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DATA NOTE

The genome sequence of the large yellow underwing, Noctua

pronuba (Linnaeus, 1758) [version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual female *Noctua pronuba* (the large yellow underwing; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 529 megabases in span. The complete assembly is scaffolded into 32 chromosomal pseudomolecules, with the W and Z sex chromosome assembled. The mitochondrial genome was also assembled and is 15.3 kilobases in length.

Keywords

Noctua pronuba, large yellow underwing, genome sequence, chromosomal, Lepidoptera



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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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Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Noctuinae; Noctuini; Noctua; *Noctua fimbriata* (Linnaeus, 1758) (NCBI:txid214277).

Background

Noctua pronuba (large yellow underwing) is a widespread noctuid moth found across Eurasia and North Africa, and one of the most familiar moths in the UK with hundreds of adults commonly caught in a single light trap. The larvae are polyphagous, feeding on a wide range of grasses and herbaceous plants, and have bright green colouration in early instars, changing to brown in later instars. The moth was recorded in Canada in 1979 and has spread south since its accidental introduction; *N. pronuba* is now an occasional pest of commercial crops in the United States, where the larvae are known as winter cutworm (Difonzo & Russell, 2010). In a major outbreak in Michigan in 2007, vast numbers of larvae caused defoliation of fields of alfalfa, rye, oat and winter wheat, and reached public nuisance proportions (Difonzo & Russell, 2010).

The forewings of the adult moth may be light brown, ochreous or dark-purplish brown, with colour controlled genetically through polymorphism at unknown loci modified by sex-linked genes (Cook & Sarsam, 1981). The hindwings, which are completely hidden at rest, are bright orange-yellow with a black band. When disturbed and the moth takes flight, the yellow hindwings are suddenly revealed, plausibly acting as 'flash colouration' to startle predators. In Europe, N. pronuba is a strongly migratory species: vertical-looking entomological radars sited in the UK have detected large numbers of individuals flying north in spring or south in autumn (Chapman et al., 2010). Indeed, in experiments using moths tethered to a flight mill N. pronuba was found to be one of the most mobile noctuid species as measured by both maximum flight speed and total distance covered in one night (Jones et al., 2015). Migration direction is thought to be affected by use of magnetic compass sense, coupled with the ability to detect and utilise favourably-directed winds (Baker & Mather, 1982; Reynolds et al., 2010).

The genome of *N. pronuba* 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 *N. pronuba*, based on one female specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from a single female *N. pronuba* collected from Wytham Woods, Oxfordshire, UK (latitude 51.772, longitude -1.338) (Figure 1). A total of 47-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 71-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 21 missing/misjoins and removed 6 haplotypic duplications, reducing the assembly length by 0.02% and the scaffold number by 25.76%, and increasing the scaffold N50 by 1.57%.

The final assembly has a total length of 529 Mb in 49 sequence scaffolds with a scaffold N50 of 17.9 Mb (Table 1). Of the assembly sequence, 99.89% was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes



Figure 1. Image of the ilNocPron1 specimen taken during preservation and processing.

Table 1. Genome data for *Noctua pronuba*, ilNocPron1.1.

Project accession data	
Assembly identifier	ilNocPron1.1
Species	Noctua pronuba
Specimen	ilNocPron1
NCBI taxonomy ID	NCBI:txid214277
BioProject	PRJEB43815
BioSample ID	SAMEA7519837
Isolate information	Female, whole organism
Raw data accessions	
PacificBiosciences SEQUEL II	ERR6590587
10X Genomics Illumina	ERR6054676-ERR6054679
Hi-C Illumina	ERR6054680-ERR6054682
Genome assembly	
Assembly accession	GCA_905163415.1
Accession of alternate haplotype	GCA_905220345.1
Span (Mb)	529
Number of contigs	74
Contig N50 length (Mb)	16.2
Number of scaffolds	49
Scaffold N50 length (Mb)	17.9
Longest scaffold (Mb)	21.7
BUSCO* genome score	C:98.9%[S:98.3%,D:0.5%], F:0.2%,M:0.9%,n:5286

*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/ ilNocPron1.1/dataset/CAJMZM01/busco (numbered by sequence length), and the W and Z sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO (Manni *et al.*, 2021) completeness of 98.9% 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.

