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Genome Resources

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A genome assembly for mule deer, *Odocoileus hemionus*, from Southern California

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Abstract

Mule deer (*Odocoileus hemionus*) is an ecologically and economically important cervid species which is widely distributed across western North America. Their broad range and use of diverse habitats, including those linked to historical glacial refugia, have made them an ideal model for studying the effects of climatic fluctuations and environmental heterogeneity on lineage diversification. The effects of this complex evolutionary history on the genome are further complicated by evidence of hybridization with other members of the genus, leading to extreme cases of mito-nuclear discordance. More recently, the influence of specific gene variants on disease dynamics, particularly the progression and morbidity of chronic wasting disease, has become an important topic of genomic research for this taxon. Here, we present and evaluate a new chromosome-level genome assembly of a representative mule deer from across the species' diverse range as part of the California Conservation Genomics Project. We assembled a genome *de novo* utilizing Pacific Biosciences HiFi long-read and Omni-C chromatin-proximity sequencing data. The assembly consisted of 814 scaffolds and 901 contigs representing a contig N50 of 46.68 Mb and a scaffold N50 of 63.29 Mb. Lastly, our benchmarking universal single-copy ortholog completeness score was 96.3%. This genome represents one of the most complete *Odocoileus* assemblies and will further our understanding of the comparative genomic architecture of cervids.

Key words: California Conservation Genomics Project, CCGP; Cervidae, mammal

Introduction

Ubiquitous throughout western North America, mule deer (*Odocoileus hemionus*) represents a highly managed ecologically and economically important member of Cervidae. Mule deer occupy a wide range of environments such as alpine communities, temperate rainforests, high deserts, and urban centers throughout their distribution, which spans the Yukon Territory through to northern Mexico (Heffelfinger and Latch 2023). This broad ecological and geographic breadth has resulted in extensive research into how historical and contemporary environmental variation have shaped evolutionary trajectories (Latch et al. 2009, 2014; Pease et al. 2009; Alminas et al. 2021; Kessler et al. 2023). However, strong signatures of mito-nuclear discordance (Carr et al. 1986; Cronin 1991; Derr 1991; Cathey et al. 1998; Klicka et al. 2024) and hybridization (Carr et al. 1986; Bradley et al. 2003; Latch et al. 2011; Haines et al. 2019; Russell et al. 2021; Wright et al. 2023) have complicated the assessment of evolutionary relationships and blurred taxonomic boundaries.

This uncertainty is evident in California and throughout the American southwest where intraspecific taxonomy has been contentious due to extensive phenotypic variation (e.g. coat patterning including tail coloration, metatarsal gland size differences, and overall body size; Sheldon 1933; Cowan 1936; Dasmann 1975; Anderson and Wallmo 1984). Molecular evidence reveals broad genetic structure among mule deer in this region (Latch et al. 2009, 2014; Pease et al. 2009), but current subspecies boundaries are inconsistent with these genetic patterns with few exceptions (Alminas et al. 2021). Despite this taxonomic uncertainty, with several subspecies designations considered invalid or reflective of ecotypic variation rather than long-term separation in glacial refugia, both molecular and morphological evidence consistently support

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the existence of two distinct lineages: black-tailed deer (*O. h. columbianus*: Columbian black-tailed and *O. h. sitkensis*: Sitka black-tailed) and mule deer (*O. h. hemionus*: Rocky Mountain; with additional subspecies proposed which garner varying levels of genetic support, including *O. h. californicus*: California; *O. h. fuliginatus*: Southern; *O. h. inyoensis*: Inyo; *O. h. eremicus*: Burro; *O. h. peninsulae*: Peninsula; *O. h. cerrosensis*: Cedros Island; *O. h. sheldoni*: Tiburón Island; reviewed in Latch and Heffelfinger 2022). Although these subspecific designations could arguably represent over-splitting, we refer here to the more traditional taxonomic understanding to remain consistent due to the lack of any formal taxonomic revision.

Assembled as part of the California Conservation Genomics Project (CCGP; Shaffer et al. 2022), we report the first chromosome-level genome assembly for a representative of *O. hemionus* from southern California within the proposed range of the original *O. h. californicus* designation (Caton 1876; Cowan 1936). While previous *O. hemionus* genomes have been assembled (Russell et al. 2019; Lamb et al. 2021), none currently represent the taxonomically uncertain and environmentally diverse region of the California Floristic Province. This gap is particularly significant given ongoing conservation and management challenges facing mule deer within the state. The proliferation of chronic wasting disease among North American cervid populations has led to extensive monitoring programs and management policy aimed at *O. hemionus* (reviewed in RG et al. 2025). The growing collection of mule deer genome assemblies from disparate populations will enhance our understanding of how hybridization and evolutionary history influence disease and population dynamics within the genus, ultimately informing more effective management policies.

