



Genome Resources

A genome assembly for a textbook mammalian study species, the California ground squirrel (*Otospermophilus beecheyi*)

Matthew L. Holding^{1,2,*} , Sree Rohit Raj Kolora³ , Jennifer E. Smith⁴ , Shana L. McDevitt⁵ , Peter H. Sudmant³ , Rebecca D. Tarvin^{3,6} 

¹Life Sciences Institute, University of Michigan, Ann Arbor, MI, United States, ²Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, United States, ³Department of Integrative Biology, University of California Berkeley, Berkeley, CA, United States, ⁴Biology Department, University of Wisconsin-Eau Claire, Eau Claire, WI, United States, ⁵California Institute of Quantitative Biosciences (QB3), University of California Berkeley – QB3 Genomics, Berkeley, CA, United States, ⁶Museum of Vertebrate Zoology, University of California Berkeley, Berkeley, CA, United States

*Corresponding author: Life Sciences Institute, University of Michigan, Ann Arbor, MI, United States. Email: matthewholding28@gmail.com

Abstract

Squirrels (Rodentia; Sciuridae) are a well-known and diverse group of rodents, including the charismatic ground-dwelling members of the Tribe Marmotini. In particular, the California ground squirrel (*Otospermophilus beecheyi*) is an emerging model system for the study of social and risk-sensitive behaviors in a rapidly changing world, as well as the physiology of resistance to snake venoms. To complement extensive natural history information for *O. beecheyi*, we provide a chromosome-scale genome to facilitate molecular studies focused on the genetic basis of ecologically important traits, population genetics, comparative genomics, and social evolution. The final scaffolded genome was 2.27 Gb contained in 9,960 contigs and placed into 1,383 scaffolds. The scaffold N50 was just more than 125 Mb. We used the presence of 10,248 complete genes detected by BUSCO v5 specifically to compare broad patterns of chromosomal synteny between chromosomal scaffolds for *O. beecheyi* and two other sciurid rodents. The recovered pattern of synteny suggests several fusion and fission events for *O. beecheyi* in relation to the other two species. Taken together, this new information should advance our understanding of *O. beecheyi* and comparative studies of mammalian genomic biology and evolution.

Key words: genome, mammal, Marmotini, Sciuridae

Introduction

Squirrels (Rodentia; Sciuridae) are extremely well-known and charismatic animals. Our familiarity with squirrels stems from the visibility of diurnal species and their propensity to live among us in neighborhoods and parks (Parker and Nilon 2008). Squirrels are hunted by humans, can cause damage by chewing or burrowing behaviors and through consumption of crop plants, and are zoonotic reservoirs of bubonic plague (Smith et al. 2016). Many basic biological features of squirrels have garnered significant research attention as well, where the diverse and often accessible squirrel species can be observed through the lens of urban vs. natural ecology. Squirrels are a diverse group of rodents with nearly 300 species distributed worldwide (Rick et al. 2024), occupying most biomes and therefore environmental extremes from tundra and deserts to rainforests (Menéndez et al. 2021).

Tribe Marmotini includes the burrow-dwelling marmots, chipmunks, prairie dogs, and ground squirrels. This clade of squirrels includes emerging model systems in several aspects of wild mammal biology. For example, ground squirrel adaptation to extremes, particularly long bouts of hibernation in cold climates, has garnered attention from a basic and biomedical perspective. Adaptations to lowered body temperature in 13-lined ground squirrels (*ictidomys*

tridecemlineatus) include several modulations to blood physiology, producing leads toward better cold storage of blood platelets (Cooper et al. 2012). In Arctic ground squirrels (*Urocyon parryi*), hibernation studies have increased our understanding of molecular basis of bone loss prevention during long-term immobility, which could inform treatment of immobilized patients (Wojda et al. 2016). Black-tailed prairie dogs (*Cynomys ludovicianus*) have been heavily studied at the interface of population biology, predation, disease epidemiology, and conservation, because plague infections cause population cycles that threaten both prairie dogs and the endangered black-footed ferrets that feed on them (Keuler et al. 2020). Finally, behavior in marmots, prairie dogs, and ground squirrels has received significant attention in testing of fundamental hypotheses in the evolution of sociality (Wey and Blumstein 2010) and the ecology of fear (Blumstein et al. 2009).

The California ground squirrel (*Otospermophilus beecheyi*; Richardson et al. 1829) is a social mammal native to California whose range extends from the San Francisco Bay to the northern regions of the Baja peninsula, and east through the Sierra Nevada to the border of the Great Basin Desert (Phuong et al. 2014; Smith et al. 2016; Holding et al. 2021). Whereas not under imminent conservation

