

FY2023 Annual Report (April 2023-March 2024)

Molecular Genetics Unit

Professor Daniel Rokhsar (Adjunct; Visiting)

Abstract

Genomic perspective on the evolution of plants and animals

The comparative study of genomes provides a window into the origin and evolution of plant and animal diversity. Contemporary genomes evolved by descent with modification from earlier sequences, accumulating both neutral and adaptive mutations, with varying genomic impact ranging from small changes in sequence to radical changes in chromosome number and gene content. We apply the general comparative principle: features that are shared by two or more genomes were either present in their common ancestor, or evolved convergently. This principle allows us, for example, to infer the gene content and even genome structure of early vertebrate by comparing genomes of appropriately chosen living species, and to discern macro-evolutionary trends. Similarly, analysis of genetic variation within individual species or between closely related populations sheds light on the evolutionary mechanisms underlying the origin and diversification of new species. The dynamic nature of genomes, including the waxing and waning of transposable elements that generate complex repetitive patterns, also provide opportunities for inferring past events that shaped the diversity of life.

1. Staff

- Dr. Gustavo Sanchez, postdoctoral researcher
- Ms. Lin Zhang, technician
- Dr. Ferdinand Marletaz, visiting researcher
- Dr. Chikatoshi Sugimoto, visiting researcher
- Ms. Kiyoko Yamada, research unit administrator

2. Collaborations

2.1 Theme: The evolution of metazoan genomes

- Type of collaboration: Collaboration
- Researchers
 - Prof. Chris Lowe (Hopkins Marine Station, Stanford University, USA)
 - Dr. Ferdinand Marletaz (University College, London, UK)
 - Dr. Laurent Formery (Hopkins Marine Station, Stanford University, USA)
 - Prof. Oleg Simakov (University of Vienna, Austria)
 - Dr. Darrin Schultz (University of Vienna, Austria)
 - Dr. Steven Haddock (Monterey Bay Aquarium Research Institute, USA)
 - Prof. Jeremiah Smith (U. Kentucky, USA)
 - Prof. Dario Lupianez (Centro Andaluz de Biología del Desarrollo, Seville, Spain)
 - Prof. Richard E. Green (University of California, Santa Cruz, USA)

2.2 Theme: Evolution and diversity of cephalopods

- Type of collaboration: Collaboration
- Researchers
 - Prof. Oleg Simakov (University of Vienna, Austria)
 - Dr. Carrie Albertin (Marine Biological Laboratory, Woods Hole, USA)
 - Dr. Joshua Rosenthal (Marine Biological Laboratory, Woods Hole, USA)
 - Dr. Spencer Nyholm (University of Connecticut, Connecticut, USA)
 - Dr. Margaret McFall-Ngai (Carnegie Institution for Science, California, USA)
 - Dr. Michele Nishiguchi (University of California Merced, Merced, USA)
 - Dr. Elizabeth Heath-Heckman (Michigan State University, Michigan, USA)
 - Dr. Fernando Fernández-Álvarez (Marine Science Institute of Barcelona ICM-CSIC, Spain)
 - Prof. Sam Reiter (Computational Neuroethology Unit, OIST)
 - Dr. Tomoyuki Mano (Computational Neuroethology Unit, OIST)

2.3 Theme: Origins and diversity of citrus

- Type of collaboration: Collaboration
- Researchers
 - Prof. Fred Gmitter (University of Florida, USA)
 - Dr. Albert Wu (DOE Joint Genome Institute, USA)
 - Dr. Hideyasu Kinjyo (Okinawa)
 - Prof. Manuel Talon (Instituto Valenciano de Investigaciones Agrarias, Spain)
 - Prof. Masashi Yamamoto (Kagoshima University, Japan)

3. Activities and Findings

3.1 Genomic perspectives on early animal evolution

The nature of the earliest diverging branch of animals has been controversial, with some studies preferring sponges (poriferans) and others comb jellies (ctenophores). Since sponges lack neurons and muscle but comb jellies have these cell types, the branching order of these and other groups has implications for the evolution of nervous systems, a key animal innovation. Previously we had found extensive conservation of conservation of synteny (that is, chromosomal gene linkage without regard to collinearity) across diverse bilaterians, cnidarians, and sponges. In a recent study, we extended this analysis to include ctenophores and several unicellular eukaryote lineages most closely related to animals. Remarkably, we find that ctenophores and non-metazoan eukaryotes share ancestral patterns of synteny, and sponges shared derived patterns with bilaterians and cnidarians. The nature of these derived changes makes it very unlikely that they are convergent. Our findings provide strong evidence for the early branching of ctenophores, implying that the most recent common ancestor of all living animals had neurons and muscle, which were then lost in sponges. In other work relevant to the evolution of echinoderms (sea stars, sea urchins, sea cucumbers, and their relatives), we used RNA tomography

and *in situ* hybridization to map the bilaterian anterior-posterior axis onto the pentaradial echinoderm body plan, with implications for the evolution of the echinoderm phylum.

