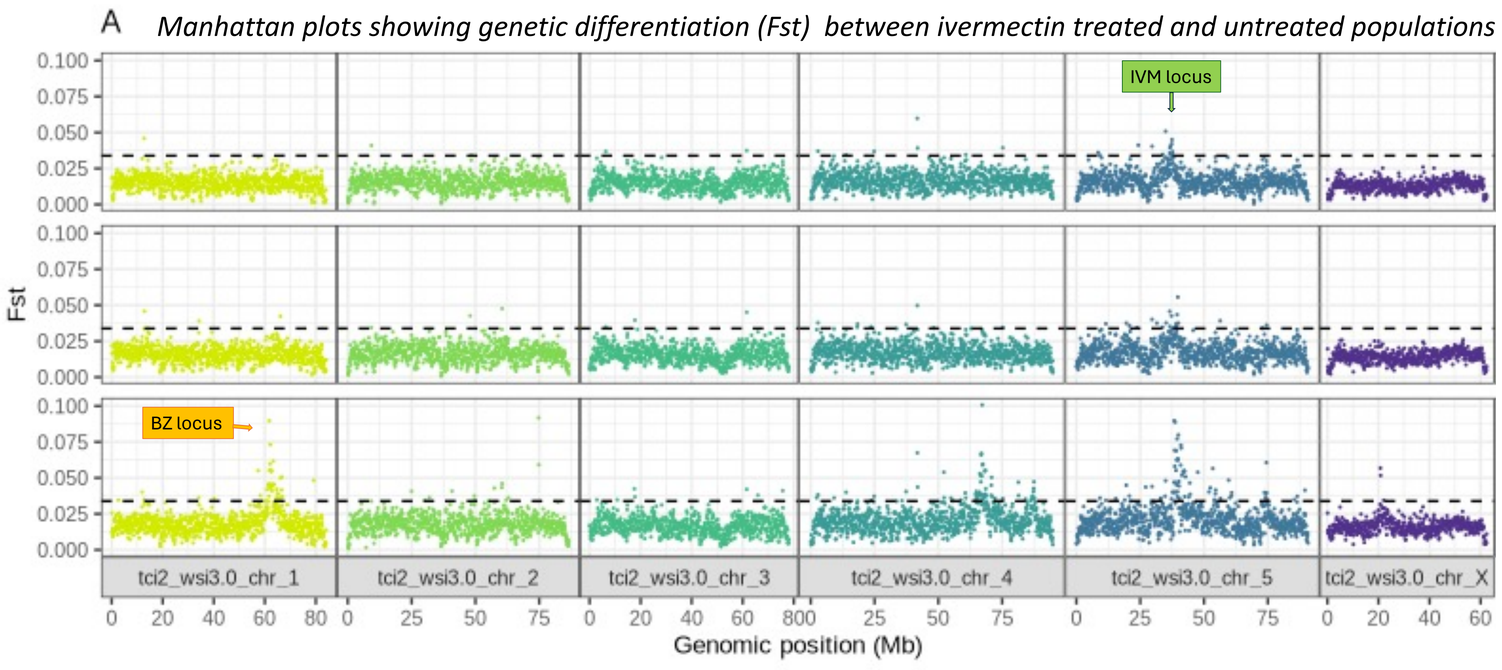
Table legend:
a. Genome completeness was assessed using BUSCO (version 5.6.1) with the Nematoda lineage dataset.
b. The number of genes and transcripts for the DNAZOO assembly were obtained from the original publication as reported by the authors, as no annotation was publicly available. Because of this, protein BUSCO scores for the protein sequences were not determined.
c. Caenorhabditis elegans gene count is based on "protein_coding_primary_transcript" from its GFF annotation from WormBase ParaSite.

Current genome assembly following our work



3. We finalised the annotation using BRAKERv3, including RNA-Seq from *T. circumcincta* and protein information from *Haemonchus contortus,* a related nematode, as input, followed by further manual curation of 3000+ genes in Apollo





UK Farm 1: Pre vs Post

UK Farm 2: Pre vs Post

Between UK Farms: Post 1 vs Post 2

Re-analysis of Choi et al 2017 data: IVM-treated males of NZ triple resistant backcross vs NZ sensitive isolate

Next, we investigated the genetic basis of anthelmintic resistance using Pool-Seq:

1. Locus under ivermectin selection on Chr V
2. Different genes to the *H. contortus* ivermectin XQTL locus (Doyle et al 2022, Cell Reports)
3. Historical selection with benzimidazoles; a Chr I locus containing *beta-tubulin isotype-1* (comparing between farms)
4. Re-analysis of NZ backcross data (Choi et al 2017, PLOS Genetics) further identified a peak on Chr X, containing *acr-8*, which may be related to levamisole resistance

Check out:
- *Benedict's poster on T. circumcincta, IVM-R and TST #81*
- *Sam's poster on in vitro culture of T. circumcincta #44*
- *Paul's poster on IVM-R in cattle GINs #34*
- *Sirapat's poster on diagnostics for cattle lungworm #96*

Conclusions

- Region under ivermectin selection on Chr V is the same on two different UK farms
- Genetic basis of ivermectin resistance appears to differ between *H. contortus* and *T. circumcincta*
- Choi backcross may have bottlenecked the population too severely (very broad peaks)

What could you do with the genome? What could you do with your species genome?

Thanks for reading!
Github: SheepwormJM , stephenrdoyle

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