Methods

Biological materials

We collected and preserved whole blood in EDTA from an adult male during a California Department of Fish and Wildlife (CDFW) capture event in San Bernardino National Forest, California, located at the eastern extent of the Transverse Mountain Range (Coordinates: 34.335231, −116.939902). This capture was conducted under the department's jurisdiction as the trustee for wildlife management in California, in accordance with CA Fish & Game Code § 1802 (2015).

HiFi library preparation and sequencing

We isolated high-molecular-weight genomic DNA (gDNA) from whole blood and preserved it in EDTA (sample accession no. ODHE-2022-008-006). We mixed 1.5 ml of blood with 4.5 ml of RBC lysis solution (Qiagen, Valencia, CA; Cat #158445) and incubated the reaction at room temperature for 5 min. We centrifuged the sample at 2,000 × *g* for 5 min to pellet white blood cells. Next, we discarded the supernatant and added 2 ml of lysis buffer containing 100 mM NaCl, 10 mM Tris–HCl pH 8.0, 25 mM EDTA, 0.5% (w/v) SDS, and 100 μg/ml Proteinase K to the cell pellet. We incubated the reaction at room temperature for several hours until the solution was homogenous. We then treated the lysate with 20 μg/ml RNase A at 37 °C for 30 min and cleaned with equal volumes of phenol/chloroform using phase lock gels (Quantabio, Beverly, Massachusetts; Cat #2302830). We precipitated the DNA by adding 0.4× volume of 5 M ammonium acetate and 3× volume of ice-cold ethanol. We washed the DNA pellet twice with 70% ethanol and resuspended in an elution buffer (10 mM Tris, pH 8.0). We accessed purity of gDNA using NanoDrop ND-1000 spectrophotometer where 260/280 ratio of 1.85 and 260/230 ratio of 2.35 was observed. DNA yield was 21 μg as quantified by Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA). Then, we verified the integrity of the high molecular weight gDNA on a Femto pulse system (Agilent Technologies, Santa Clara, CA) where 91% of DNA was observed in fragments above 100 kilobases (kb).

We constructed the HiFi SMRTbell library using the SMRTbell prep kit 3.0 (Pacific Biosciences [PacBio], Menlo Park, CA; Cat #102-182-700) according to the manufacturer's instructions. We sheared HMW gDNA to a target DNA size distribution between 15 and 18 kb using Diagenode's Megaruptor 3 system (Diagenode, Belgium; Cat #B06010003). Next, we concentrated the sheared gDNA using 1× of SMRTbell cleanup beads provided in the SMRTbell prep kit 3.0 for the repair and a-tailing incubation at 37 °C for 30 min and 65°C for 5 min, followed by ligation of overhang adapters at 20 °C for 30 min, clean-up using 1× SMRTbell cleanup beads, and nuclease treatment at 37 °C for 15 min. We used 3.1× of 35% v/v diluted AMPure PB beads (PacBio; Cat #100-265-900) for our size selection step of the SMRTbell library to progressively remove SMRTbell templates < 5 kb. We sequenced the 15 to18-kb average HiFi SMRTbell library at UC Davis DNA Technologies Core (Davis, CA) using three 8 M SMRT cell (PacBio, Menlo Park, CA; Cat #101-389-001), Sequel II sequencing chemistry 2.0, and 30-h movies each on a PacBio Sequel IIe sequencer.

Omni-C library preparation and sequencing

We prepared Omni-C libraries with whole blood (sample ID: ODHE-2022-008-006) using Dovetail Omni-C Kit (Dovetail Genomics, Scotts Valley, CA) according to the manufacturer's protocol with slight modifications. Briefly, we fixed chromatin in place in the nucleus and digested fixed chromatin with DNase I followed by extraction. We repaired chromatin ends and ligated a biotinylated bridge adapter followed by proximity ligation of adapter containing ends. After proximity ligation, we reversed cross-links and purified DNA from proteins. We treated purified DNA to remove biotin which was not internal to ligated fragments. Next, we used a New England Biolabs Ultra II DNA Library Prep kit (NEB, Ipswich, MA) with an Illumina-compatible y-adaptor to generate an NGS library. We then captured biotin-containing fragments using streptavidin beads. The postcapture product was split into two replicates prior to PCR enrichment to preserve library complexity with each replicate receiving unique dual indices. Finally, we sequenced the library at the Center for Advanced Technologies at the University of California, San Francisco (UCSF CAT) on an Illumina NovaSeq X platform (Illumina, CA) and generated approximately 100 million 2 × 150 bp read pairs per giga base of genome size.

