DATA NOTE

The genome sequence of the European robin, *Erithacus rubecula* Linnaeus 1758 [version 1; peer review: 2 approved with reservations]

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Abstract

We present a genome assembly from an individual female *Erithacus rubecula* (the European robin; Chordata; Aves; Passeriformes; Turdidae). The genome sequence is 1.09 gigabases in span. The majority of the assembly is scaffolded into 36 chromosomal pseudomolecules, with both W and Z sex chromosomes assembled.

Keywords

Erithacus rubecula, European robin, genome sequence, chromosomal

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ? ?

	1	2
version 1 02 Jul 2021	? view	? view

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2. **Martin Stervander**, Natural History Museum, Hertfordshire, UK

Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: Jonathan Threlfall was employed by F1000 Research Limited until January 2021.

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Species taxonomy

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda; Coelurosauria; Aves; Neognathae; Passeriformes; Turdidae; Erithacus; *Erithacus rubecula* Linnaeus 1758 (NCBI:txid37610).

Introduction

The European robin, *Erithacus rubecula*, is a small, insectivorous, partially migratory bird native to Europe, western Russia and Siberia, North Africa and the Middle East. Adults are predominantly brown with a characteristic red/orange breast; juveniles have spotted plumage and lack the red/orange breast. Robin populations are increasing both in the Atlantic archipelago of the United Kingdom (where it is the national bird) and Ireland, and worldwide (British Trust for Ornithology, 2019).

The robin is notable for being the first species in which the use of the earth's magnetic field for compass orientation during migration was described (Wiltschko & Wiltschko, 1972). The European robin continues to serve as an iconic model organism for migratory birds. Although the exact mechanism by which this magnetoreception occurs is not yet understood, two main complementary hypotheses are currently discussed. One is based on magnetite particles in the beak area of the bird and is mostly discussed in a map sense, and another hypothesis is based on a light-mediated biochemical reaction scheme (radical-pair reaction) that could mediate directional information provided by the earth's magnetic field into directional information for migratory journeys. The most promising receptor candidate for the latter light-mediated mechanism at current is cryptochrome 4, a blue light receptor molecule in the birds' eye (Günther *et al.*, 2018). The availability of a high quality annotated assembly of the robin's genome sequence will therefore enable researchers to investigate in more detail the genetic factors, such as cryptochrome 4, which drive robins to migrate and direct them where to go. As a model organism for behavioural research, the information deduced from the genetics of *E. rubecula* can then be used to understand the migratory behaviours of other bird species.

Genome sequence report

The reference genome was sequenced from one female *E. rubecula* collected from Eagle, Lincolnshire, UK. A total of 46-fold coverage in Pacific Biosciences single-molecule long reads (N50 19 kb) and 47-fold coverage in 10X Genomics read clouds (from molecules with an estimated N50 of 68 kb) were generated. Primary assembly contigs were scaffolded with chromosome conformation HiC data. Manual assembly curation corrected 110 missing/misjoins and removed 20 haplotypic duplications, reducing the scaffold number by 9.2%, increasing the scaffold N50 by 112.7% and decreasing the assembly length by 0.4%. The final assembly has a total length of 1.087 Gb in 1,120 sequence scaffolds with a scaffold N50 of 46.6 Mb (Table 1). The majority, 91.6%, of the assembly sequence was assigned to 36 chromosomal-level scaffolds representing 34 autosomes (numbered by sequence length), and the W and Z sex chromosomes (Figure 1–Figure 4; Table 2). The assembly has a BUSCO (Simão *et al.*, 2015) v5.0.0 completeness of 96.2% using the aves_odb10 reference set. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Table 1. Genome data for *Erithacus rubecula* bEriRub2.2.