3.2 Genomic perspectives on vertebrate evolution

Deciphering the early evolution of vertebrates from invertebrate ancestors is challenging in part due to the limited fossil record in the Cambrian and Ordovician Eras. As the only surviving lineages of jawless fishes, hagfishes and lampreys provide a crucial window into early vertebrate evolution. We investigated the complex history, timing and functional role of genome-wide duplications and programmed DNA elimination in vertebrates in the light of a chromosome-scale genome sequence for the brown hagfish *Eptatretus atami*. Combining evidence from syntenic and phylogenetic analyses, we established a comprehensive picture of vertebrate genome evolution, including an auto-tetraploidization (1R_V) that predates the early Cambrian cyclostome–gnathostome split, followed by a mid–late Cambrian allo-tetraploidization (2R_{JV}) in gnathostomes and a prolonged Cambrian–Ordovician hexaploidization (2R_{CV}) in cyclostomes. Subsequently, hagfishes underwent extensive genomic changes, with chromosomal fusions accompanied by the loss of genes that are essential for organ systems (for example, genes involved in the development of eyes and in the proliferation of osteoclasts); these changes account, in part, for the simplification of the hagfish body plan. We characterized programmed DNA elimination in hagfish, identifying protein-coding genes and repetitive elements that are deleted from somatic cell lineages during early development. The elimination of these germline-specific genes provides a mechanism for resolving genetic conflict between soma and germline by repressing germline and pluripotency functions, paralleling findings in lampreys. In other work on vertebrate genome evolution, we showed that frog genomes are remarkably static, while chromosomes rearrangements in the little skate likely altered gene regulation to give rise to expanded pectoral fins in this lineage.

3.3 Genomics of cephalopod diversity

Coleiod cephalopods are known for their large nervous systems, complex behaviors and morphological innovations. Worldwide there are over 300 species of octopodiforms (octopuses) and 500 species of decapodiforms (squid and cuttlefish), including commercially important species in Okinawa with limited molecular characterization. We have been investigating the genomic variation among coleoids to better understand their ecological and morphological diversity. This past year we found that *Thysanoteuthis rhombus*, previously thought to be a single species with extensive distribution across different oceans, consists of three cryptic species. One of these cryptic species has a high commercial value in Okinawa, highlighting the relevance of our study for future sustainability of this species for local fisheries. We have also extensively characterized cryptic species of oceanic squids in the Atlantic Ocean a lineage long believed to be well-mixed because the open ocean does not impose barriers to their populations. Cephalopod genomics is contributing to the analysis of adaptation, speciation, and ecology. We are currently investigating the genomic signatures of the high diversity and adaptation of decapodiform cephalopods as identified in our previous studies, combining genome sequencing of local species with the genomes of other species obtained through a grant funded by the Wellcome Sanger Institute.

3.4 Cellular organization of cephalopod brains

The complex nervous systems of cephalopods and vertebrates are a canonical example of convergent morphological evolution. We are using genomics, single cell, and spatial transcriptomics to study the evolution and development of the optic and vertical lobes of several cephalopods, including two local species, the bobtail

squid *Euprymna berryi* and octopus *O. laqueus*. Our goal is to characterize the cell types of various brain areas and compare them among squid and octopus. Previously we found an unexpected diversity of neural types in the optic lobe, dominated by dopamine, and previously uncharacterized glial cells. Ongoing work on the vertical lobe, a center for memory in coleoids whose structure varies across lineages, is a collaboration with the OIST Computational Neuroethology Unit. We are continuing to pursue understanding of the role and regulation of the extensive mRNA editing in the coleoid nervous system, focusing on the cellular level.