Nuclear genome assembly

We assembled the *O. hemionus* genome following the CCGP assembly pipeline, which uses PacBio HiFi reads and Omni-C data to produce high-quality and highly contiguous genome assemblies (Table 1). First, we removed the remnants adapter

Table 1. Assembly pipeline and software used.

Assembly step	Software and any nondefault options	Version	Reference
Initial assembly			
Filtering PacBio HiFi adapters	HiFiAdapterFilt	Commit 64d1c7b	Sim et al. 2022
K-mer counting	Meryl (k = 21)	1	https://github.com/marbl/meryl
Estimation of genome size and heterozygosity	GenomeScope	2	Ranallo et al. 2020
De novo assembly (contiging)	HiFiasm (Hi-C Mode, –primary, output hic.hap1.p_ctg, hic.hap2.p_ctg)	0.19.5-r592	Cheng et al. 2022
Scaffolding			
Omni-C data alignment	*Arima Genomics Mapping Pipeline*	Commit 2e74ea4	https://github.com/ArimaGenomics/mapping_pipeline
Arima Genomics Mapping Pipeline (AGMP)	BWA-MEM	0.7.17-r1188	Li 2013
	samtools	1.11	Danecek et al. 2021
	filter_five_end.pl (AGMP)	Commit 2e74ea4	https://github.com/ArimaGenomics/mapping_pipeline
	two_read_bam_combiner.pl (AGMP)	Commit 2e74ea4	https://github.com/ArimaGenomics/mapping_pipeline
	picard	2.27.5	https://broadinstitute.github.io/picard/
Omni-C Scaffolding	SALSA (-DNASE, –i 20, –p yes)	2	Ghurye et al. 2017, Ghurye et al. 2019
Omni-C Contact map generation			
Short-read alignment	BWA-MEM (-5SP)	0.7.17-r1188	Li 2013
SAM/BAM processing	samtools	1.11	Danecek et al. 2021
SAM/BAM filtering	pairtools	0.3.0	Open2C et al. 2024
Pairs indexing	pairix	0.3.7	Lee at al 2022
Matrix generation	cooler	0.8.10	Abdennur and Mirny 2020
Matrix balancing	hicExplorer (hicCorrectmatrix correct –filterThreshold –2 4)	3.6	Ramírez et al. 2018
Contact map visualization	HiGlass	2.1.11	Kerpedjiev et al. 2018
	PretextMap	0.1.4	https://github.com/wtsi-hpag/PretextView
	PretextView	0.1.5	https://github.com/wtsi-hpag/PretextMap
	PretextSnapshot	0.0.3	https://github.com/wtsi-hpag/PretextSnapshot
Manual curation tools	Rapid curation pipeline (Wellcome Trust Sanger Institute, Genome Reference Informatics Team)	Commit 7acf220c	https://gitlab.com/wtsi-grit/rapid-curation
Genome quality assessment			
Basic assembly metrics	QUAST (–est-ref-size)	5.0.2	Gurevich et al. 2013
Assembly completeness	BUSCO (–m geno, –l mammalia)	5.0.0	Manni et al. 2021
	Merqury	2020 January 29	Rhie et al. 2020
Contamination screening			
Local alignment tool	BLAST+ (-db nt, -outfmt '6 qseqid staxids bitscore std', -max_target_seqs 1, -max_hsps 1, -evalue 1e-25)	2.15	Camacho et al. 2009
General contamination screening	BlobToolKit (HiFi coverage, BUSCO = mammalia, NCBI Taxa ID = 9872)	2.3.3	Challis et al. 2020
Mitochondrial assembly			
Mitochondrial genome assembly	*MitoHiFi* (-r, -p 90, -o 1) Reference: *Odocoileus hemionus* (NCBI:NC_020729.1)	2.2	Uliano-Silva et al. 2023
MitoHiFi: Mitochondrial genome annotation	MitoFinder (MitoHiFi pipeline parameters)	1.4	Allio et al. 2020

sequences from the PacBio HiFi dataset using HiFiAdapterFilt (Sim et al. 2022) and generated the initial phased diploid assembly using HiFiasm (Cheng et al. 2021) on Hi-C mode, with the filtered PacBio HiFi reads and the Omni-C dataset. We aligned the Omni-C data to each assembly following the Arima Genomics Mapping Pipeline (https://github.com/ArimaGenomics/mapping_pipeline) and then scaffolded them with SALSA (Ghurye et al. 2017, 2019).

