DATA NOTE

The genome sequence of a hoverfly, Xanthogramma pedissequum (Harris, 1776) [version 1; peer review: 2 approved]

Olga Sivell1, Duncan Sivell1, Natural History Museum 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

1Department of Life Sciences, Natural History Museum, London, UK

Abstract
We present a genome assembly from an individual male Xanthogramma pedissequum (Arthropoda; Insecta; Diptera; Syrphidae). The genome sequence is 977 megabases in span. The majority of the assembly (95.94%) is scaffolded into six chromosomal pseudomolecules, with the X and Y sex chromosomes assembled.

Keywords
Xanthogramma pedissequum, genome sequence, chromosomal, Diptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✔ ✔

version 1
02 Feb 2022

1. Emma Bailey1, Rothamsted Research, Harpenden, UK

2. Ching-Ho Chang2, Fred Hutchinson Cancer Research Center, Seattle, USA

Any reports and responses or comments on the article can be found at the end of the article.
Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha; Syrphoidea; Syrphidae; Syrphinae; Syrphini; Xanthogramma; Xanthogramma pedissequum (Harris, 1776) (NCBI:txid414876).

Background
Xanthogramma pedissequum is a black and yellow wasp-mimic from the family Syrphidae (Diptera), commonly known as hoverflies. Xanthogramma pedissequum is found in grassland habitats, woodland rides and in suburban gardens. It occurs predominantly in southern Britain with a few scattered records reaching Scotland (Ball et al., 2015; Speight, 2017). The flight period is from May to September, peaking in late June to early July (Speight & Sarthou, 2017; Stubbs et al., 2002). Adults are often found flying low among tall plants or sitting on low growing vegetation. They feed on flowers of umbellifers and yellow composites. The larvae are predators of root aphids tended by Lasius ants (Hymenoptera: Formicidae, Lasius Fabricius, 1804) (Speight, 2017).

This species strongly resembles Xanthogramma stackelbergi, which was reported in Britain for the first time in 2012 (Stubbs, 2012b)). Keys separating those two species have been provided by Speight (2010), Speight & Sarthou (2017) and Stubbs (2012a). Both species have a yellow vertical stripe on the side of the thorax and an absence of other markings in this area would indicate X. pedissequum, but this character is variable and some X. pedissequum have additional yellow markings, similar to X. stackelbergi. Any Xanthogramma specimens with 3–5 yellow markings on the side of the thorax will therefore need to be identified using other characters, many of which are also variable (Stubbs, 2012a).

The three species within the Xanthogramma pedissequum group: X. dives (predominantly Mediterranean), X. pedissequum and X. stackelbergi cannot be separated using COI sequences. However, X. pedissequum can be distinguished based on ITS2 sequence (Nedeljković et al., 2018).

The high-quality genome sequence described here is the first one reported for Xanthogramma pedissequum and has been generated as part of the Darwin Tree of Life project. It will aid research on the taxonomy, biology and ecology of the species.

Genome sequence report
The genome was sequenced from a single female X. pedissequum (Figure 1) collected from Wigmore Park, Luton, UK (latitude 51.88378, longitude -0.36861422). A total of 36-fold coverage in Pacific Biosciences single-molecule long reads and 57-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 501 missing/misjoins and removed 14 haplotypic duplications, reducing the assembly size by 0.59% and the scaffold number by 23.90%, and increasing the scaffold N50 by 166.84%.