Received on 15 February 2025; revised on 24 June 2025; accepted on 25 June 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of The American Genetic Association. All rights reserved. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

threat, the many interesting features of *O. beecheyi* include complex social behaviors, engineering ecosystems through deep burrows and adapting to resulting hypoxic underground conditions, hibernation, coevolution with venomous snakes, and acting as a bubonic plague (*Yersinia pestis*) reservoir. Of particular note, *O. beecheyi* have served a textbook study system for understanding variation in the psychology of fear as it relates to predator presence, resource availability, and sociality (Coss and Owings 1978). The pioneering long-term studies by Richard Coss and Donald Owings unveiled the multifaceted and variable behavioral responses of *O. beecheyi* to rattlesnake predators. *O. beecheyi* were eventually found to respond acutely to rattlesnakes at a physiological level by warming their tails before tail-flagging displays (Rundus et al. 2007), which was later revealed to be an honest signal of squirrel vigilance (Putman and Clark 2015). Moreover, *O. beecheyi* back up their anti-snake behaviors by being up to hundreds of times more resistant to rattlesnake venom than a mouse (Poran et al. 1987). Recent and ongoing work in *O. beecheyi* has highlighted coevolved variation in venom resistance (Holding et al. 2016), its physiological constraints (Holding et al. 2020), and molecular basis (Biardi et al. 2011; Gibbs et al. 2020), whereas ongoing studies of *O. beecheyi* behavioral ecology continue to elucidate the interplay of predation risk (Smith et al. 2023), stress (Hammond et al. 2019), parasite loads (Smith et al. 2021), kinship, and the formation of social networks in human-influenced systems (Smith et al. 2018; Person et al. 2024; Ortiz-Jimenez et al. 2025).

Genomic resources can enhance the potential for discovery in ground squirrels and their relatives. At present, NCBI Genbank houses 21 genome assemblies from 11 species within Marmotini, of which only three species' genomes are assembled at the chromosome level. Despite being the focus of many research programs, there has been no genome assembly for *O. beecheyi*. To this end, we present here a chromosome-scale genome for *O. beecheyi* that should powerfully advance studies of their sociality, physiology, demography, and molecular coevolution with venomous rattlesnakes, while increasing the potential for comparative genome biology among squirrel species.

Methods

Biological materials

The genome animal was an adult female live-trapped at Hastings Natural History Reservation, Monterey, California under the California Department of Fish and Wildlife Scientific Collection Permit S-190320013-19228-001 and University of California IACUC protocol AUP-2019-08-12453-1. The specimen was deposited in the Museum of Vertebrate Zoology at UC Berkeley as MVZ:Mamm:239690.

Nucleic acid library preparation

High molecular weight (HMW) DNA was extracted from muscle tissue by phenol chloroform and ethanol precipitation. Tissue was pulverized in liquid nitrogen and homogenized in Qiagen Buffer ATL and Proteinase K (Qiagen, Germantown, MD) for 4 h on a thermal mixer set at 56 °C and 400 rpm. The then clear homogenate was mixed 1:1 with phenol chloroform isoamyl (PCI) alcohol (Sigma-Aldrich, Burlington, MA) for 10 min on a lab rotator, transferred into a MaXtract High

Density phase lock tube (Qiagen, Germantown, MD) and centrifuged at max speed for 5 min. The top aqueous layer was washed with PCI a second time, followed by a third 1:1 chloroform isoamyl (CI) alcohol wash. 2.5× volume ice-cold 100% ethanol was added to the final aqueous layer, inverted 20 times, and stored at −20 °C for 1 h before being pelleted at 16,000 × *g* in a 4 °C centrifuge. The pellet was washed with room-temperature 70% ethanol until clear and subsequently air dried to remove residual ethanol. HMW genomic DNA (gDNA) was resuspended in PacBio Elution Buffer (PacBio, Menlo Park, CA), placed overnight at 4 °C, and then quantified with Quant-It Qubit reagents (Thermo Fisher Scientific, Pleasanton, CA), and checked for purity on a Nanodrop (Thermo Fisher Scientific, Pleasanton, CA). gDNA was then subjected to a high-salt PCI wash and final AMPure PB bead clean after the PacBio recommended protocol. The initial 260/230 Nanodrop purity score increased from 1.7 to 2.09. The cleaned gDNA was evaluated on a Femto Pulse (Genomic DNA 165 kb Kit, Agilent, Santa Clara, CA) showing an average size of 93 kilobases (kb).

The PacBio sequencing library was then generated by ligating SMRTbell hairpin adaptors to the cleaned gDNA (PacBio SMRTbell Template Prep Kit 1.0, PacBio, Menlo Park, CA). Pre size selection Femto Analysis results show an average library size of 48 kb with 72% of fragments more than 20 kb. The final library was generated by size selecting 30 kb or larger library molecules on a Blue Pippin (PAC30KB—0.75% Agarose, High Pass, PacBio >30 kb templates, U1 Kit, Sage Science, Beverly, MA).

Sequel I DNA polymerase was bound to the final PacBio library after the manufacturer's recommendations (Sequel Binding Kit 2.1, PacBio, Menlo Park, CA) and sequenced at the Vincent J Coates Genomics Sequencing Lab at UC Berkeley to generate continuous long reads (CLR) on 8 Sequel 1 M SMRTcell 10-h movies (Sequel Sequencing Kit 2.1, PacBio, Menlo Park, CA). All sequencing runs combined resulted in more than 11 million reads with an average length of 19.56 kb, generating 215,500,012,650 total bases. We also generated Hi-C reads with the Dovetail Genomics Omni-C protocol using the manufacturer's protocol (Cantata Bio, Scotts Valley, CA) and sequenced on a 150 bp paired-end S4 Illumina NovaSeq6000 flowcell (Illumina, Inc., San Diego, CA).

