DATA NOTE

The genome sequence of the drone fly, *Eristalis tenax* (Linnaeus, 1758) [version 1; peer review: 1 approved, 1 approved with reservations]

William Hawkes¹, Karl Wotton ¹,
University of Oxford and Wytham Woods Genome Acquisition Lab,
Darwin Tree of Life Barcoding collective,
Wellcome Sanger Institute Tree of Life programme,
Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective,
Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

¹Centre for Ecology and Conservation, University of Exeter, Penryn, UK

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Abstract

We present a genome assembly from an individual female *Eristalis tenax* (the drone fly; Arthropoda; Insecta; Diptera; Syrphidae). The genome sequence is 487 megabases in span. The majority of the assembly (96.50%) is scaffolded into six chromosomal pseudomolecules, with the X sex chromosome assembled.

Keywords

Eristalis tenax, drone fly, genome sequence, chromosomal, Diptera

This article is included in the [Tree of Life](#) gateway.

Open Peer Review

Approval Status ? ✓

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version 1	?	✓
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1. Kamil S. Jaron , University of Edinburgh, Edinburgh, UK		
2. Massimiliano Virgilio , Royal Museum for Central Africa, Tervuren, Belgium		
Sam Vanbergen , Royal Museum for Central Africa, Tervuren, Belgium		

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Darwin Tree of Life Consortium (mark.blaxter@sanger.ac.uk)

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Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha; Syrphoidea; Syrphidae; Eristalinae; Eristalini; *Eristalis*; *Eristalis tenax* (Linnaeus 1758) (NCBI:txid198635).

Background

The dronefly, *Eristalis tenax* (Figure 1), is perhaps the most widespread of the hoverflies, with a cosmopolitan distribution. It is common in the United Kingdom and Ireland, where it can be found in the springtime following the emergence of females from overwintering in sheltered cavities in caves or buildings (Ball & Morris, 2013). They are at their most numerous in the UK during the late summer and autumn as the local population is augmented by migratory influxes from mainland Europe. Large southward migrations have been observed during the autumn in the UK, Europe and North America (Aubert *et al.*, 1976; Owen, 1956; Shannon, 1926). *E. tenax* is a fairly large hoverfly separated from others in the *Eristalis* genus by the presence of a

broad, black longitudinal face-stripe, longitudinal dark stripes on their eyes, and a distinctly enlarged and curved hind tibia. Like their sister species *Eristalis pertinax*, *E. tenax* is a visual, acoustic and behavioural mimic of the honeybee, *Apis mellifera* (Golding *et al.*, 2001; Moore & Hassall, 2016). Males are territorial, aggressively defending patches of flowers from conspecifics and other flying insects (Wellington & Fitzpatrick, 1981). Their larvae, colloquially known as rat-tailed maggots, live in a wide array of organically rich pools, feeding on decaying organic matter. They therefore play an important ecological role in terms of decomposition (Hurtado *et al.*, 2008). In addition, *E. tenax* is an important pollinator, visiting a wide range of crops and wild plants, while its role as a migratory pollinator may be particularly important for geographically isolated plant populations (Doyle *et al.*, 2020; Pérez-Bañón *et al.*, 2007; Rader *et al.*, 2020). *E. tenax* can be reared and has been the subject of numerous investigations into its biology, including studies on population structure, flight, mimicry, vision and behaviour (Francuski & Milankov, 2015; Golding *et al.*, 2001; Lunau *et al.*, 2018; Nicholas *et al.*, 2018; Straw *et al.*, 2006). This is the first production of a high quality *Eristalis tenax* genome, and we believe that the sequence described here, generated as part of the Darwin Tree of Life project, will further aid understanding of the biology and ecology of this hoverfly.

Genome sequence report

The genome was sequenced from a single female *E. tenax* collected from Wytham Great Wood, Oxfordshire, UK (latitude 51.769, longitude -1.33). A total of 36-fold coverage in Pacific Biosciences single-molecule long reads (N50 12 kb) and 60-fold coverage in 10X Genomics read clouds (from molecules with an estimated N50 of 60 kb) were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 71 missing/misjoins and removed 11 haplotypic duplications, reducing the assembly length by 1.92% and the scaffold number by 31.28%, and increasing the scaffold N50 by 72.23%.