Project accession data	
Assembly identifier	bEriRub2.2
Species	*Erithacus rubecula*
Specimen	bEriRub2
NCBI taxonomy ID	txid37610
BioProject	PRJEB38658
BioSample ID	SAMEA4760689
Isolate information	Female, blood
Raw data accessions	
PacificBiosciences SEQUEL I	ERX3338814, ERX3338816-ERX3338823
10X Genomics Illumina	ERX3341631-ERX3341634
Hi-C Illumina	ERX5308916
Genome assembly	
Assembly accession	GCA_903797595.2
Accession of alternate haplotype	GCA_903797565.1

Genome assembly	
Span (Mb)	1,087
Number of contigs	2,109
Contig N50 length (Mb)	5.59
Number of scaffolds	1120
Scaffold N50 length (Mb)	46.56
Longest scaffold (Mb)	112.1
BUSCO* genome score	C:96.2%[S:95.8%,D:0.4%],F:0.6%,M:3.2%,n:10,844

* BUSCO scores based on the aves_odb10 BUSCO set using v5.0.0. 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/Erithacus%20rubecula/dataset/CAIGJR02/busco.

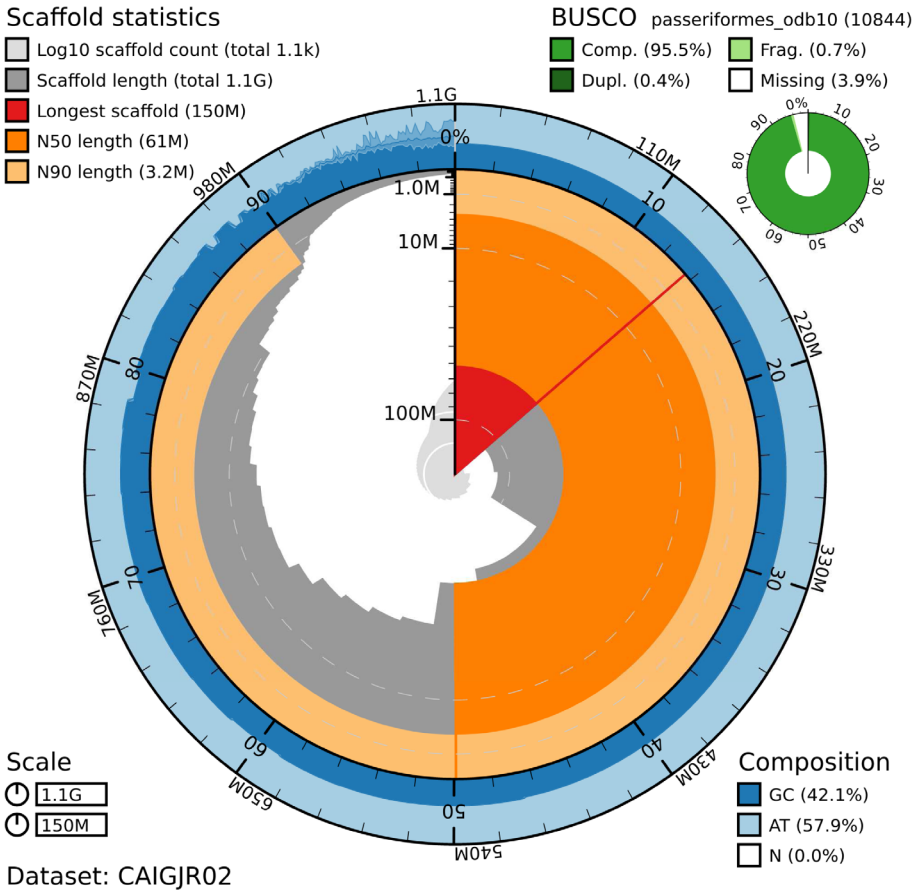

Figure 1. Genome assembly of *Erithacus rubecula*, bEriRub2.2. BlobToolKit Snailplot. The plot shows N50 metrics for bEriRub2 and BUSCO scores for the Passiformes set of orthologues. Interactive version available at https://blobtoolkit.genomehubs.org/view/Erithacus%20rubecula/dataset/CAIGJR02/snail.

Methods

A blood sample was taken from the brachael vein of a live bird during routine health checks of populations in Eagle, Lincolnshire, UK (latitude 53.193716, longitude -0.689135). Blood was collected through a glass capillary tube and stored at -20°C. The sample was taken under Home Office (ASPA) license number PB0AED9B7; birds were caught and handled under a British Trust for Ornithology ringing licence.