The final assembly has a total length of 977 Mb in 484 sequence scaffolds with a scaffold N50 of 248.7 Mb (Table 1). The majority, 95.94%, of the assembly sequence was assigned to six chromosomal-level scaffolds, representing four autosomes (numbered by sequence length), and the X and Y sex chromosome (Figure 2–Figure 5; Table 2). Chromosome 1 contains a large, heterochromatic region of low confidence at approximately 113.44–242.17 Mb. This block consists of numerous scaffolds with high repeat content that can be localised to chromosome one but their order and orientation is unsure. Hi-C
data indicates that the region 32.39–38.38Mb on Chromosome X has a strong association with scaffolds labelled as Chromosome Y and Y_unloc. This highly repetitive region is likely misassembled containing data from both X and Y that was unable to be separated due to the limitations of current technologies. The assembly has a BUSCO v5.1.2 (Manni et al., 2021) completeness of 95.5% (single 94.5%, duplicated 1.1%) 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 single male Xanthogramma pedissequum was collected from Wigmore Park (51.88378, -0.36861422), Percival Way, Wigmore, Luton, England, by Olga Sivell, Natural History Museum, London, using a sweep net. The morphological identification was provided by Duncan Sivell, Natural History Museum, London, based on Stubbs, Falk, and Others 2002; Ball et al., 2015; Speight, 2010; Speight & Sarthou, 2017; Stubbs, 2012a). The sample was snap-frozen using dry ice and stored in a CoolRack.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The idCorMarg1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Thorax tissue was disrupted to a fine powder using a Nippi Powermasher fitted with a BioMasher pestle. Fragment size analysis of 0.01–0.5 ng of DNA was then performed using an Agilent FemtoPulse. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. Low molecular weight DNA was removed from
Figure 2. Genome assembly of *Xanthogramma pedissequum*, idXanPedi1.1: 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 977,171,468 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 (346,874,609 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (248,688,513 and 101,208,079 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/idXanPedi1.1/dataset/CAJVC001/snail.

A 200-ng aliquot of extracted DNA using 0.8X AMPure XP purification kit prior to 10X Chromium sequencing; a minimum of 50 ng DNA was submitted for 10X sequencing. HMW DNA was sheared into an average fragment size between 12–20 kb in a Megaruptor 3 system with speed setting 30. Sheared DNA was purified by solid-phase reversible immobilisation using AMPure PB beads with a 1.8X ratio of beads to sample to remove the shorter fragments and concentrate the
DNA sample. The concentration of the sheared and purified DNA was assessed using a ThermoFisher Nanodrop spectrophotometer and Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

**Sequencing**

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries were constructed according to the manufacturers’ instructions. DNA sequencing was performed by the Scientific Operations core at the WSI...
Figure 4. Genome assembly of *Xanthogramma pedissequum*, idXanPedi1.1: 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/idXanPedi1.1/dataset/CAJVC001/cumulative.

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). 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 as described previously (Howe et al., 2021). Manual curation (Howe et al., 2021) was performed using HiGlass (Kerpedjiev et al., 2018) and Pretext. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva et al., 2021), which performed annotation using MitoFinder (Allio et al., 2020). 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.

Table 2. Chromosomal pseudomolecules in the genome assembly of Xanthogramma pedissequum, idXanPedi1.1.

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|-----|
| OU343160.1      | 1          | 346.88    | 34.5|
| OU343161.1      | 2          | 248.69    | 33.8|
| OU343162.1      | 3          | 195.21    | 33.2|
| OU343163.1      | 4          | 101.21    | 33.3|
| OU343164.1      | X          | 38.39     | 34.5|
| OU343165.1      | Y          | 2.99      | 36.1|
| OU343166.1      | MT         | 0.02      | 16.8|
| -               | Unplaced   | 43.79     | 36.0|

Figure 5. Genome assembly of Xanthogramma pedissequum, idXanPedi1.1: Hi-C contact map. Hi-C contact map of the idXanPedi1.1 assembly, visualised in HiGlass. Chromosomes are given in order of size, from left to right and top to bottom.
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            | 2.0     | Uliano-Silva et al., 2021                  |
| HiGlass             | 1.11.6  | Kerpedjiev et al., 2018                    |
| PretextView         | 0.2.x   | https://github.com/wtsi-hpag/PretextView    |
| BlobToolKit         | 2.6.4   | Challis et al., 2020                       |

Ethics/compliance issues
The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the Darwin Tree of Life Project Sampling Code of Practice. By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project. Each transfer of samples is further undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Darwin Tree of Life Partner, Genome Research Limited (operating as the Wellcome Sanger Institute), and in some circumstances other Darwin Tree of Life collaborators.