The final assembly has a total length of 487 Mb in 157 sequence scaffolds with a scaffold N50 of 77.1 Mb (Table 1). The majority, 96.50%, of the assembly sequence was assigned to 6 chromosomal-level scaffolds, representing 5 autosomes (numbered by sequence length), and the X sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO (Simão *et al.*, 2015) completeness of 96.6% using the diptera_odb10 reference set. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Methods

Sample acquisition and nucleic acid extraction

A female (idEriTena2) and a male (idEriTena3) *E. tenax* were collected from Wytham Great Wood, Oxfordshire, UK (latitude 51.769, longitude -1.33) by Will Hawkes, University of Exeter, who also identified the sample. The samples were collected using a net, snap-frozen on dry ice and stored in a CoolRack.

Figure 1. Top, image of the idEriTena2 specimen used for sequencing, with 43.9 mm FluidX sample tube for scale. Below, example image of an *Eristalis tenax* individual (not the sampled specimen) taken by William Hawkes.

Table 1. Genome data for *Eristalis tenax*, idEriTena2.1.

Project accession data	
Assembly identifier	idEriTena2.1
Species	<i>Eristalis tenax</i>
Specimen	idEriTena2 (genome assembly, Hi-C); idEriTena3 (RNA-Seq)
NCBI taxonomy ID	219539
BioProject	PRJEB43032
BioSample ID	SAMEA7520160
Isolate information	Female, head/thorax (genome assembly)
Raw data accessions	
PacificBiosciences SEQUEL II	ERR6565938
10X Genomics Illumina	ERR6054456-ERR6054459
Hi-C Illumina	ERR6054460
Illumina polyA RNA-Seq	ERR6054461
Genome assembly	
Assembly accession	GCA_905231855.1
Accession of alternate haplotype	GCA_905231845.1
Span (Mb)	487
Number of contigs	290
Contig N50 length (Mb)	6.65
Number of scaffolds	157
Scaffold N50 length (Mb)	77.07
Longest scaffold (Mb)	141
BUSCO* genome score	C:96.6%[S:95.9%,D:0.7%],F:0.8%, M:2.6%,n:3285

*BUSCO scores based on the diptera_odb10 BUSCO set using v5.1.2. C= complete [S= single copy, D=duplicated], F=fragmented, M=missing, n=number of orthologues in comparison. A full set of BUSCO scores is available at <https://blobtoolkit.genomehubs.org/view/idEriTena2.1/dataset/CAJNIU01/busco>.

DNA was extracted from the head/thorax of idEriTena2 at the Wellcome Sanger Institute Scientific Operations core from the whole organism using the Qiagen MagAttract HMW DNA kit, according to the manufacturer's instructions. RNA was extracted from head/thorax tissue of idEriPert3 in the Tree of Life Laboratory at the Wellcome Sanger Institute using TRIzol (Invitrogen), according to the manufacturer's instructions. RNA was then eluted in 50 µl RNase-free water and its concentration assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit RNA Broad-Range (BR) Assay kit. Analysis of the integrity of the RNA was done using Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics Chromium read cloud sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II (HiFi), Illumina HiSeq X (10X) and Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were generated from the abdomen tissue of idEriTena2 using the Arima v1 Hi-C kit and sequenced on HiSeq X.

Scaffold statistics

- Log10 scaffold count (total 160)
- Scaffold length (total 490M)
- Longest scaffold (140M)
- N50 length (77M)
- N90 length (66M)

BUSCO diptera_odb10 (3285)

- Complete (96.6%)
- Fragmented (0.8%)
- Duplicated (0.7%)
- Missing (2.6%)

Figure 2. Genome assembly of *Eristalis tenax*, idEriTena2.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 487,019,564 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (140,698,496 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (77,068,984 and 65,930,618 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated and missing BUSCO genes in the diptera_odb10 set is shown in the top right. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/idEriTena2.1/dataset/CAJNIU01/snail>.

Figure 3. Genome assembly of *Eristalis tenax*, idEriTena2.1: GC coverage. BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/idEriTena2.1/dataset/CAJNIU01/blob>.

Genome assembly

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021); haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020) with the -e flag. One round of polishing was performed by aligning 10X Genomics read data to the assembly with longranger align, calling variants with

freebayes (Garrison & Marth, 2012). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using SALSA2 (Ghurye *et al.*, 2019). The assembly was checked for contamination and corrected using the gEVAL system (Chow *et al.*, 2016) as described previously (Howe *et al.*, 2021). Manual curation was performed using gEVAL, HiGlass

Figure 4. Genome assembly of *Eristalis tenax*, idEriTena2.1: cumulative sequence. BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/idEriTena2.1/dataset/CAJNIU01/cumulative>.