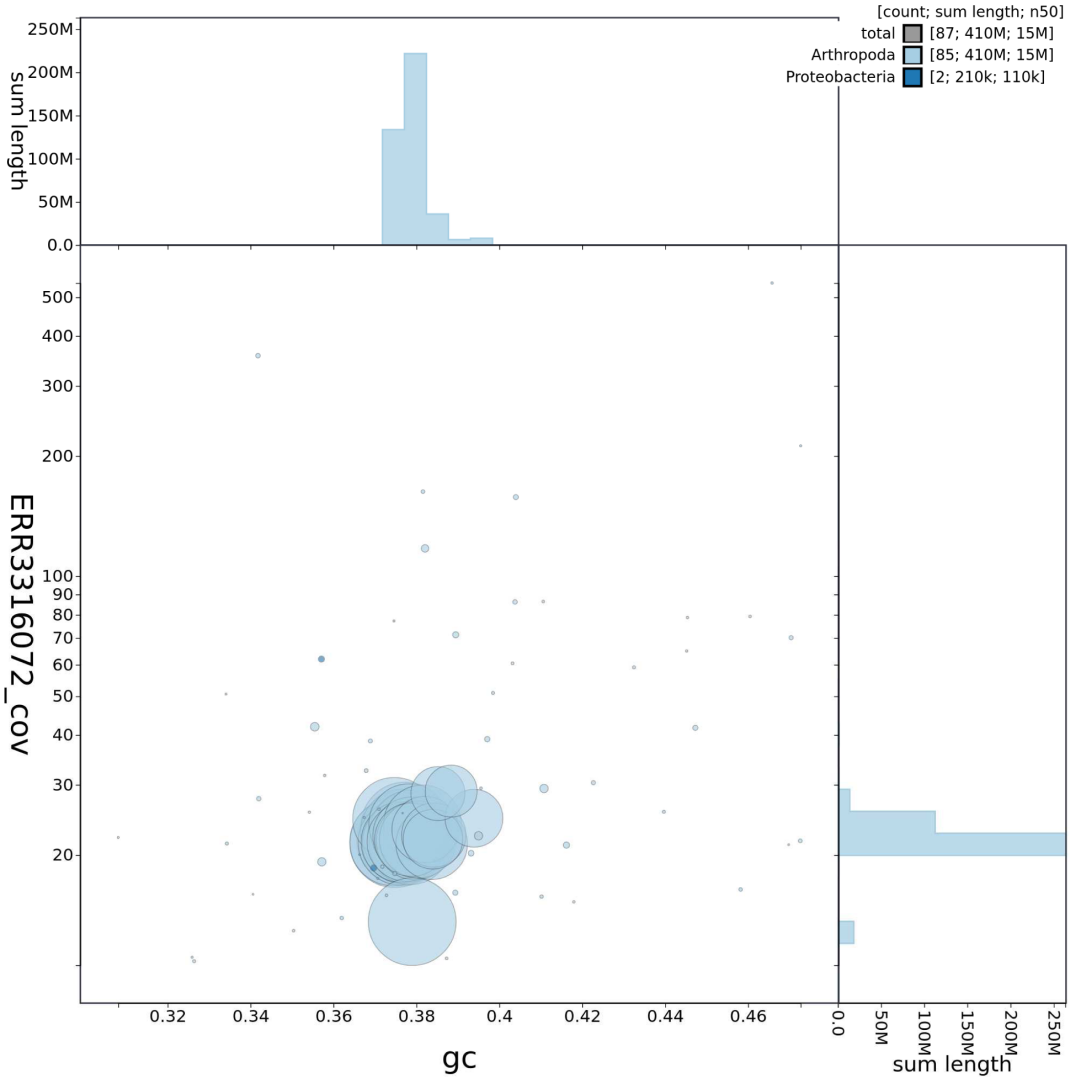

Figure 2. Genome assembly of *Erithacus rubecula*, bEriRub2.2. BlobToolKit GC-coverage plot. Interactive version available at https://blobtoolkit.genomehubs.org/view/Erithacus%20rubecula/dataset/CAIGJR02/blob?plotShape=circle.

