FY2022 Annual Report (April 2022-March 2023)

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
- 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: Origins and diversity of mandarin 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)

2.2 Theme: The evolution of metazoan genomes

- Type of collaboration: Collaboration

- Researchers
    - Prof. Jeramiah Smith (U. Kentucky, USA)
    - Prof. Oleg Simakov (University of Vienna, Austria)
    - Prof. Richard E. Green (University of California, Santa Cruz, USA)
    - Prof. Chris Lowe (Hopkins Marine Station, Stanford University, USA)
    - Prof. Rob Steele (University of California, Irvine, USA)
    - Dr. Steven Haddock (Monterey Bay Aquarium Research Institute, USA)
    - Prof. Charles Davis (Ludwig Maximilians University, Munich, Germany)

2.3 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)
    - Prof. Cliff Ragsdale (University of Chicago, USA)
    - Dr. Joshua Rosenthal (Marine Biological Laboratory, Woods Hole)

3. Activities and Findings

3.1 Development of model cephalopod systems

Cephalopod research remains limited by the inability to culture species under laboratory conditions for multiple generations to provide continuous access to animals at all stages of the life cycle. We recently reported describe a multi-generational laboratory culture system for two emerging cephalopod models: the hummingbird or Berry's bobtail squid (*Euprymna berryi*) and Morse's bobtail squid (*Euprymna morsei*), which are primarily found off mainland Japan. *E. berryi* wild adults were spawned and raised to the third filial generation, and *E. morsei* wild adults were spawned and raised to the second filial generation in a closed system at 20°C. We found that *E. berryi* and *E. morsei* grew exponentially during the first 90 and 60 days post-hatching, respectively, and females spawned days 112 and 71 days post-hatching, respectively, with high survivorship at first spawning (77-90%). We studied the life history of each species and how to distinguish sexes. By surmounting the challenges of cephalopod culture, we and others can now use these small cephalopods as laboratory models.

3.2 Genomics of cephalopods

Cephalopods are known for their large nervous systems, complex behaviors and morphological innovations. To investigate the genomic underpinnings of these features, we assembled the chromosomes of the Boston market squid, *Doryteuthis (Loligo) pealeii*, and the California two-spot octopus, *Octopus bimaculoides*, and compared them with those of the Hawaiian bobtail squid, *Euprymna scolopes*. The genomes of the soft-bodied (coleoid) cephalopods are highly rearranged relative to other extant molluscs, indicating an intense, early burst of genome restructuring. The coleoid genomes feature multi-megabase, tandem arrays of genes associated with brain development and cephalopod-specific innovations. We found that a known coleoid hallmark, extensive A-to-I mRNA editing, displays two fundamentally distinct patterns: one exclusive to the nervous system and concentrated in genic sequences, the other widespread and directed toward repetitive elements. We conclude that coleoid novelty is mediated in part by substantial genome reorganization, gene family expansion, and tissue-dependent mRNA editing. Work reporting the genome of *Euprymna berryi* has been submitted for publication and is available in preprint form.

3.3 Single cell analysis of cephalopod visual system

Although the camera-type eyes of cephalopods and vertebrates are a canonical example of convergent morphological evolution, the cellular and molecular mechanisms underlying this convergence remain obscure. We used genomics and single cell transcriptomics to study these mechanisms in the visual system of the bobtail squid *Euprymna berryi*, an emerging cephalopod model. Analysis of nearly 100,000 cellular transcriptomes from the squid visual and nervous system identified dozens of cell types that cannot be placed in simple correspondence with those of vertebrate or fly visual systems, as proposed by Ramón y Cajal and J.Z. Young. Instead, we found an unexpected diversity of neural types, dominated by dopamine, and previously uncharacterized glial cells. Surprisingly, we observed changes in cell populations and neurotransmitter usage during maturation and growth of the visual systems from hatchling to adult. Together these genomic and cellular findings shed new light on the parallel evolution of visual system complexity in cephalopods and vertebrates. Ongoing work focused on transcriptome diversity and analysis of other elements of the nervous system.

3.4 Evolution and diversity of citrus

The origin and dispersal of cultivated and wild mandarin and related citrus are poorly understood, in part due to their propensity for hybridization and asexual reproduction by nucellar embryony (a form of apomixis). We have developed a new evolutionary framework for the genus Citrus that accounts for primary citrus species and their role in producing admixed or hybrid taxa. Our recent analyses of east Asian mandarins revealed a previously unrecognized wild sexual species native to the Ryukyu Islands, which we named *Citrus ryukyuensis*. With this new species the taxonomic complexity of east Asian mandarins collapses to a satisfying simplicity, accounting for shiikuwasha, tachibana, and other traditional Ryuykuan types as hybrid species that reproduce clonally by seed via nucellar embryony. We traced the origin of citrus apomixis alleles to mangshanyeju wild mandarins, which played a central role in citrus domestication via adaptive wild introgression. Our results provide a coherent biogeographic framework for understanding the diversity and domestication of mandarin-type citrus through speciation, admixture, and rapid diffusion by apomictic reproduction. In ongoing work we are developing a formal botanical description of *C. ryukyuensis* and further characterizing the diversity of Okinawan shiikuwasha, as well as analyzing the diversity of sour oranges, lemons, and other citrus hybrids.