DNA sequencing and genome assembly

The resulting library was sequenced on the PacBio Sequel I at the Vincent J Coates Genomics Sequencing Lab at UC Berkeley to generate CLR. We also generated Hi-C reads with the Dovetail Genomics Omni-C protocol and sequenced on an Illumina NovaSeq6000 platform. The contig-level primary assembly was generated in a phase-aware manner using Falcon-Unzip V1.3.7 followed by a round of polishing using Arrow algorithm from SMRT Link software V6.0.0. Juicer v1.6 (Durand et al. 2016) was used to align the Hi-C reads to the primary assembly, and the contigs were sorted based on Hi-C contacts with the 3d-DNA (Dudchenko et al. 2017) “run-asm-pipeline.sh” with the early-exit flag. Chromosome-level scaffolds were grouped and sorted based on visualized Hi-C contacts with Juicebox Assembly Tools (JBAT) v1.11.08 (<https://github.com/aidenlab/Juicebox>). Scaffold arrangements were corrected via “bow-tie motifs” and based on information from the comparative order of orthologous loci on *O. beecheyi* contigs and those in the chromosome-level genomes

of the gray squirrel (*Sciurus carolinensis*; mSciCar1.2) and Siberian chipmunk (*Tamias sibiricus*; ASM2559416v1). Orthologous loci were identified using BUSCO (Manni et al. 2021) and the glires_odb10 database. Areas of broad mismatch between the two genomes were visually examined in JBAT for evidence of mis-assembly and corrected, and final chromosomal contact map for the assembly was saved. The final assembly was quality controlled with the NCBI Foreign Contamination Screen toolkit (Astashyn et al. 2024), resulting in the hard-masking of a single 31 bp PacBio adapter and a lack of evidence for foreign contaminant scaffolds. Further details on assembly software can be found in Table 1.

Genome annotation and analysis

The protein-coding regions of the genome were annotated using braker v.3.0.8 (Gabriel et al. 2024). Protein hints were generated via a concatenated file containing the vertebrata_odb10 protein sequences from OrthoDB (Kriventseva et al. 2019), while *O. beecheyi* liver, kidney, large intestine, small intestine, spleen, thyroid, and skeletal muscle from NCBI PRJNA804109 (Ochoa et al. 2023) were utilized as RNA hints. RNA-seq reads were aligned to the genome assembly with B v.2.2.1 (Kim et al. 2019), and these resulting alignments were provided to as input to braker. We added functional annotations to the de novo gene models initially by conducting a diamond v.2.11 (Buchfink et al. 2021) search of each gene model against the vertebrata_orthodb11 protein set with an e-value cutoff 10^{-11} and set to “more-sensitive”. Next, we used InterProScan v5.68-100.0 (Jones et al. 2014) to search for protein domains in the predicted primary amino acid sequences, and only retained annotations with detected domains.

A de novo repeat database for our genome assembly was generated with RepeatModeler v. 2.0.4 (Flynn et al. 2020), and we concatenated this repeat library with the RepBase (Bao et al. 2015) library for vertebrata, downloaded 18 February 2024. This concatenated library was used to annotate and soft-mask repeats in the genome using RepeatMasker v4.1.5 (Smit et al. 1996).

Genome completeness was assessed with both BUSCO v5.8 (Manni et al. 2021) and Compleasm (Huang and Li 2023); the unique and shared detected genes for each program were joined to generate a report of single copy, duplicated, fragmented, and missing genes. BEDtools v2.29.2 (Quinlan and Hall 2010) was used to calculate Guanine + Cytosine (GC) content, gene density, and repeat density in 500 kB sliding windows, which were later visualized these along chromosomes using circlize v0.4.16 (Gu et al. 2014). ChromoSyn v1.6.1 pipeline (Edwards et al. 2022) was used to visualize chromosomal synteny between *O. beecheyi*, *T. sibiricus*, and *S. carolinensis*.

Results

The final scaffolded genome was 2.27 Gb contained in 9,960 contigs and placed into 1,383 scaffolds. The scaffold N50 was just more than 125 Mb (Table 2). Of these scaffolds, 20 were greater than 10 Mb in length, which we suggest represent 19 autosomes and the X chromosome for this individual (Fig. 1; Supplementary Information). These chromosome-scale scaffolds comprise 91.7% of the total assembly, whereas the remainder represents small unplaced contigs and scaffolds.

The content of the assembly is largely consistent with that of other ground squirrel taxa. For example, we recovered an overall GC content for *O. beecheyi* at 38.6%, which is comparable to Gunnison’s prairie dog (40.1%). Repeat content was modestly higher for *O. beecheyi* (41.6%) than the comparative analyses reported previously where species ranged from 30% to 35% (Tsuchiya et al. 2020). Finally, after filtering based on the presence of protein domains, we identified 20,301 genes in our assembly. A considerable portion of the genic content is unfortunately missing, because 19.2% of the genes in the Glires geneset from OrthoDb10 are missing. This inconsistency is likely reflective of an incomplete and more fragmented assembly generated by our use of earlier generations of PacBio sequencing technology that preceded HiFi reads and lack of complementary whole genome short-read sequencing. ShinyGO v0.77 (Ge et al. 2020) analysis of the missing BUSCO content revealed a lack of bias in GO Biological Processes or sequence length, but a strong bias toward higher GC content of missing genes (Supplementary Fig. S1), consistent with increased difficulty of during primary assembly (Chen et al. 2013). We do not expect the levels of missingness to impact our conclusions about chromosome number or comparative analysis of synteny, given those inferences are based on the present, rather than absent, gene blocks.

