



DATA NOTE

The genome sequence of the Cinnabar Moth, *Tyria jacobaeae* (Linnaeus, 1758)

[version 1; peer review: 1 approved]

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Abstract

We present a genome assembly from an individual male *Tyria jacobaeae* (the Cinnabar Moth; Arthropoda; Insecta; Lepidoptera; Erebididae). The genome sequence is 589.7 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.74 kilobases in length.

Keywords

Tyria jacobaeae, Cinnabar Moth, genome sequence, chromosomal, Lepidoptera



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1

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1. **Helen White-Cooper** , Cardiff University, Cardiff, UK

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

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Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Noctuoidea; Erebidae; Arctiinae; Callimorphini; *Tyria*; *Tyria jacobaeae* (Linnaeus, 1758) (NCBI:txid179666).

Background

An evolutionary arms race can occur between plants and herbivores. Many plants produce toxic chemicals to deter predators, but some insects have evolved mechanisms to detoxify chemicals or sequester and deploy them for their own use. For example, moths of the subfamily Arctiinae (family Erebidae) can sequester pyrrolizidine from food plants to use as a defence. The chemistry of this phenomenon has been studied in the cinnabar moth *Tyria jacobaeae*.

Tyria jacobaeae is an easily recognised moth found across Europe and Asia: the conspicuous red and black adults are a familiar sight in well-drained grassland, heathland, woodland rides and occasionally gardens in spring and summer, flying from May to July in southern England (Waring *et al.*, 2017). The adults are primarily active at night, but they are easily disturbed in the daytime and the species is often incorrectly described as a day-flying moth. The yellow and black striped larvae are equally distinctive, feeding openly on ragwort (*Senecio jacobaea*) and grousel (*S. vulgaris*). Writing seventy years ago, Ford said that “country folk” refer to the striped larvae as “wort maggots with football jerseys on” (Ford, 1952).

The food plants produce toxic pyrrolizidine alkaloids as defence chemicals. Analysis has shown that *T. jacobaeae* larvae take up and store many of these alkaloids, which are then retained in the bodies of the larvae, pupae and adults (Ehmke *et al.*, 1990). This retention requires the larva to express an enzyme (senecionine N-oxygenase) for converting the toxic chemicals into N-oxide forms that are non-toxic to insects; a gene encoding this enzyme has been cloned (Naumann *et al.*, 2002; Sehlmeier *et al.*, 2010). Pupae and adults of *T. jacobaeae* (but not larvae) also contain an additional alkaloid, not present in the food plant, given the name ‘callimorphine’ (Edgar *et al.*, 1980). Around the larval-pupal transition stage, *T. jacobaeae* synthesise callimorphine from smaller breakdown products of plant-derived alkaloids (Ehmke *et al.*, 1990). The genes encoding the enzymatic pathway have not been characterised. Adult and larval cinnabar moths are avoided or rejected by most vertebrate predators (Aplin *et al.*, 1968); juvenile cuckoos are an exception and feed extensively on cinnabar moth larvae (Mills *et al.*, 2020).

Ragwort has become invasive in several countries causing problems to livestock farming due to its toxic effects. The Cinnabar Moth was introduced as a biological control agent in New Zealand in 1929, on the west coast of the United States in 1959 and in Canada in the 1960s; it is now well established

in these countries and has proved successful at ragwort control (Frick & Holloway, 1964; Harris *et al.*, 1975; Harman *et al.*, 1990; Markin & Littlefield, 2008).

The genome sequence of *Tyria jacobaeae* was determined as part of the Darwin Tree of Life project. The assembled genome sequence will aid development of genetic markers for monitoring population spread and greatly facilitate research into the biochemical pathways underpinning toxin chemistry in insects.

Genome sequence report

The genome was sequenced from one male *Tyria jacobaeae* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, -1.33). A total of 31-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 24 missing joins or mis-joins and removed 4 haplotypic duplications, reducing the assembly length by 0.2% and the scaffold number by 11.11%.

The final assembly has a total length of 589.7 Mb in 55 sequence scaffolds with a scaffold N50 of 21.0 Mb (Table 1). Most (99.8%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 2–Figure 5; Table 2). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission.



Figure 1. Photograph of the *Tyria jacobaeae* (ilTyrJaco4) specimen used for genome sequencing.