3.5 Evolution and diversity of citrus

The genus *Citrus* arose in south east Asia over 8 million years ago and diversified into numerous wild species, from which cultivated types were domesticated by selection and hybridization. We have developed an evolutionary framework for citrus based on comprehensive genome analysis that accounts for primary citrus species and their role in producing admixed or hybrid taxa. Among these primary species is a previously unrecognized wild sexual species native to the Ryukyu Islands, which we named *Citrus ryukyuensis*. *C. ryukyuensis* is an ancestor of shiikuwasha, tachibana, and other traditional Ryukyuan hybrid types that reproduce clonally by seed via nucellar embryony. In ongoing work, we are developing a formal botanical description of *C. ryukyuensis* and further characterizing the diversity of *C. ryukyuensis* and Okinawan shiikuwasha, as well as analyzing the genetic diversity of sour oranges, lemons, and other citrus hybrids.

4. Publications

4.1 Journals

1. **Marlétaz F**, Timoshevskaya N, Timoshevskiy VA, Parey E, Simakov O, **Gavriouchkina D**, Suzuki M, Kubokawa K, Brenner S, Smith JJ, **Rokhsar DS**. "The hagfish genome and the evolution of vertebrates." *Nature*. (2024 Jan 23, Online ahead of print). DOI: 10.1038/s41586-024-07070-3.
2. Bredeson JV, Mudd AB, Medina-Ruiz S, Mitros T, Smith OK, Miller KE, Lyons JB, Batra SS, Park J, Berkoff KC, Plott C, Grimwood J, Schmutz J, Aguirre-Figueroa G, Khokha MK, Lane M, Philipp I, Laslo M, Hanken J, Kerdivel G, Buisine N, Sachs LM, Buchholz DR, Kwon T, Smith-Parker H, Gridi-Papp M, Ryan MJ, Denton RD, Malone JH, Wallingford JB, Straight AF, Heald R, Hockemeyer D, Harland RM, **Rokhsar DS**. "Conserved chromatin and repetitive patterns reveal slow genome evolution in frogs." *Nature Communications* 15(1), 579. (2024) DOI: 10.1038/s41467-023-43012-9.
3. Deville D, Mori S, Kawai K, Escáñez A, Macali A, Lishchenko F, Braid H, Githaiga-Mwiciigi J, Sajikumar KK, Mohamed KS, Bolstad KSR, Miyahara K, **Sugimoto C**, Fernández-Álvarez FA, **Sanchez G** (2023). "Cryptic biodiversity in the commercial diamondback squid *Thysanoteuthis rhombus* Troschel 1857." *Reviews in Fish Biology and Fisheries* 14: 1 – 21 (2023) DOI: 10.1007/s11160-023-09813-3.
4. Fernández-Álvarez FA, **Sanchez G**, Deville D, Taite M, Villanueva R, Allcock AL (2023). "Atlantic Oceanic Squids in the Grey Speciation Zone." *Integrative & Comparative Biology*, icad116 (2023) DOI: 10.1093/icb/icad116.