3.5 Evolution and agronomic features of greater yam

The nutrient-rich tubers of the greater yam, Dioscorea alata L., provide food and income security for millions of people around the world. Despite its global importance, however, greater yam remains an orphan crop. We addressed this resource gap by presenting a highly contiguous chromosome-scale genome assembly of *D. alata* combined with a dense genetic map derived from African breeding populations. The genome sequence revealed an ancient allotetraploidization in the *Dioscorea* lineage, followed by extensive genome-wide reorganization. Using the genomic tools, we found quantitative trait loci for resistance to anthracnose, a damaging fungal pathogen of yam, and several tuber quality traits. Genomic analysis of breeding lines revealed both extensive inbreeding as well as regions of extensive heterozygosity that may represent interspecific introgression during domestication. These tools and insights will enable yam breeders to unlock the potential of this staple crop and take full advantage of its adaptability to varied environments. In ongoing work, we are characterizing the germplasm diversity of greater yam using high throughput genotyping and sequencing.

3.6 The evolution of metazoan genomes

We are developing new methods for analyzing genome evolution at the chromosome scale and are using these methods to explore animal diversity. Comparing chromosome-scale genome sequences of diverse bilaterians, cnidarians, and sponges we find extensive conservation of synteny (that is, gene linkage without regard to collinearity). These patterns of conservation allow us to reconstruct ancestral chromosomes across major animal groups, and the transitions between them. Comparisons among diverse metazoans show that he past 500 million years of animal chromosome evolution have been dominated by arm-scale fusions and fissions as well as the previously unappreciated process of fusion-with-mixing. Fusion-with-mixing is an irreversible process in which genes from two ancestral syntenic groups are combined and intermingled on a single chromosome. Remarkably, we also find evidence of conserved synteny between metazoans and related unicellular eukaryotes, suggesting a very deep ancestry for certain gene linkages. These findings extend our previous studies focused on the evolution of vertebrate chromosomes, in which we used fusions-with-mixing to prove that jawed vertebrates share a common allotetraploid history that is not shared with jawless vertebrates. We continue to apply these methods to other animal and non-animal groups.

4. Publications

4.1 Journals

1. Jolly, J., Hasegawa, Y., Sugimoto, C., Zhang, L., Kawaura, R., Sanchez, G., Gavriouchkina, D., Marlétaz, F., Rokhsar, D. 2022. Lifecycle, culture, and maintenance of the emerging cephalopod models *Euprymna berryi* and *Euprymna morsei.* Front. Mar. Sci. 9:1039775. doi: 10.3389/fmars.2022.1039775
2. Albertin, C.B., Medina-Ruiz, S., Mitros, T., Schmidbaur, H., Sanchez, G., Wang, Z.Y., Grimwood, J., Rosenthal, J.J.C., Ragsdale, C.W., Simakov, O., Rokhsar, D.S. 2022. Genome and transcriptome mechanisms driving cephalopod evolution. Nat Commun. 13(1):2427. doi: 10.1038/s41467-022-29748-w.
3. Bredeson, J.V., Lyons, J.B., Oniyinde, I.O., Okereke, N.R., Kolade, O., Nnabue, I., Nwadili, C.O., Hřibová, E., Parker, M., Nwogha, J., Shu, S., Carlson, J., Kariba, R., Muthemba, S., Knop, K., Barton, G.J., Sherwood, A.V., Lopez-Montes, A., Asiedu, R., Jamnadass, R., Muchugi, A., Goodstein, D., Egesi, C.N., Featherston, J., Asfaw, A., Simpson, G.G., Doležel, J., Hendre, P.S., Van Deynze, A., Kumar, P.L., Obidiegwu, J.E., Bhattacharjee, R., Rokhsar, D.S. 2022. Chromosome evolution and the genetic basis of agronomically important traits in greater yam. Nat Commun. 13(1):2001 doi:10.1038/s41467-022-29114-w.

4.2 Books and other one-time publications

Nothing to report

4.3 Oral and Poster Presentations

1. Sanchez, G. *The diversity and evolution of the bobtail squid, a promising model organism*. 第五回イカ･タコ研究会 (The 5th Squid and Octopus Research Meeting) Shizuoka, Japan (October 28-29, 2022)
2. Sugimoto, C. *Origin and diversification of Ryukyuan citrus by hybridization and apomixis.* Tohoku University – OIST 3rd Joint Workshop on Biodiversity: From Genes and Species to Ecosystem Services and Resilience, Online, Japan (October 25, 2022)
3. Sanchez, G. *Bobtail squid diversity and evolution.* Tohoku University – OIST 3rd Joint Workshop on Biodiversity: From Genes and Species to Ecosystem Services and Resilience, Online, Japan (October 24, 2022)
4. Sanchez, G. *Reference genome assembly of the Humboldt squid Dosidicus gigas.* CIAC (Cephalopod International Advisory Council) 2022, Sesimbra, Portugal (April 2, 2022)

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

6. Meetings and Events

6.1 Seminar by Prof. Michele Nishiguchi

- Date: February 27, 2023
- Venue: OIST Campus Lab3, C700
- Speaker: Prof. Michele Nishiguchi, Professor, Director, INSITE, MPI URISE@UCMerced, Associate Dean of Equity, Diversity, Justice, and Inclusion (EDJI)
- Title: Interpreting the road map between ecological and molecular boundaries using a squid-bacterial mutualism

7. Other

Nothing to report.