Genomic DNA was extracted using an agarose plug extraction from a blood sample following the Bionano Prep Animal Tissue DNA Isolation Soft Tissue Protocol. Pacific Biosciences CLR long read and 10X Genomics read cloud sequencing libraries were constructed according to manufacturers' instructions. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL I and Illumina HiSeq X instruments. Hi-C data were generated using the Dovetail HiC library preparation kit at the Wellcome Sanger Institute and sequenced using Illumina HiSeq X.

Assembly was carried out following the Vertebrate Genome Project pipeline v1.6 (Rhie *et al.*, 2020), without the use of Bionano data. Assembly was performed using Falcon-unzip (Chin *et al.*, 2016), haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020) and a first round of scaffolding carried out with 10X Genomics read clouds using scaff10x. Scaffolding with Hi-C data (Rao *et al.*, 2014) was carried out with SALSA2 (Ghurye *et al.*, 2019). The Hi-C scaffolded assembly was polished with arrow using the PacBio data, then polished with the 10X Genomics Illumina data by aligning to the assembly with longranger align, calling variants with freebayes (Garrison & Marth, 2012) and applying homozygous non-reference edits using bcftools consensus. Two rounds of the Illumina polishing were applied. 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 gEVAL, HiGlass and Pretext. Figure 1–Figure 3 and BUSCO scores were generated using BlobToolKit (Challis *et al.*, 2020). Software versions are given in Table 3.

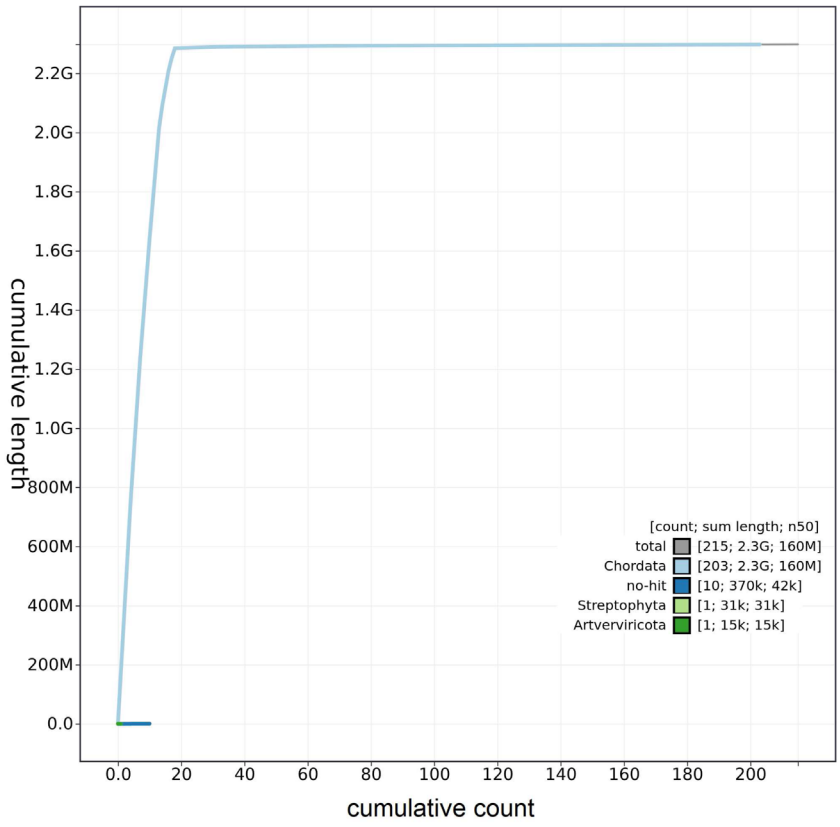

Figure 3. Genome assembly of *Erithacus rubecula*, bEriRub2.2: BlobToolKit Cumulative sequence plot. Interactive version available at https://blobtoolkit.genomehubs.org/view/Erithacus%20rubecula/dataset/CAIGJR02/cumulative.

