DATA NOTE

The genome sequence of the red admiral, *Vanessa atalanta* (Linnaeus, 1758) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual female *Vanessa atalanta* (the red admiral; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The genome sequence is 370 megabases in span. The majority of the assembly (99.44%) is scaffolded into 32 chromosomal pseudomolecules, with the W and Z sex chromosome assembled. Gene annotation of this assembly on Ensembl has identified 12,493 protein coding genes.

Keywords

Vanessa atalanta, red admiral, genome sequence, chromosomal, Lepidoptera

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This article is included in the Tree of Life gateway.
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Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Papilionoidea; Nymphalidae; Nymphalinae; Vanessa; Vanessa atalanta (Linnaeus, 1758) (NCBI:txid42275).

Background
The red admiral, Vanessa atalanta (Linnaeus, 1758), earns its name due to the majesty of its colours: striking orange, dark brown and white. It has a disjunct distribution in the Holarctic, occurring in the west Palearctic (up to 85 degrees longitude approximately) and in North and Central America (Williams, 1930). The American populations differ slightly in appearance to the Eurasian populations, and are referred to subspecies rubria (Fruhstorfer, 1909; Vane-Wright & Hughes, 2007). The sister species of V. atalanta is the kamehameha butterfly (V. tameamea), an endemic species from Hawaii (Wahlberg & Rubinoff, 2011). Red admirals are well known for their migratory movements: they migrate latitudinally in Europe and North America between the southern parts of their range, where the majority of individuals overwinter as adult and/or larva, and the northern areas, that are colonized during spring and summer (Brattström et al., 2010; Brattström et al., 2018; Scott, 1992; Walker, 2001; Williams, 1930). The species is listed as “Least concern” according to the IUCN Red List (Europe) (van Swaay et al., 2010). (Roy & Sparks, 2000) report changes in migration time and length due to global warming, which could be resulting in a northward shift of overwintering latitudes (Fox et al., 2010). The red admiral is polyvoltine across its migratory range. Females lay their eggs on nettles in the genera Urtica, Boehmeria, Laportea, and Parietaria. Males are markedly territorial, especially during late afternoon. The genome will further aid evolutionary studies of behavioral traits such as migration or male territoriality and the evolution of diapause. Vanessa atalanta has 31 pairs of chromosomes and an estimated genome size of 326 Mb (Mackintosh et al., 2019).

Genome sequence report
The genome was sequenced from a single female V. atalanta (Figure 1A, B) collected from Carrifran Wildwood, Dumfries and Galloway, Scotland (latitude 55.400132, longitude -3.3352). A total of 34-fold coverage in Pacific Biosciences single-molecule long reads (N50 11 kb) and 95-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 16 missing/misjoins and removed 59 haplotype duplications, reducing the assembly size by 0.27% and scaffold number by 32.86%, and increasing the scaffold N50 by 1.71%.

The final assembly has a total length of 370 Mb in 142 sequence scaffolds with a scaffold N50 of 13 Mb (Table 1). Of the assembly sequence, 99.44% was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes (numbered by sequence length), and the W and Z sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO (Simão et al., 2015) v5.1.2 completeness of 98.8% (single 98.7%, duplicated 0.1%) 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.

DNA was extracted from whole organism tissue of iVanAtal1 at the Wellcome Sanger Institute (WSI) Scientific Operations core from the whole organism using the Qiangen MagAttract HMW DNA kit, according to the manufacturer’s instructions. RNA was extracted from whole organism tissue of iVanAtal2 in the Tree of Life Laboratory at the WSI 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 head tissue using the Arima v1 Hi-C kit and sequenced on HiSeq X.

Genome assembly
Assembly was carried out with HiCanu (Nurk et al., 2020). Haplotype duplication was identified and removed with purger_dups (Quan 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.
Figure 1. Fore and hind wings of *Vanessa atalanta* specimens from which the genome was sequenced. (A) Dorsal surface view of wings from specimen SC_VA_1209 (iIVanAtal1) from Carrifran Wildwood, Scotland, UK, used to generate Pacific Bioscience, 10X genomics and HiC data. (B) surface view of wings from specimen SC_VA_1209 (iIVanAtal1) from Carrifran Wildwood, Scotland, UK, used to generate Pacific Biosciences, 10X genomics and HiC data. (C) Dorsal surface view of wings from specimen SC_VA_1219 (iIVanAtal2) from Carrifran Wildwood, Scotland, UK, used to generate RNASeq data. (D) surface view of wings from specimen SC_VA_1219 (iIVanAtal2) from Carrifran Wildwood, Scotland, UK, used to generate RNASeq data.
Manual curation was performed using gEV AL, HiGlass (Kerpedjiev et al., 2018) and Pretex. 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 1. Genome data for Vanessa atalanta, ilVanAtal1.2.