We used the presence of 10,248 complete genes detected by BUSCO v5 specifically to compare broad patterns of chromosomal synteny between our *O. beecheyi* chromosomal scaffolds and those of two other chromosome-scale genomes: the Siberian chipmunk (*Tamias sibiricus*; a distantly related Marmotini-clade member; Li et al. 2022) and the eastern gray squirrel (*S. carolinensis*; a tree squirrel; tribe Sciurini; Mead et al. 2020). The resulting synteny plot suggests several fusion and fission events can be inferred for *O. beecheyi* in relationship to the other two species (Fig. 1C), which is surprising given the closer relationship of *Tamias* and *Otospermophilus*. The second largest *O. beecheyi* scaffold (Chr_2) represents a fusion of Chr8 and Chr17 both present in the other two species. Conversely, two of the larger chromosomes present in *S. carolinensis* (Chr_1 and Chr_3) and *T. sibiricus* (Chr_2 and Chr_4, respectively) have each undergone fission to produce four *O. beecheyi* chromosomes. These include two of the smallest chromosomes-level scaffolds in *O. beecheyi* (Chr_15 and Chr_17) as well as the medium-sized Chr_11 and Chr_12.

Discussion

Chromosome numbers in several North American ground squirrel taxa have been characterized through direct observation of dividing cells (Nadler 1966a, 1966b), where diploid counts range from 30 to 50 chromosomes. Thus, a chromosome count of 20 ($2n=40$) for *O. beecheyi* is well within the known range for the group. Interestingly, Nadler (1966b) reported a cytogenetic estimate of $2n=38$ for *O. beecheyi*, differing from our estimate by one. Based on the locality information, the samples from Nadler’s cytogenetic work were from north of the San Francisco Bay and San Joaquin River Delta, meaning they are within the highly divergent northern lineage (Phuong et al. 2014; Holding et al. 2021) that has been recently reassigned to the resurrected Douglas’ ground squirrel (*Otospermophilus douglasii*) taxon (Long and Smith 2023). We do not see evidence from the Hi-C contact

Table 1. Assembly strategy and software.

Assembly		
Primary assembly	Falcon-Unzip	1.3.7
Polishing	Arrow	SMRT Link software V6.0.0
Hi-C read alignment	Juicer	1.6
Hi-C scaffolding	3d-DNA run-asm-pipeline.sh	180 922
Manual scaffold editing	Juicebox Assembly Tools	1.11.08
Content and completeness		
Gene content	BUSCO + Glires_Odb10	5.8
RNA-alignment	HISAT2	2.2.1
De novo gene annotation	Braker	3.0.8
Functional annotation	Diamond	2.11
Repeat library generation	RepeatModeler	2.0.4
Repeat annotation	RepeatMasker	4.1.5
GC content	Bedtools	2.29.2