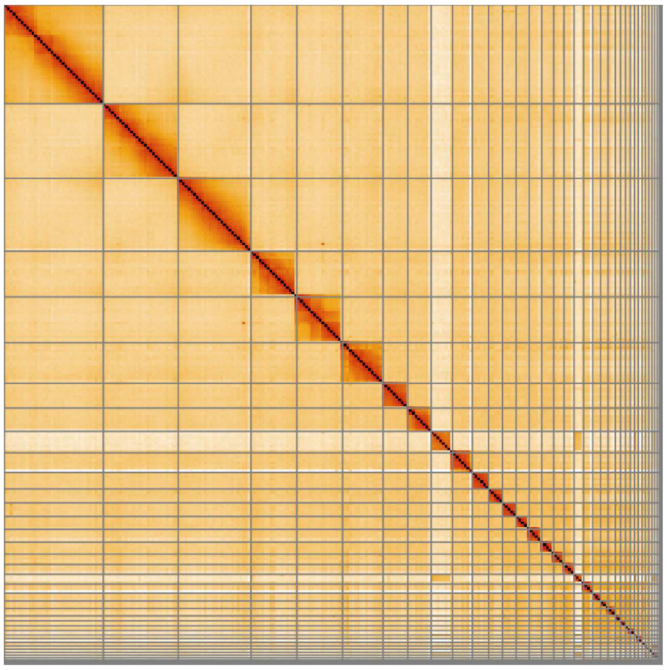

Figure 4. Genome assembly of *Erithacus rubecula*, bEriRub2.2: Hi-C contact map. Hi-C contact map of the bEriRub2.2 assembly, visualized in HiGlass (Kerpedjiev *et al.*, 2018).

Table 2. Chromosomal pseudomolecules in the genome assembly of *Erithacus rubecula* bEriRub2.2.

INSDC accession	Chromosome	Size (Mb)	GC%
LR812103.1	1	112.10	39.3
LR812104.1	2	109.05	39.7
LR812105.2	3	148.24	39.2
LR812106.1	4	68.60	39.9
LR812107.1	5	68.52	39.2
LR812108.1	6	60.68	41
LR812110.1	8	37.15	41.3
LR812111.1	9	34.93	41.8
LR812113.1	10	29.52	42.1
LR812114.1	11	24.63	43.1
LR812115.1	12	20.59	42.9
LR812116.1	13	20.40	44
LR812117.1	14	19.45	43.3
LR812118.1	15	19.11	43.5
LR812119.1	16	17.82	45
LR812120.1	17	15.59	45.4
LR812121.1	18	14.76	46.8
LR812122.1	19	13.42	46.4
LR812123.1	20	11.84	47.3
LR812124.1	21	11.12	48.5
LR812125.1	22	10.97	47.5
LR812126.1	23	7.61	50.5
LR812127.1	24	7.50	48.2
LR812128.1	25	7.13	49.1
LR812129.1	26	6.52	51.9
LR812131.1	27	5.43	52.2
LR812132.1	28	5.34	53
LR812133.1	29	4.77	50.2
LR812135.1	31	2.33	56.4
LR812130.2	W	4.15	44.6
LR812137.1	33	2.04	53.3
LR812112.1	Z	31.99	39.7
LR812138.1	34	0.96	49.3
	Unplaced	131.30	46

Table 3. Software tools used.

Software tool	Version	Source
Falcon-unzip	falcon-kit 1.2.2	(Chin *et al.*, 2016)
purge_dups	1.0.0	(Guan *et al.*, 2020)
scaff10x	4.2	https://github.com/wtsi-hpag/Scaff10X
arrow	GenomicConsensus 2.3.3	https://github.com/PacificBiosciences/GenomicConsensus
longranger align	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
freebayes	v1.1.0-3-g961e5f3	(Garrison & Marth, 2012)
bcftools consensus	1.9	http://samtools.github.io/bcftools/bcftools.html
gEVAL	2016	(Chow *et al.*, 2016)
HiGlass	1.11.6	(Kerpedjiev *et al.*, 2018)
PretextView	0.0.4	https://github.com/wtsi-hpag/PretextView
BlobToolKit	2.5	(Challis *et al.*, 2020)

Data availability

Underlying data

European Nucleotide Archive: Erithacus rubecula (European robin). Accession number PRJEB38659.

The genome sequence is released openly for reuse. The *E. rubecula* genome sequencing initiative is part of the Wellcome Sanger Institute's "25 genomes for 25 years" project. It is also part of the Vertebrate Genomes Project (VGP) ordinal references programme and the Darwin Tree of Life (DToL) project. All raw 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.

