The genome sequence of the peach blossom moth, *Thyatira batis* (Linnaeus, 1758) [version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual male *Thyatira batis* (the peach-blossom moth; Arthropoda; Insecta; Lepidoptera; Drepanidae). The genome sequence is 315 megabases in span. The majority of the assembly (99.68%) is scaffolded into 31 chromosomal pseudomolecules, with the Z sex chromosome assembled. The mitochondrial genome was also assembled and is 15.4 kilobases in length. Gene annotation of this assembly on Ensembl has identified 12,238 protein coding genes.

Keywords

*Thyatira batis*, peach-blossom, genome sequence, chromosomal

This article is included in the Tree of Life gateway.
Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glos-sata; Ditrysia; Drepanoidea; Drepanidae; Thyatirinae; Thyatira; Thyatira batis (Linnaeus, 1758) (NCBI:txid721163).

Introduction
Thyatira batis (peach-blossom) is one of the most striking moths in the UK, with the forewings marked with bright pink blotches resembling the petals of peach tree flowers. The species has been used as a model to study the effectiveness of disruptive coloration for predator avoidance (Schaefer & Stobbe, 2006). T. batis is common in woodland habitats in Britain and Ireland, and found across the palearctic, from Europe to Japan. The genome of T. batis was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all of the named eukaryotic species in the Atlantic Archipelago of Britain and Ireland. Here we present a chromosomally complete genome sequence for T. batis, based on one male specimen from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK.

Genome sequence report
The genome was sequenced from a single male T. batis collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.772, longitude -1.337) (Figure 1). A total of 29-fold coverage in Pacific Biosciences single-molecule long reads (N50 13 kb) and 122-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 36 missing/misjoins and removed six haplotypic duplications, reducing the assembly size by 2.57% and scaffold number by 38.55%, and increasing the scaffold N50 by 8.70%.

The final assembly has a total length of 315 Mb in 52 sequence scaffolds with a scaffold N50 of 11 Mb (Table 1). Of the assembly sequence, 99.7% was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes (numbered by sequence length), and the Z sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO (Simão et al., 2015) v5.1.2 completeness of 99.0% (single 98.7%, duplicated 0.3%, fragmented 0.3%, missing 0.8%) using the lepidoptera_odb10 reference set. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Gene annotation
The Ensembl gene annotation system (Aken et al., 2016) was used to generate annotation for the Thyatira batis assembly (GCA_905147785.1, see https://rapid.ensembl.org/Thyatira_batis_GCA_905147785.1/; Table 1). The annotation was created primarily through alignment of transcriptomic data to the genome, with gap filling via protein-to-genome alignments of a select set of proteins from UniProt (UniProt Consortium, 2019) and OrthoDB (Kriventseva et al., 2008). Prediction tools, CPC2 (Kang et al., 2017) and RNAsamba (Camargo et al., 2020), were used to aid determination of protein coding genes.

Methods
A male T. batis (iThyBati1) and a second sample of unknown sex were collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.772, longitude -1.337) by Douglas Boyes in July 2019 (iThyBati1) and July 2020 (iThyBati2). The samples were snap-frozen on dry ice and stored using a CoolRack.

DNA was extracted from thorax/abdomen tissue of iThyBati1 and a second sample of unknown sex were collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.772, longitude -1.337) by Douglas Boyes in July 2019 (iThyBati1) and July 2020 (iThyBati2). The samples were snap-frozen on dry ice and stored using a CoolRack.
(BR) Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

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

**Figure 2. Genome assembly of Thyatira batis, ilThyBati1.1: 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 314,798,901 bp assembly. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly (15,095,819 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (11,052,377 and 7,688,443 bp), respectively. The pale grey spiral shows the cumulative chromosome 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/ilThyBati1.1/dataset/CAJHWV01/snail.
Figure 3. Genome assembly of *Thyatira batis*, ilThyBati1.1: 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/ilThyBati1.1/dataset/CAJHWV01/blob.

Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were generated from head tissue of ilThyBati1 using the Arima v1.0 kit and sequenced on HiSeq X.