Assemblies were manually curated by iteratively generating and analyzing their corresponding Omni-C contact maps. To

generate the contact maps, we aligned the Omni-C data with BWA-MEM (Li 2013), identified ligation junctions, and generated Omni-C pairs using pairtools (Abdennur et al. 2024). We generated a multiresolution Omni-C matrix with cooler (Abdennur and Mirny 2020) and balanced it with hicExplorer (Ramírez et al. 2018). We used HiGlass (Kerpedjiev et al. 2018) and the PretextSuite (https://github.com/wtsi-hpag/PretextView; https://github.com/wtsi-hpag/PretextMap; https://github.com/wtsi-hpag/PretextSnapshot) to visualize the contact maps where we identified misassemblies and misjoins, and finally modified the assemblies using the Rapid Curation pipeline from the Wellcome Trust Sanger Institute, Genome Reference Informatics Team (https://gitlab.com/wtsi-grit/rapid-curation). Some of the remaining gaps (joins generated during scaffolding and/or curation) were closed using the PacBio HiFi reads and YAGCloser (https://github.com/merlyescalona/yagcloser). Finally, we checked for contamination using the BlobToolKit Framework (Challis et al. 2020) and FCS-GX (https://github.com/ncbi/fcs; Astashyn et al. 2024) upon submission of the genome assemblies to the National Center of Biotechnology Information (NCBI).

Genome size estimation and quality assessment

We generated *k*-mer counts from the PacBio HiFi reads using meryl (https://github.com/marbl/meryl). The *k*-mer counts were then used in GenomeScope 2.0 (Ranallo et al. 2020) to estimate genome features including genome size, heterozygosity, and repeat content. For contiguity metrics, we ran QUAST (Gurevich et al. 2013). To evaluate genome quality and functional completeness, we used BUSCO (Manni et al. 2021) with the Mammalian ortholog database (mammalia_odb10) which contains 9,226 genes. We assessed base level accuracy (QV) and *k*-mer completeness using the previously generated meryl database and merqury (Rhie et al. 2020). We further estimated genome assembly accuracy via BUSCO gene set frameshift analysis using the pipeline (Korlach et al. 2017). Measurements of the size of the phased blocks are based on the size of the contigs generated by HiFiasm on HiC mode. We followed the quality metric nomenclature (Rhie et al. 2021), with the genome quality code *x.y.P.Q.C*, where, $x = \log_{10}$[contig NG50]; $y = \log_{10}$[scaffold NG50]; $P = \log_{10}$ [phased block NG50]; Q = Phred base accuracy QV (quality value); C = % genome represented by the first "n" scaffolds, following a karyotype of 2n = 70, known for the number of chromosomes for this species (Genome on a Tree—GoaT; tax_tree (*O. hemionus*); Wurster and Benirschke 1967a, 1967b). Quality metrics for the notation were calculated on the primary haplotype assembly. Primary and alternative haplotype classifications were determined by BUSCO completeness scores with the higher of the two scores designated as primary.

Mitochondrial genome assembly

We assembled the mitochondrial genome of *O. hemionus* from the PacBio HiFi reads using the reference-guided pipeline MitoHiFi (Allio et al. 2020; Uliano-Silva et al. 2023). The mitochondrial sequence of an existing *O. hemionus* (NCBI:NC_020729.1; Hassanin et al. 2012) was used as the starting reference sequence. After completing the nuclear genome assembly, we used BLAST+ (Camacho et al. 2009) to identify regions of high similarity between the mitochondrial assembly and the nuclear genome. Contigs and scaffolds from the nuclear genome showing >99% sequence identity to the mitochondrial assembly and smaller in size were filtered.

Odocoileus genome comparisons

Contiguity metrics of available *Odocoileus* spp. genomes were generated to evaluate assembly quality. We downloaded four *O. hemionus* genomes from NCBI GenBank which represent two mule deer [PRJNA752226, accessed 14 June 2023 (Lamb et al. 2021); PRJNA417092, accessed 24 Mar 2025 (Russell et al. 2019)] and two black-tailed deer [PRJNA476345, accessed 24 Mar 2025 (*O. h. sitkensis*); PRJNA1252274, 24 Mar 2025 (*O. h. columbianus*)]. We also included two *O. virginianus* (white-tailed deer) assemblies [PRJNA782613, 24 Mar 2025 (London et al. 2022); PRJNA317745, 5 Mar 2023 (Seabury et al. 2020)]. We ran QUAST with an estimated genome size of 2.98 Gb and calculated BUSCO scores for downloaded genomes against mammalia_odb10 database.

Results

Sequencing data

The Omni-C library generated 289.88 million read pairs and the PacBio SMRTBell library generated 6.06 million HiFi reads. The PacBio HiFi reads yielded ~34× genome coverage and had an N50 read length of 17,622 bp, a minimum read length of 111 bp, a mean read length of 16,734 bp, and a maximum read length of 56,721 bp (See Supplementary Fig. 1 for read length distribution). Based on the PacBio HiFi data, Genomescope 2.0 estimated to have a genome size of 2.98 Gb, a 0.462% heterozygosity rate, and a 0.132% sequencing error rate. The *k*-mer spectrum shows a bimodal distribution with a major peak at ~32-fold coverage and a minor peak at ~16-fold coverage (Fig. 2A).