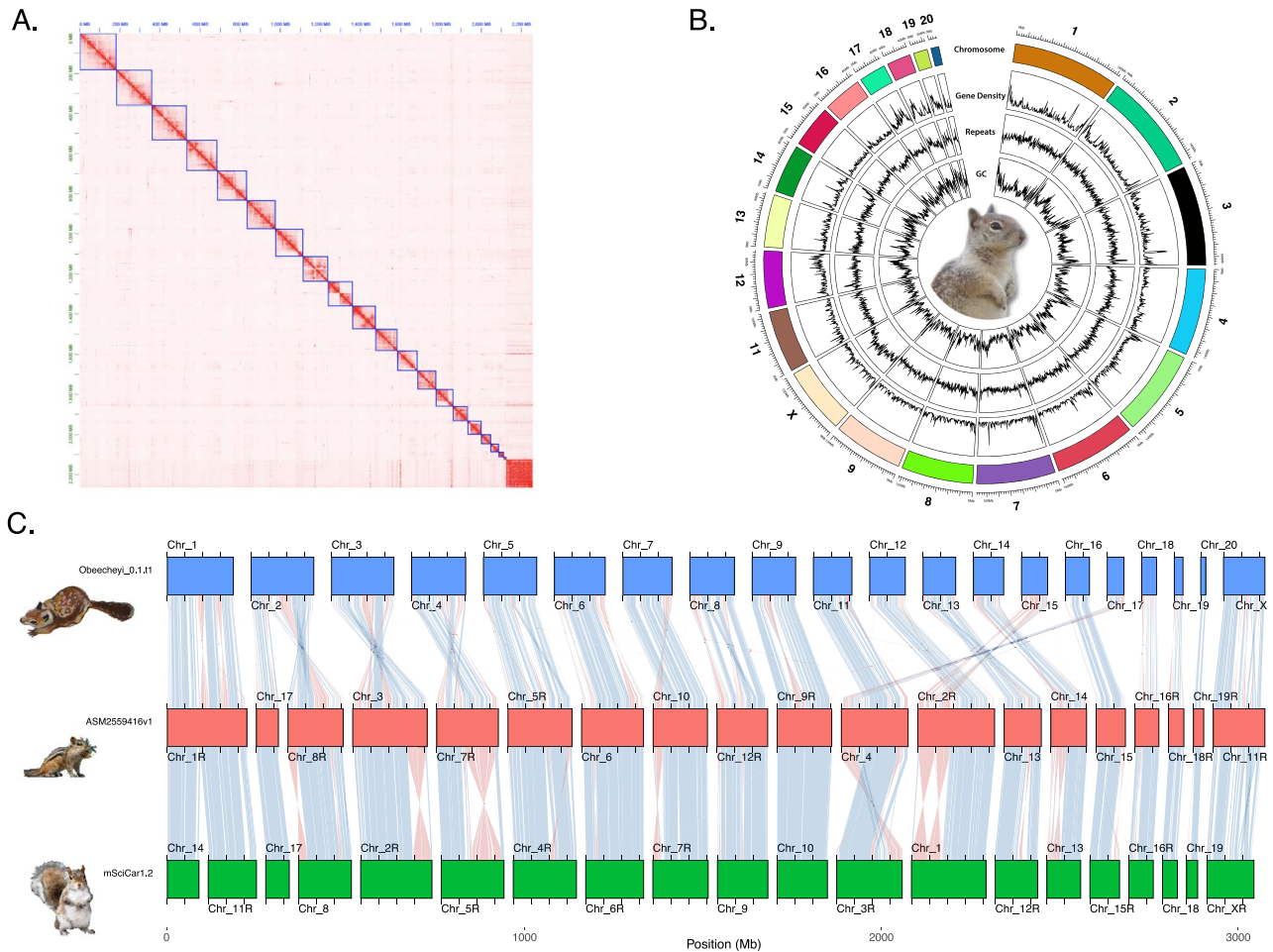


Fig. 1. A chromosome-scale genome assembly for the California ground squirrel (*O. beecheyi*). (A) The final Hi-C contact map for the genome assembly, with 20 chromosome-scale scaffolds boxed in blue lines. (B) A circular representation of the 20 chromosomes showing 500 kb sliding windows for the gene density, repeat content, and GC content as line plots. (C) Resolved synteny between the *O. beecheyi* chromosomes and the chromosomes of two other Sciuridae, the Siberian chipmunk (*T. sibiricus*; assembly ASM2559416v1) and the eastern gray squirrel (*S. carolinensis*; assembly mSciCar1.2). The lines connecting scaffolds indicate the locations of orthologous BUSCO (Manni et al. 2021) genes between the assemblies.

map (Fig. 1A) to suggest that any two of our scaffolds should be combined to reach the lower our chromosome count to that of Nadler's. We hypothesize that genetic divergence between the ecologically and morphologically similar *O. beecheyi* and *O. douglasii* could be associated with this difference in chromosome numbers as a prezygotic barrier to gene flow. This

hypothesis is consistent with the apparent tendency toward rapid change in chromosome number among other closely related ground squirrels taxa (Nadler 1966a; Nadler et al. 1971). Meanwhile, broad synteny of *Tamias* and *Sciurus* (one fusion event) and chromosome count variation among taxa such as *Cynomys* and *Marmota* (Tsuchiya et al. 2020)

Table 2. Quantification of contiguity, content, and gene completeness for the California ground squirrel genome assembly.

Assembly contiguity	
Number of contigs	9,960
Number of scaffolds	1,383
Number of scaffolds >10 M	20
Assembly size (bp)	2,269,446,536
Contig N50	425,996
Scaffold N50	124,984,502
% in chromosomal scaffold	91.7%
Assembly content	
GC content	38.6%
SINEs	7.5%
LINEs	17.5%
LTR elements	10.3%
DNA transposons	4.6%
Annotated gene count	20,301
Gene completeness	
	C: 77%; D: 3.7%; F: 2.1%; M: 19.2%

suggests that rapid chromosomal evolution in ground squirrels occurred after the divergence of *Tamias* and the bulk of tribe Marmotini (Mclean et al. 2019). As comparative genomic resources continue to build for sciurid rodents at multiple scales of evolutionary divergence, the role of chromosomal rearrangements as either a passenger or driver in the diversification process may be clarified.

We produced the first chromosome-scale reference genome for the California ground squirrel, *O. beecheyi*. From a comparative genomics perspective, we have added a new chromosome-scale assembly to the small but growing number of such resources for squirrels (Sciuridae) as a group. Squirrels are among the best studied wild rodent lineages. They have been researched from all aspects of basic biology, and current research on squirrels is becoming both increasingly integrative as well as translational in nature. This genome will be used as a basis to study the genetic architecture and population genomics of loci involved in resistance to rattlesnake venoms, as well as the role of relatedness and specific genes in the social behaviors of these mammals. We also see the potential of this genome as a resource for studies of plague resistance and in studies of adaptation to agricultural and urbanized environments. Each of these subjects, among others, can be combined with the abundance and accessibility of ground squirrel for use in an educational framework as students learn to generate and analyze the genetic data of animals captured on campus. In conclusion, the release of this genome represents another important step in the study of the California ground squirrel as a model species in diverse studies of wild mammals.