Acknowledgements

We thank Mike Stratton and Julia Wilson for their support for the 25 genomes for 25 years project.

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Publisher Full Text

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PubMed Abstract | Publisher Full Text

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 24 May 2022

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Martin Stervander
Bird Group, Natural History Museum, Hertfordshire, UK

This paper by Dunn *et al.* reports on the high-quality/high-contiguity genome assembly of the European robin, a common Eurasian muscicapid passerine bird. The rationale is well justified, not least as the European robin is a species of historic and current interest regarding orientation by magnetoreception. This report nicely sums up and presents assembly properties, although it should be noted that no annotation is included at this stage.

Overall, the report is well-written and clear, though there are a number of issues that should be addressed, and a couple of opportunities for improvement:

1. It would be prudent to insert a reference, perhaps a suitable review, following the *Introduction* statement “One is based on magnetite particles in the beak area of the bird and is mostly discussed in a map sense, and another hypothesis is based on a light-mediated biochemical reaction scheme (radical-pair reaction) that could mediate directional information provided by the earth’s magnetic field into directional information for migratory journeys.”

2. I would urge the authors to add some metadata and background of the specimen sampled.
    1. Since the sample came from a live bird in an ongoing project, it presumably has an actual identifier beyond bEriRub2, namely ring number. I would strongly suggest that this is reported, just like museum vouchers are reported for collection specimens.

    2. I suggest adding date and age to the statement “The reference genome was sequenced from one female *E. rubecula* collected from Eagle, Lincolnshire, UK.”

    3. Given that the authors presumably wanted a female (ZW), how was the sex determined?

3. It is stated that “The majority, 91.6%, of the assembly sequence was assigned to 36 chromosomal-level scaffolds representing 34 autosomes (numbered by sequence length),

and the W and Z sex chromosomes (Figure 1–Figure 4; Table 2).” However, in Table 2, 31 autosomes (chromosomal pseudomolecules 1–6, 8–29, 31, 33–34) and the two sex chromosomes are listed.

1. Which number of chromosomes is correct?

2. Table 2: Either sort the chromosomal pseudomolecules descending by size (move chr Z) or place sex chromosomes before/after autosomes (move chr Z and chr W).

4. While I appreciate that this is a brief report and that much of the biological insights will only be possible following the upcoming annotation of the genome, there are some comparisons that are possible and that would add value but seem lacking.
    1. How much of an improvement does this assembly offer compared to the previous robin genome assembly, in terms of size, contiguity, and completeness (determined with BUSCO score or otherwise)?

    2. How does the (inferred) karyotype compare to other birds? Given the conserved karyotype and synteny of birds, it would be valuable to (1) get a ‘translation key’ between chromosome designations between species (e.g., which robin chromosomal pseudomolecule number corresponds to zebra finch chromosome 4A?), and (2) get an idea of large-scale conservation. If the robin indeed has 31+2 chromosomes (see above), that would be the same number as the confamilial collared flycatcher *Ficedula albicollis* (30 + 3 linkage groups; https://www.ensembl.org/Ficedula_albicollis/Location/Genome), considerably fewer than the 40+2 reported for Swainson’s thrush *Catharus ustulatus* in the sister family Turdidae (https://www.ensembl.org/Catharus_ustulatus/Location/Genome), and one less than zebra finch (https://www.ensembl.org/Taeniopygia_guttata/Location/Genome). I would suggest that the authors run synteny analyses with collared flycatcher and zebra finch, and present the results with circle plots.

5. Table 1, BUSCO genome score: The sample size (number of genes) is incorrect, as the authors have reported the number (n:10,844) of BUSCO genes for passeriformes_odb10 BUSCO, while specifying that it is aves_odb10 BUSCO (and giving the percentages for aves_odb10 BUSCO). Thus, change to the correct sample size (n:8,338). Numbers and graphs for passeriformes_odb10 is presented in Figure 1.

6. Figure 1: What do the four shades of blue mean for the GC content track (outer segment) of the smallest contigs? The two intermediate shades are not explained (neither in the report legend nor in the interactive version).

