DATA NOTE

The genome sequence of the German wasp, *Vespula germanica* (Fabricius, 1793) [version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual female *Vespula germanica* (the German wasp; Arthropoda; Insecta; Hymenoptera; Vespidae). The genome sequence is 206 megabases in span. The majority of the assembly (98.55%) is scaffolded into 25 chromosomal pseudomolecules. The mitochondrial genome was also assembled and is 18.0 kilobases in length. Annotation of the genome assembly on Ensembl has identified 12,361 protein-coding genes.

Keywords

Vespula germanica, German wasp, genome sequence, chromosomal, Hymenoptera

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Open Peer Review

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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; Hymenoptera; Apocrita; Aculeata; Vespoidea; Vespidae; Vespinae; Vespula; *Vespula germanica* (Fabricius, 1793) (NCBI:txid30212).

Background

The German wasp, *Vespula germanica*, is a common and widespread species of social wasp throughout the palearctic. It has been introduced and become established in North America, South America, South Africa, Australia and New Zealand (Archer, 1998), where in some instances it has become a significant invasive species (Beggs *et al.*, 2011; Eloff *et al.*, 2020; Masciocchi & Corley, 2013). It is one of the most common and widespread social wasps in the UK, although populations have declined markedly since the 1970s (Archer, 2001). It is found throughout a wide variety of habitats, including urban areas. This species is eusocial, living in colonies with a reproductive queen (16–19 mm), sterile workers (12–14 mm) and reproductive males (14–17 mm) (Archer & Turner, 2014). Adults are strikingly marked yellow and black, with clear yellow genae that distinguish it from the very similar *Vespula vulgaris*. The clypeal markings varyingly consist of an incomplete dorso-ventral stripe plus two spots or three spots, unlike the anchor-like marking that is typical of *V. vulgaris*.

Colonies are annual in the UK, typically producing up to around 8000 cells (Archer & Turner, 2014). Nests are mostly constructed underground, particularly within old rodent holes, but can also be found in enclosed aerial situations such as roof spaces, sheds and thick hedges (Archer, 1998). Nests are constructed out of a paper-like substance produced from macerated wood fibres mixed with saliva. The nest consists of hexagonal cells arranged into eight to nine combs, covered by a nest envelope. The nest envelope of this species is grey in colour due to the well-weathered wood used to make the pulp.

Overwintered queens typically emerge from overwintering diapause around mid-March, nest founding occurs in May, and the first workers appear from early June. Males and new queens are produced from August, and mating occurs in September. Queens are typically polyandrous (Goodisman *et al.*, 2002). Nests usually persist until around November, although some may last through the winter until early spring. There is large variation in population sizes between years, with cyclical abundance and scarcity of individuals (Archer, 1985).

This species is generalist opportunistic predator and scavenger, with workers preying on a wide range of insect and other arthropod species and scavenging from large protein sources, such as carrion (D'adamo & Lozada, 2007). Food is malaxated before being carried back to the nest to be fed to the developing brood. Adults feed on carbohydrate rich substances including nectar, sap, honeydew and secretions from the larvae. The propensity of adults to visit flowers, particularly shallow

blooms such as ivy (*Hedera helix*) and umbellifers, means this species may act an important pollinator. Workers have been shown to exhibit limited temporal polyethism, with nest work, pulp foraging, carbohydrate foraging, and protein foraging sequentially performed by workers as they age (Hurd *et al.*, 2007).

Genome sequence report

The genome was sequenced from a single female *V. germanica* collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.770, longitude -1.339). A total of 72-fold coverage in Pacific Biosciences single-molecule long reads and 181-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 13 missing/misjoins and removed one haplotypic duplication, reducing the scaffold number by 21.74%, and increasing the scaffold N50 by 9.19%.

The final assembly has a total length of 206Mb in 36 sequence scaffolds with a scaffold N50 of 9.4 Mb (Table 1). Of the assembly sequence, 98.55% was assigned to 25 chromosomal-level scaffolds (numbered by sequence length) (Figure 1–Figure 4; Table 2). The assembly has a BUSCO v5.1.2 (Manni *et al.*, 2021) completeness of 96.4% (single 96.1%, duplicated 0.3%) using the hymenoptera_odb10 reference set (n=5991). 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 iyVesGerm1.1 genome assembly has been annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Vespula_germanica_GCA_905340365.1/). The resulting annotation includes 34,067 transcribed RNAs from 12,361 protein-coding and 4,967 non-coding genes. There are 2.26 coding transcripts per gene and 6.94 exons per transcript.

Methods

Sample acquisition and DNA extraction

A single female *V. germanica* (iyVesGerm1) was collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.770, longitude -1.339) by Liam Crowley, University of Oxford, using a net. The sample was identified by the same individual and snap-frozen on dry ice. Unfortunately, no image of the sequenced specimen is available.

DNA was extracted from head/thorax tissue of iyVesGerm1 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. RNA was extracted from remaining head/thorax tissue of iyVesGerm1 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

Table 1. Genome data for *Vespula germanica*, iyVesGerm1.1.