(Kerpedjiev *et al.*, 2018) and Pretext. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2021). The genome was analysed and BUSCO scores generated

within the BlobToolKit environment (Challis *et al.*, 2020). Table 3 contains a list of all software tool versions used, where appropriate.

Figure 5. Genome assembly of *Eristalis tenax*, idEriTena2.1: Hi-C contact map. Hi-C contact map of the idEriTena2.1 assembly, visualised in HiGlass.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Eristalis tenax*, idEriTena2.

INSDC accession	Chromosome	Size (Mb)	GC%
HG993124.1	1	140.7	41
HG993125.1	2	97.83	41.9
HG993126.1	3	77.07	42.7
HG993127.1	4	66.31	43.1
HG993128.1	5	65.93	42.7
HG993129.1	X	16.18	42.7
HG993130.1	MT	0.02	18.8
-	Unplaced	22.99	42.8

Table 3. Software tools used.

Software tool	Version	Source
Hifiasm	0.12	Cheng et al., 2021
purge_dups	1.2.3	Guan et al., 2020
SALSA2	2.2	Ghurye et al., 2019
longranger align	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
freebayes	1.3.1-17-gaa2ace8	Garrison & Marth, 2012
MitoHiFi	1	Uliano-Silva et al., 2021
gEVAL	N/A	Chow et al., 2016
HiGlass	1.11.6	Kerpedjiev et al., 2018
PretextView	0.1.x	https://github.com/wtsi-hpag/PretextView
BlobToolKit	2.6.2	Challis et al., 2020

Data availability

European Nucleotide Archive: *Eristalis tenax* (the dronefly). Accession number [PRJEB43032](#); <https://identifiers.org/ena.embl/PRJEB43032>.

The genome sequence is released openly for reuse. The *E. pertinax* genome sequencing initiative is part of the [Darwin Tree of Life](#) (DTOL) project. All raw sequence data and the assembly have been deposited in INSDC databases. The genome will be annotated using the RNA-Seq data and presented through the Ensembl pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in [Table 1](#).

Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: <https://doi.org/10.5281/zenodo.4789929>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.4893704>.

Members of the Wellcome Sanger Institute Tree of Life programme are listed here: <https://doi.org/10.5281/zenodo.5377053>.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: <https://doi.org/10.5281/zenodo.4790456>.

Members of the Tree of Life Core Informatics collective are listed here: <https://doi.org/10.5281/zenodo.5013542>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.4783559>.

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Open Peer Review

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Reviewer Report 25 April 2022

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Massimiliano Virgilio

Department of Biology, Royal Museum for Central Africa, Tervuren, Belgium

Sam Vanbergen

Royal Museum for Central Africa, Tervuren, Belgium

Technical paper presenting a first chromosome-level assembly for the cosmopolitan pollinator fly *Eristalis tenax* (Diptera, Syrphidae). Clearly presented wet- and dry-lab pipelines and robust methodological approach relying on well established procedures. A relevant contribution to genomic research on Syrphidae. Just for the sake of clarity, it would be nice to have additional info on the amount of DNA/RNA recovered and used in library prep.

Additional input from co-reviewer S. Vanbergen (RMCA):

There might be instances of mixed-up IDs/species names. It is mentioned "RNA was extracted from head/thorax tissue of idEriPert3", shouldn't this be IdEriTena3? In table 1 the IDs appear to be correct. Likewise, in the data availability section the "*E. pertinax* genome sequencing initiative" is brought up. I presume this should be the "*E. tenax* genome sequencing initiative" instead.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: molecular ecology and systematics of Diptera, integrative -omic approaches to taxonomy

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 14 April 2022

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Kamil S. Jaron

Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK

I very much welcome another dipteran genome for I was always interested in the remarkable diversity of sex determination in this taxon.

The only available information regarding *E. tenax* karyotype I found is <http://dx.doi.org/10.13005/bbra/2672>, which is suggesting *E. tenax* has XY sex determination. Perhaps I would mention that Y is missing in the assembly (given a female was sequenced). Furthermore, the same study proposes that the X chromosome is actually of a similar size to other smaller autosomes, but in this assembly, it seems to be a lot shorter (16.18 Mbp compared to 60Mbp+). Could you elaborate on the difference?

Related to this issue, there is no indication in the paper on how the X chromosome was identified in this species. How do we know which one is X?

Otherwise, It is a really interesting genome, I am very pleased to see the Chr 1 assembled in full glory in 140Mbp!

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Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Partly

Are sufficient details of methods and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Evolutionary genomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.