Nuclear genome assembly

The final genome assembly (mOdoHem1) consists of two phased haplotypes; however, the assemblies have been tagged as primary (mOdoHem1.0.p) and alternate (mOdoHem1.0.a) based on completeness and contiguity. The final haplotype assemblies are close in size to the estimated genome assembly size from GenomeScope2.0, as it has been observed in other taxa (see Pflug et al. 2020, for example), with a difference of ~610 Mb between haplotypes. This difference seems to be related to the inclusion of sex chromosome–related scaffolds to the primary haplotype.

The primary haplotype consists of 814 scaffolds spanning 3.42 Gb with a contig N50 of 46.68 Mb, a scaffold N50 of 63.29 Mb, the maximum contig size of 107.65 Mb, and maximum scaffold size of 166.98 Mb. The alternate haplotype consists of 388 scaffolds spanning 2.8 Gb with a contig N50 of 53.6 Mb, a scaffold N50 of 71.92 Mb, the largest contig size of 114.3 Mb, and the largest scaffold size of 117.36 Mb.

The primary haplotype has a BUSCO completeness score for the Mammalian gene set of 96.3%, a base pair quality value (QV) of 70.22, a *k*-mer completeness of 94.21%, and a frameshift indel QV of 44.6. The alternate haplotype has a BUSCO completeness score for the same gene set of 94.5%, a base-pair QV of 71.08, a *k*-mer completeness of 89.4%, and a frameshift indel QV of 43.73.

During manual curation we made a total of 116 joins (72 on the primary haplotype and 44 on the alternate haplotype) and 13 breaks (8 on the primary haplotype and 5 on the

Table 2. Genbank accession numbers and genome assembly statistics.

Bio Projects & vouchers	**CCGP NCBI BioProject**						**PRJNA720569**
	Genera NCBI BioProject						**PRJNA765645**
	Species NCBI BioProject						**PRJNA777200**
	NCBI BioSample						**SAMN41460070**
	Specimen identification						**ODHE-2022-008-006_CCGP**
	NCBI Genome accessions				**Primary haplotype**	**Alternate haplotype**	
	Assembly accession				**JBFCZP000000000**	**JBFCZQ000000000**	
	Genome sequences				**GCA_042768445.1**	**GCA_042767535.1**	
Genome Sequence	PacBio HiFi reads	Run			1 PACBIO_SMRT (Sequel IIe) run: 6.1 M spots, 101.4G bases, 65.8Gb		
		Accession			SRX26390780		
	Omni-C Illumina reads	Run			2 ILLUMINA (Illumina NovaSeq X) runs: 289.9 M spots, 87.6G bases, 28.4Gb		
		Accession			SRX26390781–2		
Genome Assembly Quality Metrics	Assembly identifier (Quality code[a])				mOdoHem1(7.7.P7.Q70.C77)		
	HiFi Read coverage[b]				33.95X		
					Primary haplotype	**Alternate haplotype**	
	Number of contigs				901	455	
	Contig N50 (bp)				46 676 287	53 595 885	
	Contig NG50[b]				56 557 357	52 038 776	
	Longest Contigs				107 653 483	114 301 365	
	Number of scaffolds				814	388	
	Scaffold N50				63 288 026	71 917 018	
	Scaffold NG50[b]				79 205 051	66 809 063	
	Largest scaffold				166 982 627	117 364 546	
	Size of final assembly				3 418 972 884	2 801 335 277	
	Phased block NG50[b]				52 971 770	52 038 776	
	Gaps per Gbp (# Gaps)				19 (67)	23(79)	
	Indel QV (Frame shift)				44.59693976	43.72617466	
	Base pair QV				70.22 Full assembly = 70.5206	71.0815	
	k-mer completeness				94.2147 Full assembly = 99.5271	89.4018	
	BUSCO completeness[c] (mammalia) *n* = 9226		**C**	**S**	**D**	**F**	**M**
		P[d]	96.30%	92.80%	3.50%	0.90%	2.80%
		A[d]	94.50%	91.30%	3.20%	0.90%	4.60%
	Organelles		1 complete mitochondrial sequence				CM091143

[a] Assembly quality code x.y.P.Q.C derived notation, from (Rhie et al. 2021). x = log10[contig NG50]; y = log10[scaffold NG50]; P = log10 [phased block NG50]; Q = Phred base accuracy QV (Quality value); C = % genome represented by the first "n" scaffolds, following a known karyotype for *O. hemionus* of 2n = 70 (Genome on a Tree, Challis et al. 2023). Quality code for all the assembly denoted by primary assembly (mOdoHem1.0.p). [b] Read coverage and NGx statistics have been calculated based on the estimated genome size of 2.98 Gb. [c] BUSCO Scores. Complete BUSCOs (C). Complete and single-copy BUSCOs (S). Complete and duplicated BUSCOs (D). Fragmented BUSCOs (F). Missing BUSCOs (M). [d] (P)rimary haplotype and (A)lternate haplotype assembly values.