Acknowledgments

We are grateful to QB3 Genomics UC Berkeley, Berkeley, CA, RRID:SCR_022170 and Chris Conroy at the Museum of Vertebrate Zoology, UC Berkeley who assisted with animal collection. We thank Pacific Biosciences and Dovetail Genomics for generously providing reagents.

Author contributions

Matthew L. Holding (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing—original draft), Sree Rohit Raj Kolora (Data curation,

Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing), Jennifer E. Smith (Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing—original draft), Shana L. McDevitt (Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—original draft), Peter H. Sudmant (Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing—review & editing), and Rebecca D. Tarvin (Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing—original draft, Writing—review & editing)

Supplementary material

Supplementary material is available at *Journal of Heredity* online.

Funding

This work was supported by the 2019 Berkeley Collegium Narrowing the Gap Between Teaching and Research grant from UC Berkeley to RDT and PHS.

Data availability

The sequence data can be found on NCBI under BioProject PRJNA1214846 and BioSample SAMN46388214.

References

- Astashyn A, Tvedte ES, Sweeney D, Sapojnikov V, Bouk N, Joukov V, Mozes E, Strobe PK, Sylla PM, Wagner L, et al. Rapid and sensitive detection of genome contamination at scale with FCS-GX. *Genome Biol.* 2024;25:60. <https://doi.org/10.1186/s13059-024-03198-7>
- Bao W, Kojima KK, Kohany O. Repbase update, a database of repetitive elements in eukaryotic genomes. *Mob DNA.* 2015;6:11. <https://doi.org/10.1186/s13100-015-0041-9>
- Biardi JE, Ho CYL, Marcinczyk J, Nambiar KP. Isolation and identification of a snake venom metalloproteinase inhibitor from California ground squirrel (*Spermophilus beecheyi*) blood sera. *Toxicol.* 2011;58:486–493. <https://doi.org/10.1016/j.toxicol.2011.08.009>
- Blumstein DT, Ferando E, Stankowich T. A test of the multipredator hypothesis: yellow-bellied marmots respond fearfully to the sight of novel and extinct predators. *Anim Behav.* 2009;78:873–878. <https://doi.org/10.1016/j.anbehav.2009.07.010>
- Buchfink B, Reuter K, Drost H-G. Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat Methods.* 2021;18:366–368. <https://doi.org/10.1038/s41592-021-01101-x>
- Chen Y-C, Liu T, Yu C-H, Chiang T-Y, Hwang C-C. Effects of GC bias in next-generation-sequencing data on de novo genome assembly. *PLoS One.* 2013;8:e62856. <https://doi.org/10.1371/journal.pone.0062856>
- Cooper ST, Richters KE, Melin TE, Liu ZJ, Hordyk PJ, Benrud RR, Geiser LR, Cash SE, Simon Shelley C, Howard DR, et al. The hibernating 13-lined ground squirrel as a model organism for potential cold storage of platelets. *Am J Physiol Regul Integr Comp Physiol.* 2012;302:R1202–R1208. <https://doi.org/10.1152/ajpregu.00018.2012>
- Coss RG, Owings DH. Snake-directed behavior by snake naive and experienced California ground squirrels in a simulated burrow. *Z Tierpsychol.* 1978;48:421–435. <https://doi.org/10.1111/j.1439-0310.1978.tb00268.x>
- Dudchenko O, Batra SS, Omer AD, Nyquist SK, Hoeger M, Durand NC, Shamim MS, Machol I, Lander ES, Aiden AP, et al. De novo assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. *Science.* 2017;356:92–95. <https://doi.org/10.1126/science.aal3327>

