Tohoku University - OIST 3rd Joint Workshop on Biodiversity: From Genes and Species to Ecosystem Services and Resilience

Talk Session 1

10:20~12:00, October 24, 2022 Venue: TOKYO ELECTRON House of Creativity 3F, Lecture Theater, Katahira Campus, Tohoku University

Treasure–maps, trap–jaws, and the metaverse: biodiversity science in the big data era

Evan P. Economo

Okinawa Institute of Science and Technology Graduate University

Biodiversity science is inherently data-limited, particularly for understudied but functionally important groups such as invertebrates. This is a problem as we aim to track and mitigate the ongoing biodiversity crisis. However, the "big data" era and associated advances in computation provides new avenues for mapping, understanding, and conserving biodiversity. In this talk, I will focus on two efforts to build big data resources for an ecologically dominant insect clade, ants, and use them to address problems in ecology, evolution, and conservation. First, we still need to understand the geographic distribution of invertebrate life on Earth. I discuss a project to resolve a species-level map of life for ants, and how we are using this to both investigate large-scale patterns of diversification and identify important regions for conservation. Second, organisms are more than points on a map, and I describe efforts to use 3D X-ray scanning to capture big data on organismal form and function across the ant tree of life, along with our work applying these techniques to unravel innovation and diversification of specialized mandibles in ants. Finally, I offer some ideas for how big data and new technologies can change how we interact with biodiversity.

Stoichiometry of leaf litter leachate affects microbial dynamics and heterotrophic/autotrophic activity in freshwater microbial community

Pei-Chi Ho*, Suzuna Nakajima and Jotaro Urabe

Graduate School of Life Science, Tohoku University

Dissolved nutrients released from leaf litters support and alter the structure of freshwater food webs. Dissolved organic carbon (DOC) leached from leaf litters is consumed by heterotrophic bacteria, enhancing freshwater detritus food chain. Inorganic phosphorus (P) and nitrogen (N) nutrients and micronutrients released from leaf litters during the leaching process support phytoplankton primary production and enhances the grazing food chain. In this study, we investigated how DOC, nitrogenous and phosphorus nutrients in the leachates of leaf litters influence freshwater microbial food web by incubating freshwater bacteria, protists and phytoplankton from sediment in four different leaf litter leachates. We also added micronutrients to the incubations to test the limitation of micronutrient in litter leachates. We found high density of heterotrophic protists and fungi in Japanese hemlock leachate, which contains the highest DOC but lower N and P nutrients. Significant limitation of micronutrients on autotrophic protists, phytoplanktona and cyanobacteria is found only in incubations of Siebold's beech and oak leachates. Furthermore, the heterotrophic/autotrophic biomass ratio increases with DOC in leachates, indicating that leachate DOC supports the detritus food chain. We conclude that the stoichiometry of leaf litter leachate influences microbial food web structure and heterotrophic/autotrophic production in freshwater food webs.

Genome evolution in termites

Tracy Lynn Audisio¹, Thomas Bourguignon²

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Structural changes to genomes can alter phenotypes and result in major ecological and evolutionary shifts. Termites provide an excellent model to study the evolutionary implications of genome structural variants, since different lineages are known to have divergent chromosome numbers and genome sizes. Despite these differences, the extent of variation and association with different feeding ecologies, developmental pathways, and social structures remain unknown. In this project, a hybrid sequencing and multiplatform assembly approach was used to integrate long-read, linked-read, and long-range data to generate 50 *de novo* genome assemblies that span the breadth of termite diversity. Structural variants were characterized across termite genomes and differences were assessed using a comparative phylogenetic framework to provide insight into how variants evolve, how they are maintained, and how they shape termite phenotypes.

Gonadotropin-releasing hormone, GnRH, in invertebrates -Functional evolution and diversity-