7. Figure 2: The interactive version of this plot looks completely different, presumably because the print version is based on base coverage in ERR3316072, while the interactive version is based on base coverage of ERR5528452. Presumably, I should have been able to change the source for base coverage in the interactive version (?), but it could perhaps be nice to have the same data filter as default in the interactive version as in the report.

8. Figures 2–3: I strongly suggest that the online captions for the interactive graphs are used

also for the figures included in the genome report.

9. Figure 4: I really miss an annotation of the chromosomal pseudomolecule numbers in the Hi-C contact map, and would strongly urge the authors to add these to guide the reader. This would also aid in the interpretation, which could further be facilitated by a slightly richer caption. For example, why do a couple of chromosomes appear as lighter bars, i.e. with weaker between-chromosome contact? Does the first major one (ninth in order) coincide with the Z chromosome? And is the localize high contact between the third and fifth chromosome something worth commenting on?

10. Minor copyedit issues noted:

    1. In the main text body, be consistent in referring to the species either with its common name or its scientific name, after introducing the species with both names. Currently, there is a mishmash.

    2. Table 1, Number of scaffolds: add thousands separator (1120 → 1,120)

    3. Table 1, footnote: remove extra spaces following some equal signs.

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: I have current ongoing collaborations with Miriam Liedvogel, who is a co-author of this report, but they do not include robins or orientation in migratory birds. We have not yet published together. I have reviewed this genome report with no less scrutiny than usual, and here provide a fair and impartial assessment.

Reviewer Expertise: Avian genomics, short-read sequencing, molecular evolution, differentiation, adaptation, genomic architecture

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, however I have significant reservations, as outlined above.

Reviewer Report 25 April 2022

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Toni Gossmann
Department of Evolutionary Genetics, Bielefeld University, Bielefeld, Germany

The article by Dunn *et al.* describes a haplotype resolved genome assembly of the European robin.

I think the article is clearly written and a dense compendium of genomic and assembly features. There is however another genome released (B10K 2020) of this species and some basic comparative statistics would have been nice, also to illustrate the added value of the haplotype resolution.

The importance of the genome is highlighted through the species feature to use the earth magnetic field for navigation and they highlight a receptor of particular importance.

Unfortunately, the gene location or any basic features is not referred to later on in the statistical overview or in the text. Perhaps a couple of sentences would be meaningful (e.g. differences between the two haplotypes or the other assembly specific to cryptochrome 4).

As this is a songbird genome, it might be relevant to understand that not the entire genome has been captured as blood samples were used. Torgasheva *et al* [1]have shown via cytogenetics that most songbirds possess an additional chromosome in the germline. Kinsella *et al* [2] have started to genomically assemble this for the zebra finch. A haplotype resolved genome may help to support future attempts also to obtain the GRC from the European robin.

There is no mention of the mitochondrial genome and whether it was obtained from the genomic reads. Such an assembly could be contrasted to other (mito)genome assemblies of the same species to illustrate that the individual is a common representative.

Regarding the Figures. I really like the dynamic figure associated with Fig1. The static figure, however, has some limitations. I think the abbreviations in the BUSCO legend are not clear and I find the N percentage with 0.0% not very helpful (perhaps show <0.01%). I am also unclear what the scale legend is about and find it very hard to imagine the different chromosomes here, which I assume the bigger scaffolds are almost complete chromosomes.

Regarding Figure 2, I am unsure what the legend is supposed to mean, perhaps one could add some sentences in the caption. Also, the online tool for dynamic visualization is unclear to me. I also don't understand the Figure 3 caption, which I think is comparable to Figure 2.

References

1. Torgasheva A, Malinovskaya L, Zadesenets K, Karamysheva T, et al.: Germline-restricted chromosome (GRC) is widespread among songbirds. *Proceedings of the National Academy of Sciences*. 2019; **116** (24): 11845-11850 Publisher Full Text
2. Kinsella C, Ruiz-Ruano F, Dion-Côté A, Charles A, et al.: Programmed DNA elimination of germline development genes in songbirds. *Nature Communications*. 2019; **10** (1). Publisher Full

Text

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: population genetics, genomics, avian genomics, molecular evolution

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, however I have significant reservations, as outlined above.