Table 1. Genome data for *Tyria jacobaeae*, iTyrJaco4.1.

Project accession data		
Assembly identifier	iTyrJaco4.1	
Species	<i>Tyria jacobaeae</i>	
Specimen	iTyrJaco4	
NCBI taxonomy ID	179666	
BioProject	PRJEB57422	
BioSample ID	SAMEA8603118	
Isolate information	iTyrJaco4, male: whole organism (DNA sequencing) iTyrJaco2: head (Hi-C scaffolding); thorax (RNA sequencing)	
Assembly metrics*		Benchmark
Consensus quality (QV)	67	≥ 50
<i>k</i> -mer completeness	100%	≥ 95%
BUSCO**	C:98.8%[S:98.4%,D:0.4%], F:0.2%,M:1.0%,n:5,286	C ≥ 95%
Percentage of assembly mapped to chromosomes	99.8%	≥ 95%
Sex chromosomes	Z chromosome	localised homologous pairs
Organelles	Mitochondrial genome assembled	complete single alleles
Raw data accessions		
PacificBiosciences SEQUEL II	ERR10480604	
Hi-C Illumina	ERR10489914	
PolyA RNA-Seq Illumina	ERR10489913	
Genome assembly		
Assembly accession	GCA_947561695.1	
Accession of alternate haplotype	GCA_947561705.1	
Span (Mb)	589.7	
Number of contigs	141	
Contig N50 length (Mb)	9.3	
Number of scaffolds	56	
Scaffold N50 length (Mb)	21.0	
Longest scaffold (Mb)	26.9	

* Assembly metric benchmarks are adapted from column VGP-2020 of "Table 1: Proposed standards and metrics for defining genome assembly quality" from (Rhie *et al.*, 2021).

** BUSCO scores based on the lepidoptera_odb10 BUSCO set using v5.3.2. 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/iTyrJaco4.1/dataset/CANNZZ01/busco>.

The estimated Quality Value (QV) of the final assembly is 67 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.8% (single = 98.4%, duplicated = 0.4%), using the lepidoptera_odb10 reference set (*n* = 5,286).

Metadata for specimens, spectral estimates, sequencing runs, contaminants and pre-curation assembly statistics can be found at <https://links.tol.sanger.ac.uk/species/179666>.

Methods

Sample acquisition and nucleic acid extraction

A male *Tyria jacobaeae* (specimen number Ox000516, individual iTyrJaco4) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude -1.33) on 2020-06-25. The specimen was taken from woodland habitat by Douglas Boyes (University of Oxford) using a light trap. The specimen was identified by the collector and snap-frozen on dry ice.