Assembly was carried out with HiCanu (Nurk et al., 2020). 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 and corrected using the gEVAL system (Chow et al., 2016) as described previously (Howe et al., 2021). Manual curation was performed using
Figure 4. Genome assembly of *Thyatira batis*, ilThyBati1.1: 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/ilThyBati1.1/dataset/CAJHWV01/cumulative.

gEVAL, HiGlass (Kerpedjiev et al., 2018) and Pretex. 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. The materials that have contributed to
Table 2. Chromosomal pseudomolecules in the genome assembly of *Thyatira batis*, ilThyBati1.1.

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|-----|
| LR990486.1      | 1          | 13.79     | 36.5|
| LR990487.1      | 2          | 12.99     | 35.8|
| LR990488.1      | 3          | 12.92     | 36.2|
| LR990489.1      | 4          | 12.41     | 36  |
| LR990490.1      | 5          | 12.09     | 35.4|
| LR990491.1      | 6          | 12.08     | 35.1|
| LR990492.1      | 7          | 11.78     | 35.7|
| LR990493.1      | 8          | 11.68     | 35.4|
| LR990494.1      | 9          | 11.58     | 35.4|
| LR990495.1      | 10         | 11.46     | 35.4|
| LR990496.1      | 11         | 11.23     | 35.8|
| LR990497.1      | 12         | 11.05     | 35.7|
| LR990498.1      | 13         | 10.83     | 35.4|
| LR990499.1      | 14         | 10.83     | 35.3|
| LR990500.1      | 15         | 10.56     | 35.7|
| LR990501.1      | 16         | 10.27     | 36  |

Figure 5. Genome assembly of *Thyatira batis*, ilThyBati1.1: Hi-C contact map. Hi-C contact map of the ilThyBati1.1 assembly, visualised in HiGlass. Chromosomes are arranged in size order from left to right and top to bottom.
Table 3. Software tools used.

| Software tool     | Version | Source                                                  |
|-------------------|---------|---------------------------------------------------------|
| HiCanu            | 1.0     | Nurk et al., 2020                                       |
| 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                              |
| gEVAL             | N/A     | Chow et al., 2016                                      |
| HiGlass           | 1.11.6  | Kerpedjiev et al., 2018                                |
| PreTextView       | 0.1.x   | https://github.com/wtsi-hpg/PretextView                |
| BlobToolKit       | 2.6.2   | Challis et al., 2020                                   |

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 WSI), and in some circumstances other Darwin Tree of Life collaborators.

Data availability
European Nucleotide Archive: Thyatira batis (peach blossom) genome assembly, iTHyBat1. Accession number PRJEB41953; https://identifiers.org/ena.embl/PRJEB41953.

The genome sequence is released openly for reuse. The T. batis 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. Raw data and assembly accession identifiers are reported in Table 1.

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Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.501354.

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

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The authors report a chromosome-level genome assembly of the peach blossom moth, a model species for the use of disruptive colours as anti-predator defence. This genome was produced as part of the Darwin Tree of Life project using the cutting-edge pipeline for reference genome assembly by the Wellcome Sanger Institute.

The quality of the genome is extremely high with almost all parts assigned to chromosomes and almost 100% BUSCO gene completeness. As the individual sequenced is a male, the Z chromosome is assembled, but the W chromosome is missing.

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: Speciation, 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.
The authors presented a chromosome-level haplotype genome of the peach blossom moth (Thyatira batis) widely spread from western Europe to East Asia, as a part of the Darwin Tree of Life Project. The genome has outstanding quality. Primary contigs were assembled and phased with HiFi long-reads, 10X data polished the contigs and called variants, and then Hi-C data scaffolded the contigs. N50 (one of the traditional metrics) is very long (11M), the number of scaffolds is only 52, and they annotated 12,238 protein-coding genes. BUSCO scores are also enough. Thus, we believe that they assembled the genome with recently introduced cutting-edge tools in a proper way. Our only comment is about non-coding genes, such as IncRNAs, which are also essential genes regulating protein-coding genes. We would like to recommend considering the annotation of non-coding genes if they have enough time for it.

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: Bioinformatics and 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.