Makoto Osada1 and Kazue Nagasawa1

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The regulation of reproduction, like other physiological functions, involves many intercellular signaling mechanisms. The hypothalamus-pituitary-gonadal axis in vertebrates is its central regulatory mechanism. In particular, the gonadotropin-releasing hormone GnRH, localized in the hypothalamus, is an indispensable neuropeptide hormone that functions as the starting point for reproductive control via steroid hormone biosynthesis. Over the past three decades, there has been increasing evidence strongly indicating that members of the GnRH superfamily including GnRH, adipokinetic hormone (AKH), corazonin (Crz), and adipokinetic hormone/corazonin-related peptides (ACP), are almost ubiquitous amongst bilateral animals from deuterostomes to protostomes. The ancestral molecule of the GnRH peptide is believed to be very old, and the GnRH possibly has origins in even more ancient, non-bilateral ancestors. The GnRH superfamily molecules have undergone diverse functional differentiation during their evolution, eg) reproductive control by GnRH in vertebrates, reproductive, behavioral, and metamorphosis controls by invertebrate GnRH, and black pigmentation by Crz and lipid mobilization by AKH in insects. In the phylum Mollusk, the second largest in the animal kingdom after Arthropoda, GnRH is suggested to be implicated in steroidogenesis and germ cell development. The nature of GnRH function in invertebrates will be discussed.

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Talk Session 2

13:00~14:40, October 24, 2022 Venue: TOKYO ELECTRON House of Creativity 3F, Lecture Theater, Katahira Campus, Tohoku University

Prediction of biosynthetic pathways of major marine toxins based on their structural diversity

Mari Yotsu-Yamashita¹, Yuta Kudo^{1,2}, Shigeki Tsuchiya¹, Satoshi Numano¹, Yuko Cho¹, Keiichi Konoki¹, Kazuo Nagasawa³

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Pufferfish toxin, tetrodotoxin (TTX) and paralytic shellfish toxin, saxitoxin (STX) are major marine neurotoxins. We have been studying the biosynthetic and metabolic pathways of these toxins based on the structural



diversity of their derivatives and related compounds¹⁻⁴⁾. Especially, biosynthetic genes of TTX have not been discovered yet. As an approach to solve this issue, we have attempted to obtain clues from the chemical structures of many natural TTX analogues and related compounds isolated from pufferfish and terrestrial amphibia, newts. According to the results, we predict that biosynthetic pathways towards TTX in marine and terrestrial environments are not identical because only a few TTX analogues are commonly present both in these animals. By comparison of the structures of TTX analogues from pufferfish, multi-oxidation steps were suggested to construct marine TTX, while monoterpene origin was suggested for terrestrial TTX. Regarding STX, putative biosynthetic genes have been reported in toxin producing cyanobacteria and dinoflagellates. However, the biosynthetic intermediates and the order of some biosynthetic reactions are remaining unclear. We are trying to answer to these questions using the synthetic predicted biosynthetic intermediates of STX. Recently found possible metabolites of STX in scallops will be also discussed.

References

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Microalgal environmental responses underlying molecular interactions in coral-algal symbiosis

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In oligotrophic oceans, reef-building corals depend on symbiotic algae for most of their energy and cannot survive without establishing the symbiotic relationships. Although the symbiotic algae are known to stably reside in acidic compartments within host animals, e.g. 'symbiosome' in corals, molecular interactions and carbon transfer mechanisms between symbiotic algae and their hosts remain unclear. Here we show that acidity in the environment signals the release of carbon as a form of monosaccharide from coral symbiont algae by degrading the algal cell wall. This provides a novel perspective on symbiotic interactions that acidic compartments within host animals play an important role for the energy supply form symbionts by exploiting algal responses to environmental changes.



Temporal variation of microbial community assembly is driven by drift and selection: A meta-analysis.

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² Research School of Biology, Australian National University, Acton, ACT, Australia

Microbial community assembly can be driven by neutral or selective processes. These processes can cluster the microbes homogeneously, i.e., phylogenetically similar microbes together. Or heterogeneously, which promotes divergence of community composition. The effects of these processes on microbial co-existence and interactions have been widely studied. However, there is still no consensus if similar overarching trends exist in species interaction over time and across ecosystems. Using publicly available 16S rRNA microbial time series studies, I performed a metaanalysis of bacterial communities across 6491 samples from ten studies representing aquatic, soil, and animal-associated habitats. I examined the relative importance of neutral and selective processes and quantified the influence of different selective processes, such as abiotic environmental factors. Overall, I found that microbial community assembly is driven by a combination of homogenizing selection and neutral processes conserved across studies in an ecosystem. This selection pressure on candidate taxa is mediated by pH, geography, etc., sometimes irrespective of time. These results suggest that the microbes inhabiting the same habitat are structured by varying ecological processes, which has tremendous application in advancing our understanding of trends in microbial communities across sites and time to predict ecosystem functions in the changing environment.