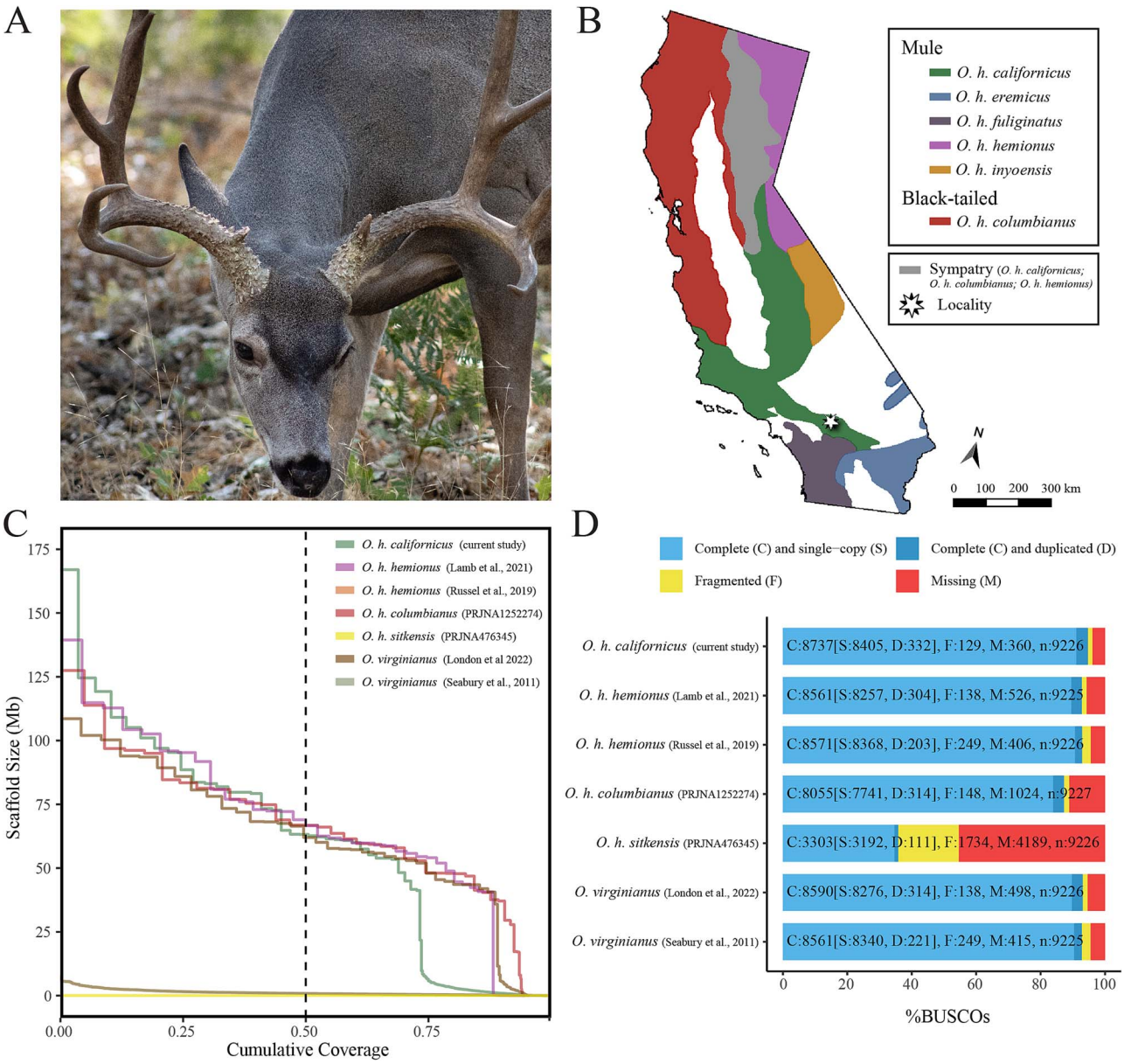

Fig. 1. A) Photo of a male mule deer, *Odocoileus hemionus* from Yosemite National Park (photo credit: Joshua Hallas). B) California distribution of *O. hemionus* highlighting the ranges of putative subspecies (Dasmann 1975). C) Nx plot of all available *Odocoileus* genomes depicting scaffold size against cumulative proportion of the total assembly. D) BUSCO scores for each available assembly.

alternate haplotype) based on the signal from the Omni-C contact maps. We filtered out 2 contigs corresponding to mitochondrial contamination (1 per haplotype). No other contigs were removed.

