DATA NOTE

The genome sequence of the orange-tip butterfly, *Anthocharis cardamines* (Linnaeus, 1758) [version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual female *Anthocharis cardamines* (the orange-tip; Arthropoda; Insecta; Lepidoptera; Pieridae). The genome sequence is 360 megabases in span. The majority (99.74%) of the assembly is scaffolded into 31 chromosomal pseudomolecules, with the W and Z sex chromosomes assembled. Gene annotation of this assembly on Ensembl has identified 12,477 protein coding genes.

Keywords

Anthocharis cardamines, orange-tip, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status  

| 1 | 2 |
|---|---|
| version 1 | ✓ | ✓ |
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Any reports and responses or comments on the article can be found at the end of the article.
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Author roles: Ebdon S: Investigation, Resources, Writing – Review & Editing; Bisschop G: Investigation, Resources, Writing – Review & Editing; Lohse K: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; Saccheri I: Writing – Original Draft Preparation, Writing – Review & Editing; Davies J: Writing – Original Draft Preparation, Writing – Review & Editing.

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**Species taxonomy**

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Papilionoidea; Pieridae; Pierinae; Anthocharis; Anthocharis cardamines (Linnaeus, 1758) (NCBI:txid227532).

**Background**

The orange-tip butterfly (*Anthocharis cardamines*) is a member of the Anthocharidini, a tribe within the Pierinae (Wahlberg et al., 2014), with a Palearctic distribution, including throughout the British Isles, where two subspecies are recognised, britannica (mainland Britain) and hibernica (Ireland and Isle of Man). The English population exhibits a reduced number of chromosomes (n = 30) compared to specimens from continental Europe (n = 31), implying a fusion event since the separation of England from the continent ~7,000 years ago (Bigger, 1978). Following range contraction on the British mainland in the late 19th century, which left disjunct populations in England and Northeast Scotland, the species began recolonizing these regions in the mid-20th century (Long, 1979), and has shown an increasing trend to the present (Fox et al., 2015). *A. cardamines* is listed as Least Concern in the IUCN Red List (Europe) (van Swaay et al., 2010). The spring flight period is phenologically responsive to temperature and climate change (Prieto & Destouni, 2015). The species is polyphagous on Brassicaceae, usually *Cardamine pratensis* and *Aliaria petiolata* in Britain (Courtney & Duggan, 1983), inhabiting flowery meadows, woodland borders, riverbanks, hedgerows and gardens. Host-plant use influences adult emergence schedule, size and dispersal behaviour (Davies & Saccheri, 2013).

**Genome sequence report**

The genome was sequenced from a single female *A. cardamines* (Figure 1) collected from Carrifran Wildwood, Scotland (latitude 55.4001, longitude -3.3352). A total of 69-fold coverage in Pacific Biosciences single-molecule circular consensus (HiFi) long reads and 97-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 50 missing/misjoins and removed 2 haplotypic duplications, reducing the assembly length by 0.53% and the scaffold number by 40.00%, and increased the scaffold N50 by 5.62%.

The final assembly has a total length of 360 Mb in 54 sequence scaffolds with a scaffold N50 of 12.5 Mb (Table 1). The majority, 99.74%, of assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 29 autosomes (numbered by sequence length), and the W and Z sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO
v5.1.2 (Manni et al., 2021) completeness of 99.0% (single 98.6%, duplicated 0.4%) using the lepidoptera_odb10 reference set (n=5,286). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

**Genome annotation report**
The ilAntCard3.1 genome has been annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Anthocharis_cardamines_GCA_905404175.1/). The resulting annotation includes 28,207 transcribed mRNAs from 12,477 protein-coding and 4,279 non-coding genes. There are 1.82 coding transcripts per gene and 8.41 exons per transcript.

**Table 1. Genome data for Anthocharis cardamines, ilAntCard3.1.**

| Project accession data | Assembly identifier | ilAntCard3.1 |
|------------------------|---------------------|--------------|
| Species                | Anthocharis cardamines |
| Specimen               | ilAntCard3 (genome assembly); ilAntCard2 (Hi-C) |
| NCBI taxonomy ID       | NCBI:txid227532 |
| BioProject             | PRJEB43792 |
| BioSample ID           | SAMEA7523110 |
| Isolate information    | Female, abdomen (ilAntCard3); male, whole organism (ilAntCard2) |

| Raw data accessions    | PacificBiosciences SEQUEL II | ERR6544655 |
|                       | 10X Genomics Illumina         | ERR6054580-ERR6054583 |
|                       | Hi-C Illumina                | ERR6054584 |

| Genome assembly        | Assembly accession           | GCA_905404175.1 |
|                       | Accession of alternate haplotype | GCA_905404305.1 |
| Span (Mb)              | 360                             |
| Number of contigs      | 114                             |
| Contig N50 length (Mb) | 6.3                             |
| Number of scaffolds    | 54                              |
| Scaffold N50 length (Mb)| 12.5                         |
| Longest scaffold (Mb)  | 17.4                            |
| BUSCO* genome score    | C:99.0%(S:98.6%,D:0.4%),F:0.2%,M:0.8%,n:5,286 |

*BUSCO scores based on the lepidoptera_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/ilAntCard3.1/dataset/CAJQEZ01/busco.

**Methods**

**Sample acquisition and nucleic acid extraction**
A single female *A. cardamines* specimen (ilAntCard3; genome assembly) and a single male *A. cardamines* specimen (ilAntCard2; Hi-C) were collected from Carrifran Wildwood, Scotland (latitude 55.4001, longitude -3.3352) using a net by Sam Ebdon, Gertjan Bisshop and Konrad Lohse (all University of Edinburgh). The samples were identified by Konrad Lohse and were snap-frozen at -80°C.