Methods

Sample acquisition, DNA extraction and sequencing

A single female *N. pronuba* (ilNocPron1) was collected from Wytham Woods, Oxfordshire, UK (latitude 51.772, longitude -1.338) by Douglas Boyes, UKCEH, and identified by the same individual. The specimen was collected using a light trap in woodland, preserved on dry ice prior to transfer to the Wellcome Sanger Institute.

DNA was extracted from whole organism tissue at the Wellcome Sanger Institute (WSI) Scientific Operations core

from the whole organism using the Qiagen MagAttract HMW DNA kit, according to the manufacturer's instructions. Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud sequencing libraries were constructed according to the manufacturers' instructions. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II and Illumina HiSeq X instruments. Hi-C data were generated from remaining whole organism tissue using the Arima Hi-C v2 kit and sequenced on HiSeq X.

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 (Garrison & Marth, 2012). The assembly was then

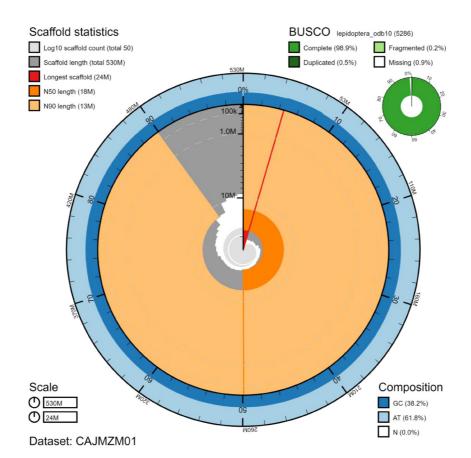


Figure 2. Genome assembly of Noctua pronuba, **ilNocPron1.1: metrics.** The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 529,194,529 bp assembly. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly (24,140,489 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (17,891,575 and 12,542,342 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/ilNocPron1.1/dataset/CA|MZM01/snail.

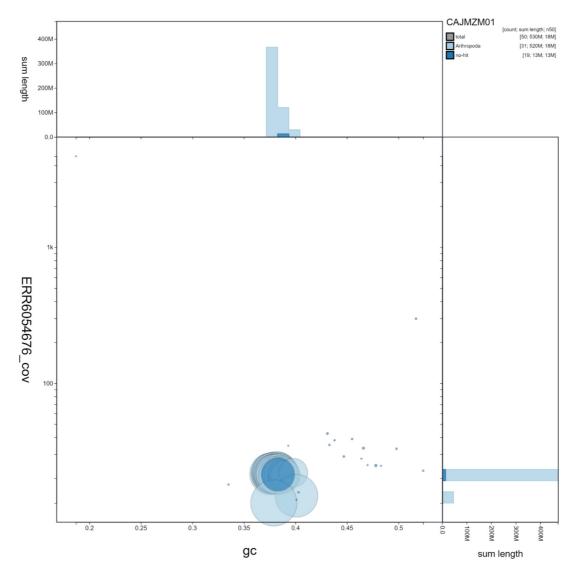


Figure 3. Genome assembly of *Noctua pronuba*, **ilNocPron1.1: GC coverage**. BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilNocPron1.1/dataset/CAJMZM01/blob.

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.

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

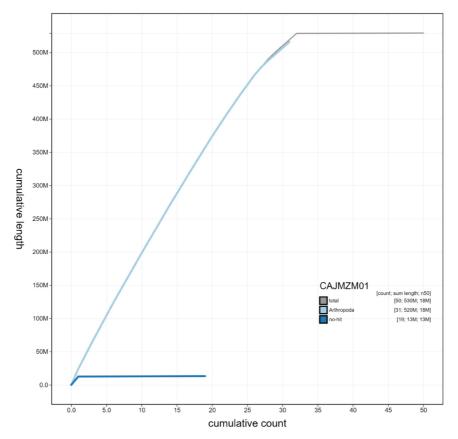


Figure 4. Genome assembly of *Noctua pronuba*, **ilNocPron1.1: cumulative sequence**. BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilNocPron1.1/dataset/CAJMZM01/cumulative.