- Durand NC, Shamim MS, Machol I, Rao SSP, Huntley MH, Lander ES, Aiden EL. Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. *Cell Syst.* 2016;3:95–98. <https://doi.org/10.1016/j.cels.2016.07.002>
- Edwards RJ, Dong C, Park RF, Tobias PA. A phased chromosome-level genome and full mitochondrial sequence for the dikaryotic myrtle rust pathogen, *Austropuccinia psidii*. *bioRxiv*. 2022. <https://doi.org/10.1101/2022.04.22.489119> preprint: not peer reviewed
- Flynn JM, Hubley R, Goubert C, Rosen J, Clark AG, Feschotte C, Smit AF. RepeatModeler2 for automated genomic discovery of transposable element families. *Proc Natl Acad Sci.* 2020;117:9451–9457. <https://doi.org/10.1073/pnas.1921046117>
- Gabriel L, Bruna T, Hoff KJ, Ebel M, Lomsadze A, Borodovsky M, Stanke M. BRAKER3: fully automated genome annotation using RNA-seq and protein evidence with GeneMark-ETP, AUGUSTUS, and TSEBRA. *Genome Res.* 2024;34:769–777. <https://doi.org/10.1101/gr.278090.123>
- Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics.* 2020;36:2628–2629. <https://doi.org/10.1093/bioinformatics/btz931>
- Gibbs HL, Sanz L, Pérez A, Ochoa A, Hassinger ATB, Holding ML, Calvete JJ. The molecular basis of venom resistance in a rattlesnake-squirrel predator-prey system. *Mol Ecol.* 2020;29:2871–2888. <https://doi.org/10.1111/mec.15529>
- Gu Z, Gu L, Eils R, Schlesner M, Brors B. Circlize implements and enhances circular visualization in R. *Bioinformatics.* 2014;30:2811–2812. <https://doi.org/10.1093/bioinformatics/btu393>
- Hammond TT, Vo M, Burton CT, Surber LL, Lacey EA, Smith JE. Physiological and behavioral responses to anthropogenic stressors in a human-tolerant mammal. *J Mammal.* 2019;100:1928–1940. <https://doi.org/10.1093/jmammal/gyz134>
- Holding ML, Biardi JE, Gibbs HL. Coevolution of venom function and venom resistance in a rattlesnake predator and its squirrel prey. *Proc R Soc B Biol Sci.* 2016;283:20152841. <https://doi.org/10.1098/rspb.2015.2841>
- Holding ML, Putman BJ, Kong LM, Smith JE, Clark RW. Physiological stress integrates resistance to rattlesnake venom and the onset of risky foraging in California ground squirrels. *Toxins.* 2020;12:617. <https://doi.org/10.3390/toxins12100617>
- Holding ML, Sovic MG, Colston TJ, Gibbs HL. The scales of coevolution: comparative phylogeography and genetic demography of a locally adapted venomous predator and its prey. *Biol J Linn Soc.* 2021;132:297–317. <https://doi.org/10.1093/biolinnean/blaa192>
- Huang N, Li H. Compleasm: a faster and more accurate reimplementation of BUSCO. *Bioinformatics.* 2023;39:brad595. <https://doi.org/10.1093/bioinformatics/btad595>
- Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, et al. InterProScan 5: genome-scale protein function classification. *Bioinformatics.* 2014;30:1236–1240. <https://doi.org/10.1093/bioinformatics/btu031>
- Keuler KM, Bron GM, Griebel R, Richgels KLD. An invasive disease, sylvatic plague, increases fragmentation of black-tailed prairie dog (*Cynomys ludovicianus*) colonies. *PLoS One.* 2020;15:e0235907. <https://doi.org/10.1371/journal.pone.0235907>
- Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol.* 2019;37:907–915. <https://doi.org/10.1038/s41587-019-0201-4>
- Kriventseva EV, Kuznetsov D, Tegenfeldt F, Manni M, Dias R, Simão FA, Zdobnov EM. OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. *Nucleic Acids Res.* 2019;47:D807–D811. <https://doi.org/10.1093/nar/gky1053>
- Li R, Zhang M, Cha M, Xiang J, Yi X. Chromosome-level genome assembly of the Siberian chipmunk (*Tamias sibiricus*). *Sci Data.* 2022;9:783. <https://doi.org/10.1038/s41597-022-01910-5>
- Long DJ, Smith JE. *Otospermophilus douglasii* (Rodentia: Sciuridae). *Mamm Species.* 2023;55:sead010. <https://doi.org/10.1093/mspecs/sead010>
- Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol Biol Evol.* 2021;38:4647–4654. <https://doi.org/10.1093/molbev/msab199>
- Mclean BS, Bell KC, Allen JM, Helgen KM, Cook JA. Impacts of inference method and data set filtering on phylogenomic resolution in a rapid radiation of ground squirrels (Xerinae: Marmotini). *Syst Biol.* 2019;68:298–316. <https://doi.org/10.1093/sysbio/syy064>
- Mead D, Fingland K, Cripps R, Portela Miguez R, Smith M, Corton C, Oliver K, Skelton J, Betteridge E, Doucan J, et al. The genome sequence of the eastern grey squirrel, *Sciurus carolinensis* Gmelin, 1788. *Wellcome Open Res.* 2020;5:27. <https://doi.org/10.12688/wellcomeopenres.15721.1>
- Menéndez I, Gómez Cano AR, Cantalapedra JL, Peláez-Campomanes P, Álvarez-Sierra MÁ, Hernández Fernández M. A multi-layered approach to the diversification of squirrels. *Mammal Rev.* 2021;51:66–81. <https://doi.org/10.1111/mam.12215>
- Nadler CF. Chromosomes and systematics of American ground squirrels of the subgenus *Spermophilus*. *J Mammal.* 1966a;47:579–596. <https://doi.org/10.2307/1377889>
- Nadler CF. Chromosomes of *Spermophilus franklini* and taxonomy of the ground squirrel genus *Spermophilus*. *Syst Biol.* 1966b;15:199–206. <https://doi.org/10.2307/sysbio/15.3.199>
- Nadler CF, Hoffmann RS, Pizzimentt JJ. Chromosomes and serum proteins of prairie dogs and a model of Cynomys evolution. *J Mammal.* 1971;52:545–555. <https://doi.org/10.2307/1378589>
- Ochoa A, Hassinger ATB, Holding ML, Gibbs HL. Genetic characterization of potential venom resistance proteins in California ground squirrels (*Otospermophilus beecheyi*) using transcriptome analyses. *J Exp Zool B Mol Dev Evol.* 2023;340:259–269. <https://doi.org/10.1002/jez.b.23145>
- Ortiz-Jimenez CA, Conroy SZ, Person ES, DeCuir J, Gall GEC, Sih A, Smith JE. Human presence shifts the landscape of fear for a free-living mammal. *Ecology.* 2025;106:e4499. <https://doi.org/10.1002/ecy.4499>
- Parker TS, Nilon CH. Gray squirrel density, habitat suitability, and behavior in urban parks. *Urban Ecosyst.* 2008;11:243–255. <https://doi.org/10.1007/s11252-008-0060-0>
- Person ES, Lacey EA, Smith JE. Space use and social networks: correlated but not congruent in California ground squirrels. *Anim Behav.* 2024;217:39–51. <https://doi.org/10.1016/j.anbehav.2024.08.009>
- Phuong MA, Lim MCW, Wait DR, Rowe KC, Moritz C. Delimiting species in the genus *Otospermophilus* (Rodentia: Sciuridae), using genetics, ecology, and morphology: species delimitation in ground squirrels. *Biol J Linn Soc.* 2014;113:1136–1151. <https://doi.org/10.1111/bij.12391>
- Poran NS, Coss RG, Benjamini E. Resistance of California ground squirrels (*Spermophilus beecheyi*) to the venom of the northern Pacific rattlesnake (*Crotalus viridis oreganus*): a study of adaptive variation. *Toxicon.* 1987;25:767–777. [https://doi.org/10.1016/0041-0101\(87\)90127-9](https://doi.org/10.1016/0041-0101(87)90127-9)
- Putman BJ, Clark RW. The fear of unseen predators: ground squirrel tail flagging in the absence of snakes signals vigilance. *Behav Ecol.* 2015;26:185–193. <https://doi.org/10.1093/beheco/aru176>
- Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics.* 2010;26:841–842. <https://doi.org/10.1093/bioinformatics/btq033>
- Richardson J, Richardson J, Swainson W, Kirby W. *Fauna boreali-americana, or, The zoology of the northern parts of British America: containing descriptions of the objects of natural history collected on the late northern land expeditions, under command of Captain Sir John Franklin, R.N.* London: J. Murray [etc.]; 1829: <https://doi.org/10.5962/bhl.title.39293>
- Rick TC, Radde HD, Teeter WG, Elliott Smith EA, Alvitre CM, Dagtas ND, Kennedy-Richardson KO, King JL, Martinez DR, Schnorr S, et al. Enhancing biodiversity: historical ecology and biogeography of the Santa Catalina Island ground squirrel, *Otospermophilus*