5. Baden T, Briseño J, Coffing G, Cohen-Bodénès S, Courtney A, Dickerson D, Dölen G, Fiorito G, Gestal C, Gustafson T, Heath-Heckman E, Hua Q, Imperadore P, Kimbara R, Król M, Lajbner Z, Lichilín N, Macchi F, McCoy MJ, Nishiguchi MK, Nyholm SV, Otjacques E, Pérez-Ferrer PA, Ponte G, Pungor JR, Rogers TF, Rosenthal JJC, Rouressol L, Rubas N, **Sanchez G**, Santos CP, Schultz DT, Seuntjens E, Songco-Casey JO, Stewart IE, Styfhals R, Tuanapaya S, Vijayan N, Weissenbacher A, Zifcakova L, Schulz G, Weertman W, Simakov O, Albertin CB (2023). "Cephalopod-omics: Emerging Fields and Technologies in Cephalopod Biology." *Integrative & Comparative Biology* 63(6), 1226-1239 (2023) DOI: 10.1093/icb/icad087.
6. Erdmann-Pham DD, Batra SS, Turkalo TK, Durbin J, Blanchette M, Yeh I, Shain H, Bastian BC, Song YS, **Rokhsar DS**, Hockemeyer D (2023). "Tracing cancer evolution and heterogeneity using Hi-C." *Nature Communications* 14(1):7111. (2023) DOI: 10.1038/s41467-023-42651-2.
7. Formery L, Peluso P, Kohnle I, Malnick J, Thompson JR, Pitel M, Uhlinger KR, **Rokhsar DS**, Rank DR, Lowe CJ (2023). "Molecular evidence of anteroposterior patterning in adult echinoderms." *Nature* 623(7987):555-561. (2023) DOI: 10.1038/s41586-023-06669-2.
8. Gutnick T, **Rokhsar DS**, Kuba MJ (2023). "Cephalopod behaviour." *Current Biology* 33(20):R1083-R1086. (2023) DOI: 10.1016/j.cub.2023.08.094.
9. Ahuja N, Hwaun E, Pungor JR, Rafiq R, Nemes S, Sakmar T, Vogt MA, Grasse B, Diaz Quiroz J, Montague TG, Null RW, Dallis DN, **Gavriouchkina D**, **Marletaz F**, Abbo L, **Rokhsar DS**, Niell CM, Soltesz I, Albertin CB, Rosenthal JJC (2023). "Creation of an albino squid line by CRISPR-Cas9 and its application for in vivo functional imaging of neural activity." *Current Biology* 33(13):2774-2783.e5. (2023) DOI: 10.1016/j.cub.2023.05.066.
10. Rouressol L, Brisenio J, Vijayan N, Chen GY, Ritschard EA, **Sanchez G**, Nyholm SV, McFall-Ngai MJ, Simakov O (2023). "Emergence of novel genomic regulatory regions associated with light-organ development in the bobtail squid." *iScience*. 26 (7), 107091 (2023) DOI: 10.1016/j.isci.2023.107091
11. Session AM, **Rokhsar DS** (2023). "Transposon signatures of allopolyploid genome evolution." *Nature Communications* 14(1):3180.(2023) DOI: 10.1038/s41467-023-38560-z.
12. Schultz DT, Haddock SHD, Bredeson JV, Green RE, Simakov O, **Rokhsar DS** (2023). "Ancient gene linkages support ctenophores as sister to other animals." *Nature* 618(7963), 110-117 (2023) DOI: 10.1038/s41586-023-05936-6.
13. Marlétaz F, de la Calle-Mustienes E, Acemel RD, Paliou C, Naranjo S, Martínez-García PM, Cases I, Sleight, VA, Hirschberger C, Marcet-Houben M, Navon D, Andrescavage A, Skvortsova K, Duckett, P.E., González-Rajal Á, Bogdanovic O, Gibcus JH, Yang L, Gallardo-Fuentes L, Sospedra I, Lopez-Rios J, Darbellay F, Visel A, Dekker J, Shubin N, Gabaldón T, Nakamura T, Tena JJ, Lupiáñez DG, **Rokhsar DS**, Gómez-Skarmeta, JL (2023). "The little skate genome and the evolutionary emergence of wing-like fins." *Nature* 616, 495–503. <https://doi.org/10.1038/s41586-023-05868-1>

4.2 Books and other one-time publications

Nothing to report

4.3 Oral and Poster Presentations

1. **Gustavo Sanchez.** "Cephalopod Diversity, Evolution, and RNA Editing." International Symposium: Past, Present, and Future of the Marine Environment and Ecosystems. Tohoku University (October 18, 2023).

4.4 Seminars

1. **Gustavo Sanchez.** "Cephalopod Diversity, Evolution, and RNA Editing." Max Planck Institute for Evolutionary Anthropology (EVA) Internal Seminar (September 28, 2023).
2. **Gustavo Sanchez.** "Cephalopod evolution: Investigating RNA Editing at the cellular level." OIST Internal Seminar Series, (July 21, 2023).

5. Intellectual Property Rights and Other Specific Achievements

5.1 External Grants

Kakenhi Grant-in-Aid for Early-Career Scientists

- Title: Adaptive RNA editing in Cephalopods
- Period: FY2022-2023
- Principal Investigator: Dr. Gustavo Sanchez

The Interdisciplinary Collaborative Research Program of the Atmosphere and Ocean Research Institute, the University of Tokyo

- Investigators: Dr. Gustavo Sanchez, Prof. Yoko Iwata, Atmosphere and Ocean Research Institute (AORI)
- Period: 2023

6. Meetings and Events

6.1 Seminar by Dr. Fernando Ángel Fernández-Álvarez

- Date: May 31, 2023
- Venue: OIST Campus Lab4, L4E01
- Speaker: Dr. Fernando Ángel Fernández-Álvarez, Institute of Marine Sciences (ICM), CSIC, Spain
- Title: The enigmatic evolutionary relationships among decapodiform cephalopod lineages

7. Other

Nothing to report