DNA was extracted at the Scientific Operations Core, Wellcome Sanger Institute. The ilAntCard3 sample was weighed and dissected on dry ice. Abdomen tissue was disrupted by
manual grinding with a disposable 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 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

Dataset: CAJQEZ01

Figure 2. Genome assembly of Anthocharis cardamines, ilAntCard3.1: 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 359,616,706 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 (17,367,467 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (12,507,586 and 8,384,805 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/ilAntCard3.1/dataset/CAJQEZ01/snail.
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 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.
Figure 4. Genome assembly of *Anthocharis cardamines*, ilAntCard3.1: 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/ilAntCard3.1/dataset/CAJQEZ01/cumulative.

Genome assembly
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.
Table 2. Chromosomal pseudomolecules in the genome assembly of Anthocharis cardamines, iAntCard3.1.

| INSDC accession | Chromosome | Size (Mb) | GC%  |
|-----------------|------------|-----------|------|
| FR989950.1      | 1          | 17.37     | 34.4 |
| FR989952.1      | 2          | 14.43     | 34.1 |
| FR989953.1      | 3          | 14.38     | 34.4 |
| FR989954.1      | 4          | 13.91     | 34.2 |
| FR989955.1      | 5          | 13.89     | 34.2 |
| FR989956.1      | 6          | 13.7      | 33.9 |
| FR989957.1      | 7          | 13.48     | 33.9 |
| FR989958.1      | 8          | 13.27     | 34.1 |
| FR989959.1      | 9          | 12.76     | 34   |
| FR989960.1      | 10         | 12.72     | 34.2 |
| FR989961.1      | 11         | 12.7      | 33.8 |
| FR989962.1      | 12         | 12.51     | 33.9 |
| FR989963.1      | 13         | 12.35     | 34.1 |
| FR989964.1      | 14         | 12.17     | 34.1 |
| FR989965.1      | 15         | 11.88     | 34.2 |

| INSDC accession | Chromosome | Size (Mb) | GC%  |
|-----------------|------------|-----------|------|
| FR989966.1      | 16         | 11.84     | 34.3 |
| FR989967.1      | 17         | 11.78     | 34.4 |
| FR989968.1      | 18         | 11.58     | 34.9 |
| FR989969.1      | 19         | 10.66     | 34.3 |
| FR989970.1      | 20         | 10.34     | 34.8 |
| FR989971.1      | 21         | 10.07     | 34.8 |
| FR989972.1      | 22         | 9.08      | 35.1 |
| FR989973.1      | 23         | 8.97      | 35.1 |
| FR989974.1      | 24         | 8.77      | 34.8 |
| FR989975.1      | 25         | 8.73      | 34.9 |
| FR989976.1      | 26         | 8.38      | 36.9 |
| FR989977.1      | 27         | 7.81      | 34.5 |
| FR989978.1      | 28         | 7.02      | 36   |
| FR989979.1      | 29         | 6.82      | 35   |
| FR989980.1      | W          | 3.64      | 37.4 |
| FR989981.1      | Z          | 16.45     | 34.1 |
| FR989982.1      | MT         | 0.02      | 19.5 |

Figure 5. Genome assembly of Anthocharis cardamines, iAntCard3.1: HI-C contact map. HI-C contact map of the iAntCard3.1 assembly, visualised in HiGlass. Chromosomes are shown in size order from left to right and top to bottom. The interactive HI-C map can be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=ds_ts81KSRO_D0mSTg7guA.
(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 (Howe et al., 2021) was performed using gEVAL, 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 3. Software tools used.

| Software tool    | Version | Source                        |
|------------------|---------|-------------------------------|
| HiCanu           | 2.1     | Nurkl et al., 2020            |
| purge_dups       | 1.2.3   | Guan et al., 2020             |
| SALSA2           | 2.2     | Ghurye et al., 2019           |
| longranger       | 2.2.2   | https://support.10xgenomics.com/ |
| align             |         | genome-exome/software/pipelines/ |
|                  |         | latest/advanced/other-pipelines |
| freebayes        | 1.3.1-17- | Garrison & Marth, 2012      |
|                  | gaa2ace8 |                               |
| 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      | 2.6.4   | Challis et al., 2020          |

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The article "The genome sequence of the orange-tip butterfly, Anthocharis cardamines (Linnaeus, 1758)" reported the first genome assembly for the orange-tip butterfly. The methods for assembly, scaffolding, and annotation are mostly well described and of a high standard. Also, the quality of the assembly is really high.

I have only two concerns about the manuscript. First, the method for genome polishing with 10x data was not well explained. It is described up to the variant calling phase, but the actual way to polish the genome should be stated. Second, the annotation quality was not confirmed. Running a BUSCO on the annotation might be a help to see how well the genome was annotated.

I would also like to ask the authors to add information about which subspecies are used for this study. According to the “Background” section, it seems it’s *britannica* but stating this clearly in the “Methods” section would help the readers to get important information about the actual samples used for this study.

**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 genome assembly and annotation. Evolutionary Ecology. Butterfly 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.

Reviewer Report 07 November 2022

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In the article "The genome sequence of the orange-tip butterfly, *Anthocharis cardamines* (Linnaeus, 1758)" the authors Ebdon *et al*. report the genome assembly and annotation of an individual orange tip butterfly from Scotland.

This effort appears to be an addition to the Darwin Tree of Life project and so it's sole focus on the generation of a high quality genome assembly is understandable. The methods used are generally considered the best available at present, and the resulting assembly and annotation conform to expectations. A brief exploration of the raw data revealed no anomalies to this reviewer.

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: Genome assembly and annotation. Butterfly, fish, and bird genomics. 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.