The Omni-C contact maps show highly contiguous assembly, suggesting that the *O. hemionus* genome is organized in 35 chromosomes based on the number of major bins along the diagonal axis of the plot (Fig. 2C and D). Assembly statistics are reported in Table 2 and represented graphically in Fig. 2C. We have deposited the genome assembly on NCBI GenBank (see Table 2 and Data Availability for details).

Mitochondrial genome assembly

We assembled a mitochondrial genome with MitoHiFi. Final mitochondrial genome is circular and spans 16 478 bp. The base composition of the final assembly version is A = 33.57%, C = 23.59%, G=, 13.3%, T = 29.54%, and consists of 22 unique transfer RNAs, 13 protein coding genes, and 2 rRNAs. Gene composition follows the animal mitochondrial genome structure (Boore 1999). We have deposited the mitochondrial genome assembly on NCBI GenBank (see Table 2 and Data Availability for details).

Odocoileus reference genome comparisons

Contiguity metrics (see Supplementary Table 1) and the Nx plot (Fig. 1C) suggest that the presented mule deer genome was comparable to other high-quality *Odocoileus* assemblies. The first 19 scaffolds comprise 50% of the cumulative assembly coverage (Fig. 1B). Given a known karyotype of 2n = 70, these scaffolds are highly concordant with chromosomes. The BUSCO completeness scores (Fig. 1D) for the primary assembly is higher than those observed in other chromosome-level *Odocoileus* assemblies.