Data availability
European Nucleotide Archive: Xanthogramma pedissequum (hoverfly). Accession number PRJEB45174; https://identifiers.org/ena.embl/PRJEB45174.

The genome sequence is released openly for reuse. The X. pedissequum 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 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 Natural History Museum Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.5746819.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.5744972.

Members of the Wellcome Sanger Institute Tree of Life programme are listed here: https://doi.org/10.5281/zenodo.5744840.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/zenodo.5746904.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5743293.

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.5638618.

References
Allio R, Schomaker-Bastos A, Romiguier J, et al.: MitoFinder: Efficient Automated Large-Scale Extraction of Mitogenomic Data in Target Enrichment Phylogenomics. Mol Ecol Resour. 2020; 20(4): 892–905. PubMed Abstract | Publisher Full Text | Free Full Text

Ball S, Ball SG, Morris R: Britain’s Hoverflies: A Field Guide - Revised and Updated Second Edition. Princeton University Press, 2015. Reference Source
Challis R, Richards E, Rajan J, et al.: BlobToolKit--Interactive Quality
Assessment of Genome Assemblies. G3 (Bethesda). 2020; 10(4): 1361–1374. PubMed Abstract | Publisher Full Text | Free Full Text
Cheng H, Concepcion GT, Feng X, et al.: Haplotype-Resolved de Novo Assembly Using Phased Assembly Graphs with Hifiasm. Nat Methods. 2021; 18(2): 170–175. PubMed Abstract | Publisher Full Text | Free Full Text
Garrison E, Marth G: Haplotype-Based Variant Detection from Short-Read Sequencing. 2012; arXiv: 1207.3907. Reference Source
Ghurye J, Rhie A, Walenz BP, et al.: Integrating Hi-C Links with Assembly Graphs for Chromosome-Scale Assembly. PLoS Comput Biol. 2019; 15(8): e1007273. PubMed Abstract | Publisher Full Text | Free Full Text
Guan D, McCarthy SA, Wood J, et al.: Identifying and Removing Haplotypic Duplication in Primary Genome Assemblies. Bioinformatics. 2020; 36(9): 2896–2898. PubMed Abstract | Publisher Full Text | Free Full Text
Howe K, Chow W, Collins J, et al.: Significantly Improving the Quality of Genome Assemblies through Curation. Gigascience. 2021; 10(1): giaa153. PubMed Abstract | Publisher Full Text | Free Full Text
Kerpedjiev P, Abdenur N, Lekchos F, et al.: HiGlass: Web-Based Visual Exploration and Analysis of Genome Interaction Maps. Genome Biol. 2018; 19(1): 125. PubMed Abstract | Publisher Full Text | Free Full Text
Manni M, Berkeley MR, Seppey M, et al.: BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. Mol Biol Evol. 2021; 38(10): 4647–4654. PubMed Abstract | Publisher Full Text | Free Full Text
Nedeljković Z, Ricarte A, Šašić Zorić L, et al.: The Genus Xanthogramma Schiner, 1861 (Diptera: Syrphidae) in Southeastern Europe, with Descriptions of Two New Species. The Canadian Entomologist. 2018; 150(4): 440–64. PubMed Full Text
Rao SSP, Hunley MH, Durand NC, et al.: A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. Cell. 2014; 159(7): 1665–80. PubMed Abstract | Publisher Full Text | Free Full Text
Speight MCD: Is Xanthogramma Stackelbergi Present in Britain? Hoverfly Newsletter. 2010; 50: 7–8. Speight MCD: Species Accounts of European Syrphidae, 2017. Syrph the Net the Database of European Syrphidae (diptera). 2017; 97: 294. Reference Source
Speight MCD, Sarthou JP: StN KEYS FOR THE IDENTIFICATION OF THE EUROPEAN SPECIES OF VARIOUS GENERA OF SYRPHIDAE, 2017 CLES StN POUR LA DETERMINATION DES ESPECES EUROPEENNES DE PLUSIEURS GENRES. 2017. Reference Source
Stubbs A: Xanthogramma Pedissequum Group. Hoverfly Newsletter. 2012a; 52: 13. Stubbs A: Xanthogramma Stackelbergi Violovitsch (Diptera, Syrphidae) in Britain. Dipterists Digest. 2012b; 19(1): 102. Stubbs AE, Falk SJ, Others: British Hoverflies: An Illustrated Identification Guide. British Entomological and Natural History Society. 2002. Reference Source
Uliano-Silva M, Ferreira Nunes JG, Krasheninnikova K, et al.: marcelauliano/MitoHiFi: mitohifi_v2.0. 2021. Publisher Full Text
Open Peer Review