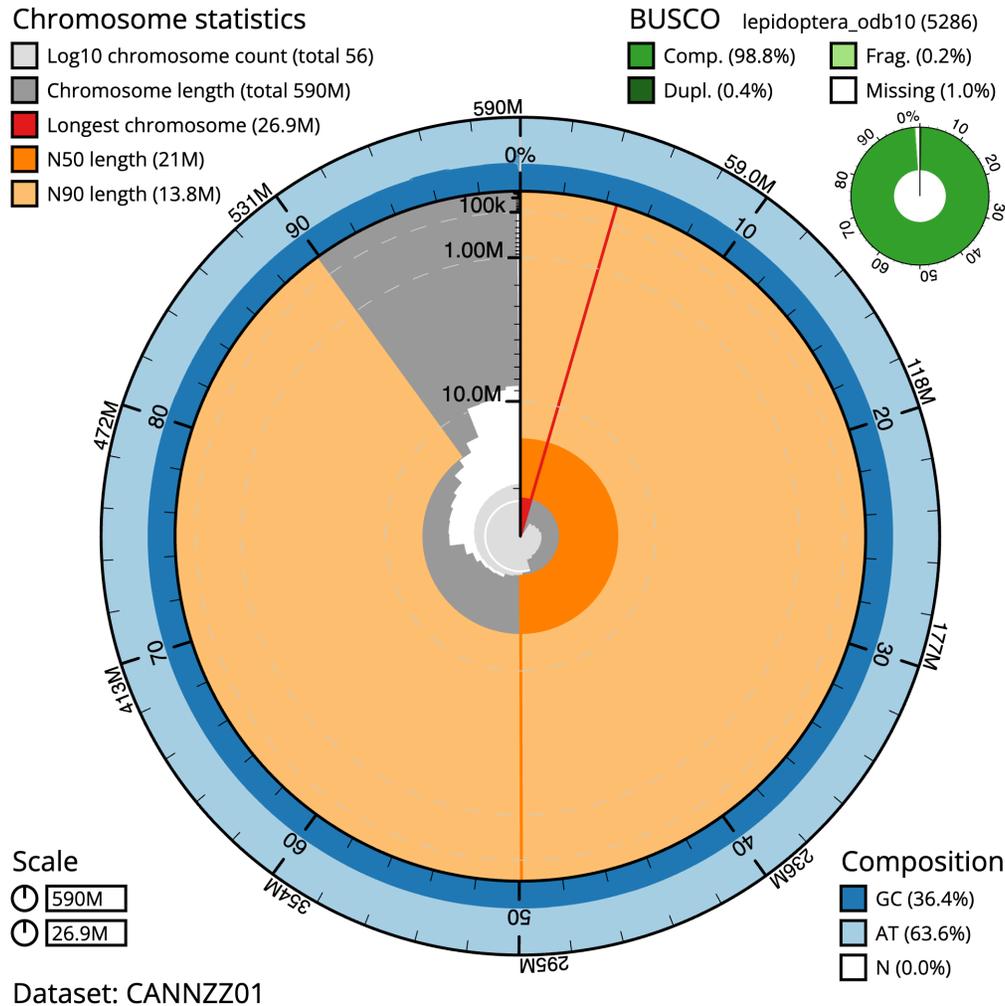


Figure 2. Genome assembly of *Tyria jacobaeae*, iTyrJaco4.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 589,695,457 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (26,932,988 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (21,035,164 and 13,819,771 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 lepidoptera_odb10 set is shown in the top right. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/iTyrJaco4.1/dataset/CANNZZ01/snail>.

A second *T. jacobaeae* specimen (specimen number NHMUK01411107, individual iTyrJaco2) was collected in Wigmore Park, Luton (latitude 51.88, longitude -1.37) on 2020-06-23 by netting. The specimen was collected and identified by Olga Sivell (Natural History Museum), and then preserved on dry ice. This specimen was used for Hi-C scaffolding and RNA sequencing.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The iTyrJaco4 sample was weighed and dissected on dry ice. Whole organism tissue was cryogenically

disrupted to a fine powder using a Covaris cryoPREP Automated Dry Pulveriser, receiving multiple impacts. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. HMW DNA was sheared into an average fragment size of 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 Nanodrop spectrophotometer and Qubit Fluorometer