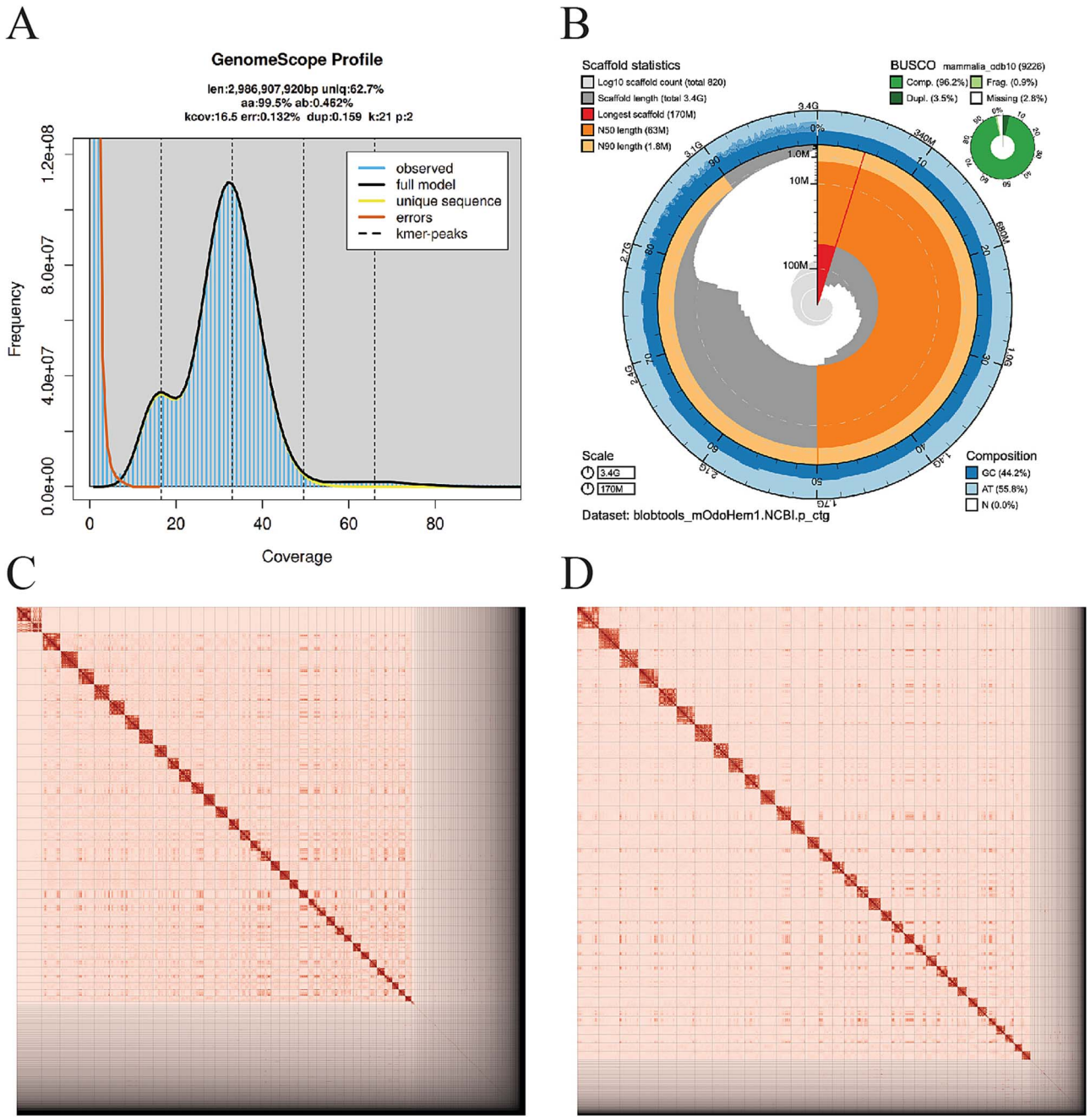

Fig. 2. Genome assembly metrics. A) Visualization of GenomeScope2.0 K-mer spectra output of PacBio HiFi data. B) Quality metrics characterized by BlobToolKit snail plot of the primary *O. hemionus* assembly (mOdoHem1). The full size of the assembly is represented by the circle in the plot, with the central plot covering length-related metrics from the inside out. The red line indicates the length of the longest scaffold, while all other scaffolds, arranged in descending size order, are shown in dark gray, starting from the outer edge of the central plot and moving clockwise. Scaffold N50 and N90 values are shown in dark and light orange, respectively. Light-gray circle shows the cumulative scaffold count using log10 scale. Proportion of GC to AC composition at 0.1% length intervals are depicted by dark and light blue, respectively. C) Haplotype 1 and D) haplotype 2 Omni-C contact maps for genome assemblies. Omni-C contact maps translate proximity of genomic regions in 3-D space to contiguous linear organization.

Discussion

Our genome assembly of a mule deer from southern California (putative *O. h. californicus*) advances the genomic resources available for the genus *Odocoileus* by improving geographic coverage. Currently, six genome assemblies are available within the genus: two for Rocky Mountain mule deer, one for Columbian black-tailed deer, one for Sitka black-tailed deer, and two for the closely related white-tailed deer. Assembly quality varies with longest scaffold and N50 values ranging from 0.11 to 167 Mb and 0.01 to 72.1 Mb (see Supplementary Table 1), respectively. Recent assemblies from Lamb et al. (2021) and London et al. (2022) represent the highest-quality assemblies for *Odocoileus*. Our assembly demonstrates comparable contiguity and BUSCO completeness, meeting the standards set by both the CCGP (Shaffer et al. 2022) and the Vertebrate Genome Project (Rhie et al. 2021).

This assembly provides a valuable addition to *Odocoileus* genomic resources, supporting both evolutionary studies and management efforts. While other assemblies of mule deer are representative of the core distribution, the interior of North America, the assembly presented here fills a critical gap by capturing undocumented single nucleotide polymorphisms and structural genomic variation from a historically under sampled population at the edge of the species' range in southern California. Together with the recent release of a Columbian black-tailed deer genome from northern California (BioProject accession: PRJNA1252274), this assembly will aid in resolving inter- and intraspecific boundaries, which have long been difficult to delineate due to factors such as mito-nuclear discordance (e.g. Wright et al. 2022; Klicka et al. 2024), hybridization (e.g. Haines et al. 2019), and extensive morphological variation (e.g. Dasmann 1975). As additional genomes from diverse populations are assembled, researchers will be able to more accurately characterize variation in *Odocoileus*, enabling the use of approaches such as pangenomes (Sherman and Salzberg 2020; Sarawad et al. 2024). This contribution is especially timely given the recent documentation of CWD in California (California Department of Fish and Wildlife 2024). As additional genetic data are compiled, researchers will be better equipped to characterize the potential impacts of the disease on naïve populations. This assembly enhances the ability of wildlife managers to assess genetic diversity, population structure, and adaptive variation, supporting more effective conservation and management strategies across the range of *O. hemionus*.

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Author contributions

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Supplementary material

Supplementary material is available at *JHERED* Journal online.

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Data availability

Data generated for this study are available under NCBI BioProject PRJNA777200. Raw sequencing data for sample ODHE-2022-008-006_CCGP (NCBI BioSample SAMN41460070) are deposited in the NCBI Short Read Archive (SRA) under SRX26390780 for PacBio HiFi sequencing data, and SRX26390781, SRX26390782 for the Omni-C Illumina sequencing data. GenBank accessions for both primary and alternate assemblies are GCA_042768445.1 and GCA_042767535.1; and for genome sequences JBFCZP000000000 and JBFCZQ000000000. The GenBank genome assembly for the mitochondrial genome is CM091143.1. Assembly scripts and other data for the analyses presented can be found at the following GitHub repository: www.github.com/ccgproject/ccgp_assembly

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