Current Peer Review Status: ✔️ ✔️

Version 1

Reviewer Report 10 February 2022

https://doi.org/10.21956/wellcomeopenres.19416.r48478

© 2022 Chang C. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Ching-Ho Chang
Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, 98109, USA

Sivell et al. report a genome assembly of a hoverfly, Xanthogramma pedissequum. The high completeness and contiguity indicate that genome assembly quality is very high. However, I feel that some extra analyses would greatly improve the significance of the study. I also found that the description of methods can be more detailed.

My major suggestion is about the chromosome evolution of the Dipteran lineage. Dipteran species have been known to have a large gene synteny, which is named Muller element, as it was first described by H. J. Muller (Muller 1940¹). Muller labeled Drosophila chromosome arms as A to F and found genes located in one Muller element seldom translocated to other Muller elements in other species. Recent studies found Muller elements are also conserved in other Dipteran species, e.g., mosquitos. The nomenclature is particularly useful for people to study chromosome evolution. Can the author also assign hoverfly’s chromosomes using Muller elements?

The other major suggestion is that sex chromosomes have turned over several times in Dipterans (Vicoso and Bachtrog, 2015⁰). Can the authors state the evolution of sex chromosomes in Xanthogramma pedissequum based on their observation?

I also have a minor question. There is no bacteria or fungus contamination in their assembly. Is that true, or did the authors use some ways to filter out their contaminating contigs?

References
1. Muller HJ: Bearings of the 'Drosophila' work on systematics. J. Huxley (ed.), The New Systematics. 1940. 185-268
2. Vicoso B, Bachtrog D: Numerous transitions of sex chromosomes in Diptera. PLoS Biol. 2015; 13 (4): e1002078 PubMed Abstract | Publisher Full Text

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:** Genomics, Genetics

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.

---

**Emma Bailey**
Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, UK

The Darwin Tree of Life project report a high quality genome assembly for a Syrphidae hoverfly (*Xanthogramma pedissequum*). This has been performed using PacBio and 10X sequencing data, with scaffolding using Hi-C data. All raw data and the genome assembly are easily accessible. This will make a useful contribution to further research of hoverflies, many of which are key pollinators and beneficial predators of crop pests.

**Background**

This gives a good overview of the biology of *X. pedissequum*, highlighting their potentially important role as aphid predators.

**Genome sequence report**

"The final assembly has a total length of 977 Mb" - Was any genome size estimate obtained for this species to reaffirm this value, using flow cytometry or K-mer analysis?

"The majority, 95.94%, of the assembly sequence was assigned to six chromosomal-level scaffolds" - Can any comment be made on whether this was the expected number of chromosomes based on closely related species?
Statistics on the repeat content of the genome would be useful, especially considering the "high repeat content" reported in chromosome 1. But perhaps this will follow with subsequent annotation.

I don't personally feel that figures 3 and 4 add much to the report. However, as they appear to be included as a standard for all DToL genome reports, I see no harm in their inclusion.

Figure 5 - it is difficult to identify individual chromosomes on the contact map, is it possible to outline these?

**Methods**

Wet lab methods are covered well. Genome assembly methods are brief, no parameters are mentioned, so I'm assuming settings were all kept as default? It might be worth mentioning this if so.

Additionally, it could be useful to include a table of assembly statistics before and after purge_dups, polishing and scaffolding, but this is not essential.

**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?**
Partly

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

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Insect genomics/bioinformatics

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.