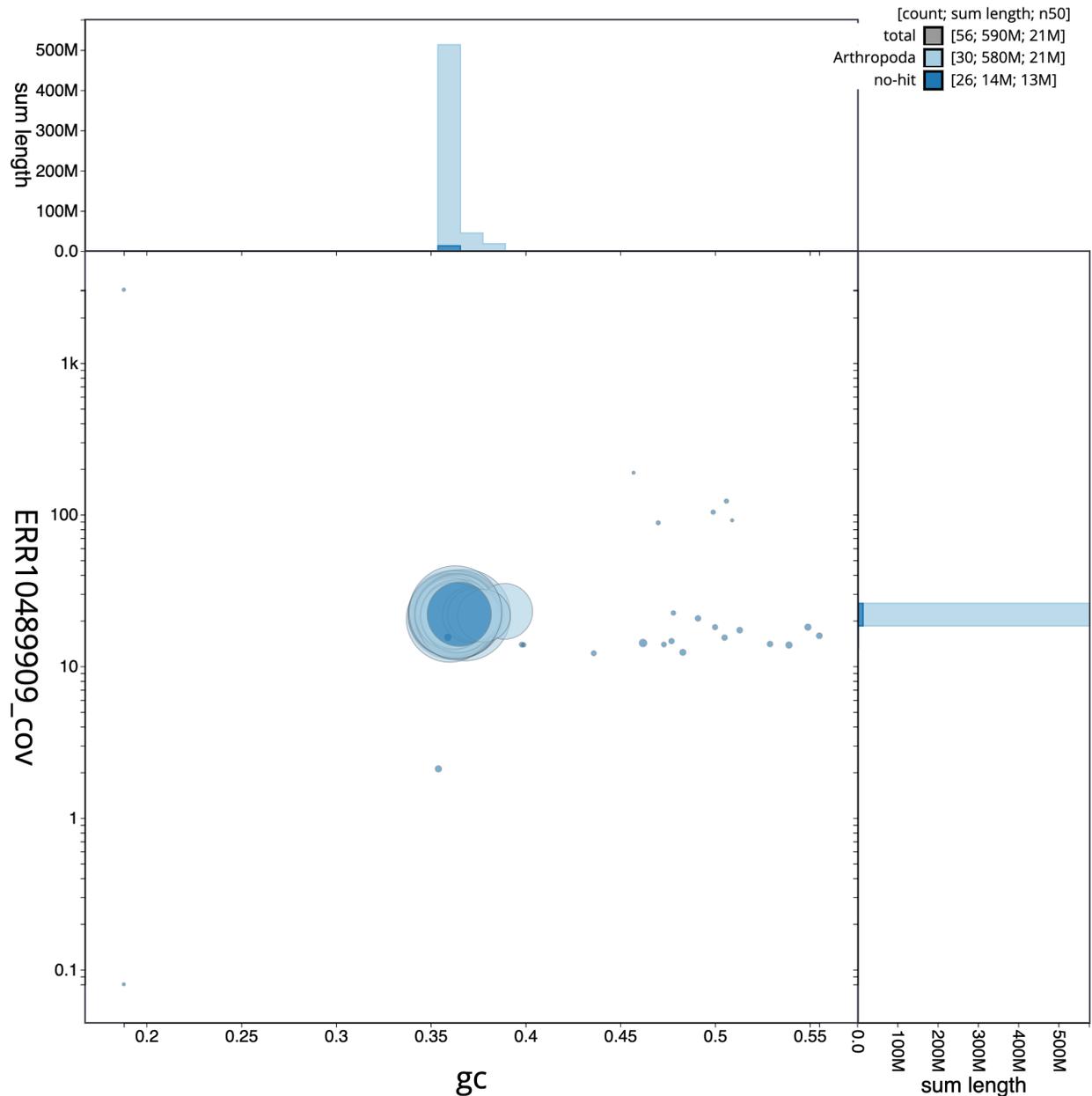


Figure 3. Genome assembly of *Tyria jacobaeae*, iTyrJaco4.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/iTyrJaco4.1/dataset/CANNZZ01/blob>.

and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from thorax tissue of iTyrJaco2 in the Tree of Life Laboratory at the WSI using TRIzol, according to the manufacturer's instructions. RNA was then eluted in 50 μ l 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 DNA 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. DNA and RNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi) and