- beecheyi nesioticus*. *R Soc Open Sci*. 2024;11:240726. <https://doi.org/10.1098/rsos.240726>
- Rundus AS, Owings DH, Joshi SS, Chinn E, Giannini N. Ground squirrels use an infrared signal to deter rattlesnake predation. *Proc Natl Acad Sci*. 2007;104:14372–14376. <https://doi.org/10.1073/pnas.0702599104>
- Smit AFA, Hubley R, Green P. *RepeatMasker Open-3.0*. 1996: <http://www.repeatmasker.org>
- Smith JE, Long DJ, Russell ID, Newcomb KL, Muñoz VD. *Otospermophilus beecheyi* (Rodentia: Sciuridae). *Mamm Species*. 2016;48: 91–108. <https://doi.org/10.1093/mspecies/sew010>
- Smith JE, Gamboa DA, Spencer JM, Travenick SJ, Ortiz CA, Hunter RD, Sih A. Split between two worlds: automated sensing reveals links between above- and belowground social networks in a free-living mammal. *Philos Trans R Soc B Biol Sci*. 2018;373:20170249. <https://doi.org/10.1098/rstb.2017.0249>
- Smith JE, Smith IB, Working CL, Russell ID, Krout SA, Singh KS, Sih A. Host traits, identity, and ecological conditions predict consistent flea abundance and prevalence on free-living California ground squirrels. *Int J Parasitol*. 2021;51:587–598. <https://doi.org/10.1016/j.ijpara.2020.12.001>
- Smith JE, Carminito C, Hamilton S, Newcomb KL, Randt C, Travenick S. Sensory integration of danger and safety cues may explain the fear of a quiet coyote. *Proc R Soc B*. 2023;290:20231812. <https://doi.org/10.1098/rspb.2023.1812>
- Tsuchiya MTN, Dikow RB, Cassin-Sackett L. First genome sequence of the Gunnison's prairie dog (*Cynomys gunnisoni*), a keystone species and player in the transmission of sylvatic plague. *Genome Biol Evol*. 2020;12:618–625. <https://doi.org/10.1093/gbe/evaa069>
- Wey TW, Blumstein DT. Social cohesion in yellow-bellied marmots is established through age and kin structuring. *Anim Behav*. 2010;79:1343–1352. <https://doi.org/10.1016/j.anbehav.2010.03.008>
- Wojda SJ, Gridley RA, McGee-Lawrence ME, Drummer TD, Hess A, Kohl F, Barnes BM, Donahue SW. Arctic ground squirrels limit bone loss during the prolonged physical inactivity associated with hibernation. *Physiol Biochem Zool*. 2016;89:72–80. <https://doi.org/10.1086/684619>