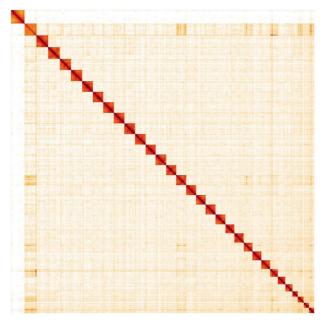


Figure 5. Genome assembly of *Noctua pronuba*, ilNocPron1.1: Hi-C contact map. Hi-C contact map of the ilNocPron1.1 assembly, visualised in HiGlass. Chromosomes are shown in order of size from left to right and top to bottom.

Table 2. Chromosomal pseudomolecules inthe genome assembly of Noctua pronuba,ilNocPron1.1.

INSDC accession	Chromosome	Size (Mb)	GC%
LR999893.1	1	20.39	38.0
LR999894.1	2	19.95	38.2
LR999895.1	3	19.85	37.6
LR999896.1	4	19.06	37.7
LR999897.1	5	18.68	38.2
LR999898.1	6	18.65	37.8
LR999899.1	7	18.55	37.7
LR999900.1	8	18.48	38.1
LR999901.1	9	18.20	38.2
LR999902.1	10	18.14	37.8
LR999903.1	11	17.91	37.8
LR999904.1	12	17.89	37.9
LR999905.1	13	17.62	38.0
LR999906.1	14	17.46	38.2
LR999907.1	15	17.25	37.9
LR999908.1	16	17.15	38.1
LR999909.1	17	17.08	38.3
LR999910.1	18	16.84	38.0
LR999911.1	19	16.20	38.4
LR999912.1	20	15.88	38.5
LR999913.1	21	15.49	38.6
LR999914.1	22	15.37	38.0
LR999915.1	23	14.84	38.2
LR999916.1	24	14.16	38.7
LR999917.1	25	12.54	38.3
LR999918.1	26	12.31	38.4
LR999919.1	27	10.04	38.8
LR999920.1	28	9.48	38.8
LR999921.1	29	9.39	39.0
LR999922.1	30	9.09	39.8
LR999892.1	W	20.55	40.1
LR999891.1	Z	24.14	37.8
LR999923.1	MT	0.02	18.9
-	Unplaced	0.55	45.4

Software tool	Version	Source
HiCanu	2.1	Nurk et al., 2020
purge_dups	1.2.3	Guan <i>et al.</i> , 2020
SALSA2	2.2	Ghurye <i>et al.</i> , 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	1	Uliano-Silva <i>et al.</i> , 2021
gEVAL	N/A	Chow <i>et al.</i> , 2016
PretextView	0.1.x	https://github.com/wtsi-hpag/PretextView
HiGlass	1.11.6	Kerpedjiev <i>et al.</i> , 2018
BlobToolKit	2.6.4	Challis <i>et al.</i> , 2020

Table 3. Software tools used.

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: Noctua pronuba (large yellow underwing). Accession number PRJEB43815; https://identifiers. org/ena.embl/PRJEB43815.

The genome sequence is released openly for reuse. The *N. pronuba* 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 University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.5746938.

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/zen-odo.6125027.

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.6125046.

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

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Joost van den Heuvel

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This data note contains the description of the chromosome level assembly of the genome of *Noctua pronuba*. Data and analysis are well suited and state of the art, resulting in a highly complete and chromosome level assembly, including two sex chromosomes and an alternate haplotype. This assembly will be useful to different fields of research as *Noctua pronuba* is an extremely common species, shows interesting phenotypes but, is also a pest species in North America since the early 80s.

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: population genetics, entomology, evolution, life histories

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 02 December 2022

https://doi.org/10.21956/wellcomeopenres.19641.r53301

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Boyd A. Mori 匝

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This data note provides the first genome assembly for the large yellow underwing moth, *Noctua pronuba*. The species is widespread throughout the Palearctic, and the larvae of which are an occasional crop pest.

This is an additional species to the Tree of Life project. The assembly uses the current leading methods in genome sequencing and assembly. Methods although sparse, are clear. One potential caveat for all of these data notes would be to include a supplementary file with the parameters used for all programs in the assembly pipeline to enhance reproducibility.

Standard metrics all indicate a high quality genome assembly.

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? $\ensuremath{\mathsf{Yes}}$

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

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: agricultural entomology, insect 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.