Project accession data	
Assembly identifier	iyVesGerm1.1
Species	<i>Vespula germanica</i>
Specimen	iyVesGerm1
NCBI taxonomy ID	NCBI:txid30212
BioProject	PRJEB43741
BioSample ID	SAMEA7520501
Isolate information	Female, head/thorax
Raw data accessions	
PacificBiosciences SEQUEL II	ERR6635596
10X Genomics Illumina	ERR6054558-ERR6054561
Hi-C Illumina	ERR6054562-ERR6054564
PolyA RNA-Seq Illumina	ERR6286717
Genome assembly	
Assembly accession	GCA_905340365.1
Accession of alternate haplotype	GCA_905340295.1
Span (Mb)	206
Number of contigs	56
Contig N50 length (Mb)	6.6
Number of scaffolds	36
Scaffold N50 length (Mb)	9.4
Longest scaffold (Mb)	20.4
BUSCO* genome score	C:96.4%[S:96.1%,D:0.3%], F:0.9%,M:2.7%,n:5991
Genome annotation	
Number of protein-coding genes	12,361
Average length of coding sequence (bp)	1,553.05
Average number of exons per transcript	6.94
Average exon size (bp)	331.04
Average intron size (bp)	1,355.58

*BUSCO scores based on the hymenoptera_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/CAJPHY01/dataset/CAJPHY01/busco>.

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

Scaffold statistics

- Log10 scaffold count (total 37)
- Scaffold length (total 210M)
- Longest scaffold (20M)
- N50 length (9.4M)
- N90 length (4.5M)

BUSCO hymenoptera_odb10 (5991)

- Complete (96.4%)
- Fragmented (0.9%)
- Duplicated (0.3%)
- Missing (2.7%)

Scale

Dataset: CAJPHY01

Composition

- GC (35.1%)
- AT (64.9%)
- N (0.0%)

Figure 1. Genome assembly of *Vespula germanica*, iyVesGerm1.1: 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 205,789,424 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 (20,389,060 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (9,441,317 and 4,478,551 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 hymenoptera_odb10 set is shown in the top right. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/CAJPHY01/dataset/CAJPHY01/snail>.

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/thorax tissue of iyVesGerm1 using the Arima v2 kit and sequenced on HiSeq X.

Figure 2. Genome assembly of *Vespula germanica*, iyVesGerm1.1: 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/CAJPHY01/dataset/CAJPHY01/blob>.

Genome assembly

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021). 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

Figure 3. Genome assembly of *Vespula germanica*, iyVesGerm1.1: 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/CAJPHY01/dataset/CAJPHY01/cumulative>.

previously (Howe *et al.*, 2021). Manual curation 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.

Figure 4. Genome assembly of *Vespula germanica*, iyVesGerm1.1: Hi-C contact map. Hi-C contact map of the iyVesCrab1.1 assembly, visualised in HiGlass. Chromosomes are shown in size order from left to right and top to bottom.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Vespula germanica*, iyVesGerm1.1.

INSDC accession	Chromosome	Size (Mb)	GC%
HG996528.1	1	20.39	35.7
HG996529.1	2	19.91	35.2
HG996530.1	3	14.87	35.8
HG996531.1	4	11.21	34.9
HG996532.1	5	10.58	35.8
HG996533.1	6	9.48	34.5
HG996534.1	7	9.47	35.8
HG996535.1	8	9.44	35.2
HG996536.1	9	9.01	35.2
HG996537.1	10	8.94	34.1
HG996538.1	11	8.41	34.8
HG996539.1	12	6.60	35.2

INSDC accession	Chromosome	Size (Mb)	GC%
HG996540.1	13	6.55	35.2
HG996541.1	14	6.12	34.5
HG996542.1	15	6.11	34.1
HG996543.1	16	5.79	36.3
HG996544.1	17	5.26	35.2
HG996545.1	18	5.24	34.1
HG996546.1	19	4.99	33.9
HG996547.1	20	4.56	34.8
HG996548.1	21	4.48	33.7
HG996549.1	22	4.34	33.5
HG996550.1	23	4.05	33.8
HG996551.1	24	3.92	36.0
HG996552.1	25	3.10	31.6
HG996553.1	MT	0.02	18.1
-	Unplaced	2.97	43.3

Table 3. Software tools used.

Software tool	Version	Source
Hifiasm	0.12	Cheng et al., 2021
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	v1.3.1-17-gaa2ace8	Garrison & Marth, 2012
MitoHiFi	1.0	https://github.com/marcelauliano/MitoHiFi
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

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 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: *Vespula germanica* (German wasp). Accession number [PRJEB43741](#); <https://identifiers.org/ena.embl/PRJEB43741>.

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

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Members of the Tree of Life Core Informatics collective are listed here: <https://doi.org/10.5281/zenodo.5743293>.

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

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Rodolpho S. T. Menezes

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This short genome report describes the genome sequencing of the German wasp, *Vespula germanica*. The work begins with a background about the natural history as well as some morphological features of this social wasp are commented. The DNA extraction, sequencing, and assembly process are well described. The genome assembly reported will be interesting for further studies.

- I think in the sections "Genome sequence report" and "Genome annotation report", information about the mitochondrial genome should be better described.
- Keywords: Please be in alphabetic order. Change "chromosomal" to "chromosome".

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?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phylogenomics, Phylogeography, and Cytogenetics.

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 16 March 2022

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Volker P. Brendel

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The Data Note provides a succinct review of the genome assembly and annotation efforts by the author group concerning the German wasp. The approaches taken are standard practice and well enough documented to serve as a template for similar genome projects. All the data (raw sequence reads and derived pseudomolecules and hypothetical annotation products) are easily accessible in public databases. The genome assembly and annotation provides a starting point for further phylogenetic and functional studies not pursued in this paper - and rightly so, as such studies should encompass multiple similar data sets.

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: computational 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.