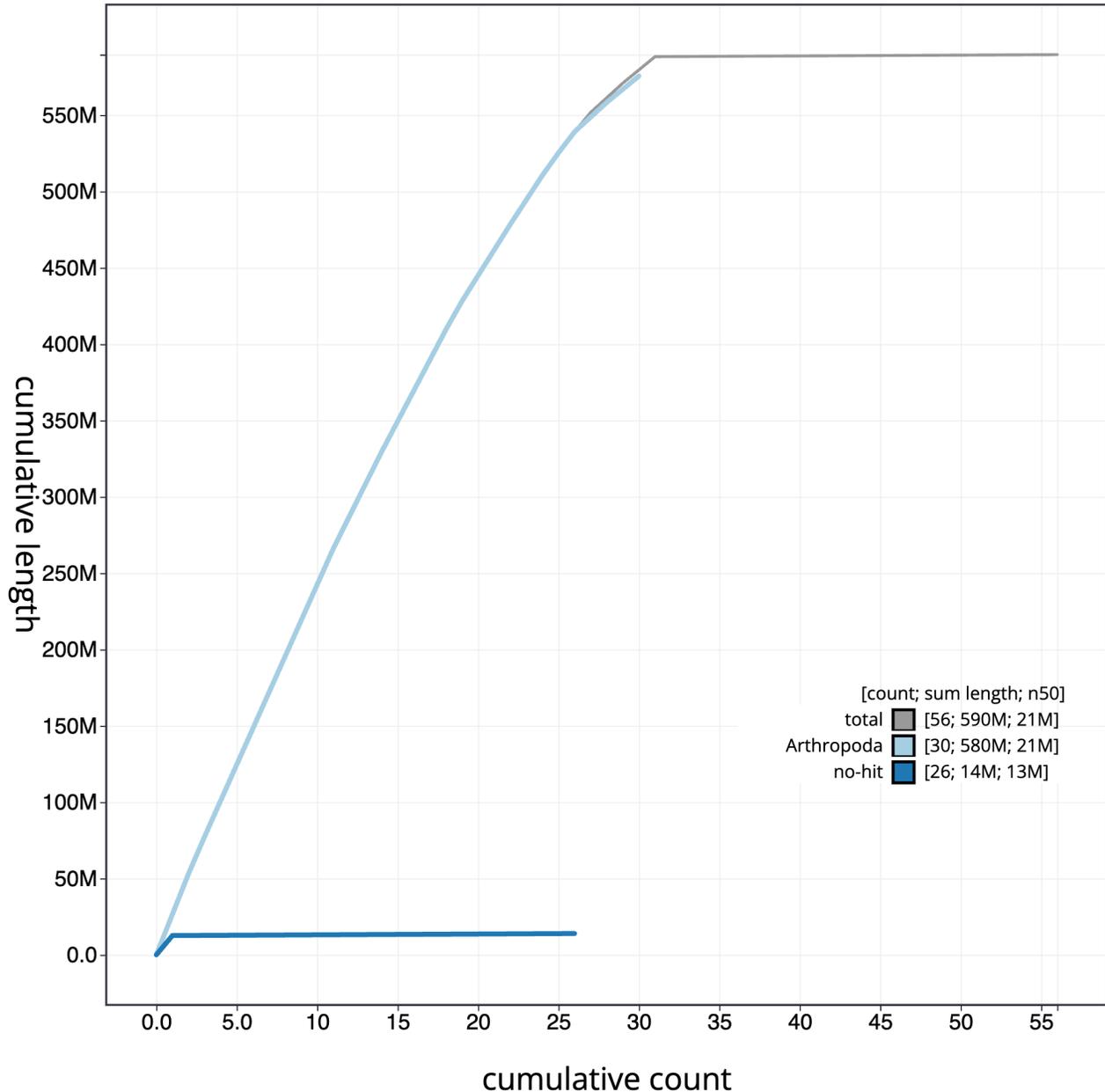


Figure 4. Genome assembly of *Tyria jacobaeae*, iTyrJaco4.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/iTyrJaco4.1/dataset/CANNZZ01/cumulative>.

Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were also generated from head tissue of iTyrJaco2 using the Arima2 kit and sequenced on the Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021) and haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using YaHS (Zhou *et al.*, 2023). The assembly was checked for contamination

and corrected as described previously (Howe *et al.*, 2021). Manual curation was performed using HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2022), which runs MitoFinder (Allio *et al.*, 2020) or MITOS (Bernt *et al.*, 2013) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format