| **Project accession data** |       |
|---------------------------|-------|
| Assembly identifier       | ilVanAtal1.2 |
| Species                   | Vanessa atalanta |
| Specimen                  | ilVanAtal1 (HiFi, 10X, Hi-C), ilVanAtal2 (RNAseq) |
| NCBI taxonomy ID          | NCBI:txid42275 |
| BioProject                | PRJEB41956 |
| BioSample ID              | SAMEA7523145 |
| Isolate information       | Female, whole organism (ilVanAtal1 and ilVanAtal2) |

| **Raw data accessions**   |       |
|---------------------------|-------|
| PacificBiosciences SEQUEL II | ERR6608650 |
| 10X Genomics Illumina     | ERR6001611, ERR6002556, ERR6002557, ERR6003033 |
| Hi-C Illumina             | ERR6002558, ERR600255, ERR6003034 |
| Illumina PolyA RNAseq     | ERR6363249 |

| **Genome assembly**       |       |
|---------------------------|-------|
| Assembly accession        | GCA_905147765.2 |
| Accession of alternate haplotype | GCA_905147705.2 |
| Span (Mb)                  | 370 |
| Number of contigs          | 159 |
| Contig N50 length (Mb)     | 12.2 |
| Number of scaffolds        | 141 |
| Scaffold N50 length (Mb)   | 12.8 |
| Longest scaffold (Mb)      | 15.0 |
| BUSCO* genome score        | C:99.1%[S:98.9%,D:0.2%], F:0.2%, M:0.7%, n:1658 |

| **Genome annotation**      |       |
|---------------------------|-------|
| Number of protein-coding genes | 12,493 |
| Average length of coding sequence (bp) | 1,728 |
| Average number of exons per transcript | 9.45 |
| Average exon size (bp)     | 203 |
| Average intron size (bp)   | 2,047 |

*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/ilVanAtal1.2/dataset/CAJHWX02/busco.

**For assembly GCA_905147765.1, ilVanAtal1.1.

(Howe et al., 2021).
Gene annotation

The Ensembl gene annotation system (Aken et al., 2016) was used to generate annotation for version 1 of the Vanessa atalanta assembly (GCA_905147785.1). 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. See https://rapid.ensembl.org/Vanessa_atalanta_GCA_905147765.1/ for further details.

**Ethical/compliance issues**
The materials that have contributed to this genome note were supplied by a Tree of Life collaborator. 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...

**Figure 3. Genome assembly of Vanessa atalanta, ilVanAtal1.2: 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/Vanessa%20atalanta/dataset/CAJHWX02/blob.
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).

**Figure 4. Genome assembly of *Vanessa atalanta*, iVanAtal1.2: 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/Vanessa%20atalanta/dataset/CAJHWX02/cumulative.
Table 2. Chromosomal pseudomolecules in the genome assembly of *Vanessa atalanta*, iiVanAtal1.2.

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|-----|
| LR990550.1      | 1          | 15.00     | 32.1|
| LR990551.1      | 2          | 14.66     | 32.6|
| LR990552.2      | 3          | 14.42     | 32.5|
| LR990553.2      | 4          | 14.40     | 32.0|
| LR990554.1      | 5          | 14.01     | 31.9|
| LR990555.1      | 6          | 13.78     | 32.6|
| LR990556.1      | 7          | 13.67     | 32.0|
| LR990557.1      | 8          | 13.65     | 32.3|
| LR990558.1      | 9          | 13.29     | 32.2|
| LR990559.2      | 10         | 13.09     | 32.1|
| LR990560.1      | 11         | 12.98     | 32.1|
| LR990561.1      | 12         | 12.80     | 32.5|
| LR990562.1      | 13         | 12.79     | 32.4|
| LR990563.1      | 14         | 12.69     | 32.2|
| LR990564.1      | 15         | 12.58     | 32.4|
| LR990565.1      | 16         | 12.15     | 32.4|

Figure 5. Genome assembly of *Vanessa atalanta*, iiVanAtal1.2: Hi-C contact map. Hi-C contact map of the iiVanAtal1.2 assembly, visualised in HiGlass.
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                          |
| PretextView      | 0.1.x   | https://github.com/wtsi-hpag/PretextView   |
| HiGlass         | 1.11.6  | Kerpdeljiev et al., 2018                   |
| BlobToolKit     | 2.6.2   | Challis et al., 2020                       |

Each transfer of samples is undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Tree of Life collaborator, Genome Research Limited (operating as the Wellcome Sanger Institute) and in some circumstances other Tree of Life collaborators.

Data availability
European Nucleotide Archive: Vanessa atalanta (red admiral)
Accession number PRJEB42064; https://identifiers.org/ena.embl/PRJEB42064.

The genome sequence is released openly for reuse. The V. atalanta 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.

Author information
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.5743293.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5638618.

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I don't want to throw you the stone because all papers describing genome annotations do the same, but I find pity that BUSCO results of the assembly completeness are always mentioned while annotation completeness (gene predictions) is never. Based on the translation of the protein coding gene, the BUSCO annotation completeness is very useful to give a picture of how complete is the annotation. The comparison between the assembly completeness score and the annotation completeness score shows the potential room for improvement of the annotation. It's easy to see the proportion of genes present in the assembly that are finally not present in the final gene build. In your case, the BUSCO for the assembly is 98.8% but the BUSCO for the annotation might be around 50%. That type of result might afraid and it is why researchers do not like to show it, but such a result does not mean that the annotation is bad. It reflects a choice in the annotation approach type. Evidence based annotations can provide really good gene models (low number of false positive and a high number of high-confidence gene models (protein coding genes as well as non-coding genes), while can miss a lot of genes. Other approaches like ab-initio approaches might miss fewer genes but can, at the same time, predict a lot of false positives. Some people do not care about false positives and want to know if they can trust the blastP on the proteome when it says that the gene/protein is missing. In such case, if we had the BUSCO annotation score, we would know if were better to tBlastn on the assembly instead of blastP the proteome.

I think that the Darwin Tree of Life Projects should include that information by default.

Best regards,

Jacques Dainat Ph.D.

Competing Interests: No competing interests were disclosed.