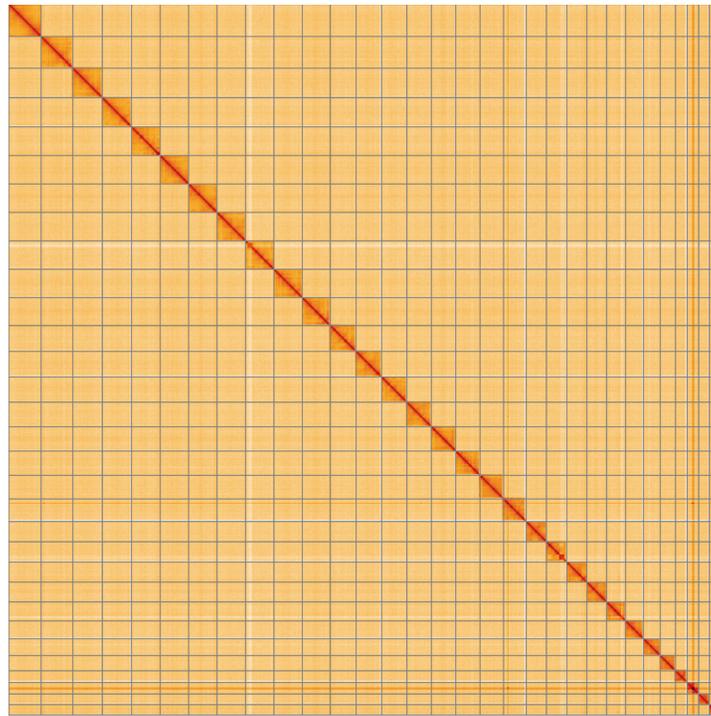


Figure 5. Genome assembly of *Tyria jacobaeae*, ilTyrJaco4.1: Hi-C contact map of the ilTyrJaco4.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at <https://genome-note-higlass.tol.sanger.ac.uk/I/?d=GLpKSBq9Q1KB5LreH8Gkjw>.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Tyria jacobaeae*, ilTyrJaco4.

INSDC accession	Chromosome	Length (Mb)	GC%
OX387208.1	1	26.18	37.0
OX387209.1	2	24.37	36.5
OX387210.1	3	24.09	36.5
OX387211.1	4	23.83	36.0
OX387212.1	5	23.57	36.5
OX387213.1	6	23.6	36.5
OX387214.1	7	23.54	36.0
OX387215.1	8	23.5	36.0
OX387216.1	9	23.4	36.0
OX387217.1	10	23.12	36.5
OX387218.1	11	21.43	36.0
OX387219.1	12	21.04	36.5
OX387220.1	13	20.86	36.5
OX387221.1	14	20.34	36.5
OX387222.1	15	20.21	36.5

INSDC accession	Chromosome	Length (Mb)	GC%
OX387223.1	16	20.02	36.5
OX387224.1	17	19.52	36.5
OX387225.1	18	18.69	36.0
OX387226.1	19	16.86	36.5
OX387227.1	20	16.76	36.5
OX387228.1	21	16.65	36.5
OX387229.1	22	16.31	36.0
OX387230.1	23	15.87	36.5
OX387231.1	24	14.7	36.5
OX387232.1	25	13.82	36.0
OX387233.1	26	12.67	36.5
OX387234.1	27	9.63	37.0
OX387235.1	28	9.56	39.0
OX387236.1	29	8.81	37.5
OX387237.1	30	8.5	38.0
OX387207.1	Z	26.93	36.5
OX387238.1	MT	0.02	19.0

(Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done using Nextflow (Di Tommaso *et al.*, 2017) DSL2 pipelines “sanger-tol/readmapping” (Surana *et al.*, 2023a) and “sanger-tol/genomenote” (Surana *et al.*, 2023b). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

Table 3 contains a list of relevant software tool versions and sources.

Wellcome Sanger Institute – Legal and Governance

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**’, which can be found in full on the Darwin Tree of Life website [here](#). 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.

Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the

nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so we align with best practice wherever possible. The overarching areas of consideration are:

- Ethical review of provenance and sourcing of the material
- Legality of collection, transfer and use (national and international)

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: *Tyria jacobaeae* (cinnabar moth). Accession number PRJEB57422; <https://identifiers.org/ena.embl/PRJEB57422>. (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Tyria jacobaeae* 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 available 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.4789928>.

Members of the Natural History Museum Genome Acquisition Lab are listed here:

<https://doi.org/10.5281/zenodo.4790042>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.4893703>.

Members of the Wellcome Sanger Institute Tree of Life programme are listed here: <https://doi.org/10.5281/zenodo.4783585>.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: <https://doi.org/10.5281/zenodo.4790455>.

Members of the Tree of Life Core Informatics collective are listed here: <https://doi.org/10.5281/zenodo.5013541>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.4783558>.

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.1.5	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
YaHS	1.1a.2	https://github.com/c-zhou/yahs

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Helen White-Cooper 

Cardiff University, Cardiff, Wales, UK

This report describes the genome sequence from a single male cinnabar moth, caught in southern England. This species is of interest due to its use in control of ragwort, and its alkaloid processing biochemical pathways. The sample extraction methods, sequencing, and processing are all clearly described. Chromosomal assemblies are high quality.

A second specimen was used for the HiC library generation (head), and for RNAseq (thorax). The sex of this individual should be mentioned in the report.

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: Drosophila genetics, with an interest in other insects including lepidoptera

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.