Extraordinary genomic turbulence in larvaceans

Nicholas M. Luscombe

Okinawa Institute of Science and Technology Graduate University

The conserved arrangement of genes on chromosomes (synteny) reflects the existence of genomic mechanisms relying on proximity and is also essential to the formation of crossovers during meiosis. Some syntenies are still visible between animals having their last common ancestor half a billion year ago. In contrast, the zooplankton Oikopleura dioica has one of the fastest-evolving animal genomes, due to its life cycle of only a few days, and the lack of the non-homologous end joining pathway (NHEJ) for repairing DNA damage such as UV-induced DNA breaks. Its genome is one of the smallest in the animal reign (65 Mbp), allowing us to sequence it at a reasonable cost. Our study shows that gene order in genome of distinct Oikopleura dioica populations is subject to considerable changes, suggesting that the regulatory circuits in Oikopleura dioica are less dependent on long-range interactions compared with other chordate animals. Even in comparison with other pairs of invertebrate species that diverged at a similar time scale, the number of breaks of syntenies in Oikopleura dioica genomes remains among the highest ever reported so far. We propose that the Oikopleura dioica genome can be used as a contrast model to better decipher the principles constraining gene order, with the perspective of applying these rules to the synthesis of artificial genomes reduced in size and designed in silico.

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Talk Session 3

14:55~16:15, October 24, 2022 Venue: TOKYO ELECTRON House of Creativity 3F, Lecture Theater, Katahira Campus, Tohoku University

Exploring the structure and dynamics of ecological systems: The Minimalist Approach

Michio Kondoh¹

¹ Graduate School of Life Sciences, Tohoku University

Ecological community is a set of species that coexist in the same habitat. A community can be viewed as a network of species that are linked each other by interspecific interactions. Community theory predicts that the structure of community network can influence population and community dynamics and therefore holds a key to understanding species coexistence and ecological stability. However, the complex and non-static nature of ecological systems makes either empirical test and mathematical modelling not straightforward. In this talk, I present a data-driven approach, which we call "Minimalist Approach." In this approach we do not make an a priori assumption about the equations that govern the ecological dynamics or utilize the behavioral, individual-level interactions as a proxy for population-level interactions that drive population dynamics. Instead, we start with a set of timeseries, such as those of population dynamics, and make interspecific interactions inferred from those data. The inferred community network and its relations to dynamics is identified by model-free approach that does not assume any specific forms of community dynamical equations. Some findings we have made by applying the approach to a wide variety of organisms, such as fish, phytoplankton, insect and soil microbial community, will be presented.



Fig. The structure of community network inferred from the 14-year monitoring data. (Ushio et al. 2018, Nature)

How physical forces shape microbial biodiversity

Simone Pigolotti

Biological Complexity Unit, Okinawa Institute of Science and Technology.

Many microbial communities are extremely diverse. Their diversity can be deeply affected by the physical forces; understanding the link between these forces and the consequent biodiversity patterns is a fundamental challenge. Physical forces can influence ecosystems on a vast range of spatial scales. In this talk, I will present two case studies at the extremes of this range. I will show how competition between strains of E.coli in a microchannel is affected by the channel walls. I will then discuss how turbulent currents shape biodiversity of protists in the oceans.

Toward prediction of impact of large and infrequent disturbances on systems

Aiko Iwasaki¹

Research Center for Marine Biology, Graduate School of Life Sciences Tohoku University

Large and infrequent disturbances, which rarely occur but impact in large spatial scale with strong intensity, have a potential to restructure ecosystems. However, little is known about general patterns of the ecological impact and recovery process of populations which experienced such disturbance events due to the lack of a way to comprehensively evaluate individual cases. Here, I propose a framework for evaluating the resistance and resilience to such disturbances across different kinds of disturbances and species based on disturbance intensity, which enables a better understanding of which species are vulnerable and recover fast to which kind of disturbance. This framework revealed that algae and sessile animals in rocky intertidal shore showed high resistance to tsunami induced by the 2011 off the Pacific coast of Tohoku Earthquake through the comparison of impact of 27 events for five kinds of disturbance on 50 wildlife populations.

Patterns and drivers of stability responses to environmental change

Samuel R.P-J. Ross (OIST)

Biodiversity provides resilience to environmental change. Here I discuss the stabilising effect of biodiversity through a series of short case studies, incorporating concepts, statistical models, theory, and experimental work, with the aim of moving beyond the patterns of stability towards the drivers underlying this relationship. I discuss response diversity as a potentially key driver of stability. Response diversity captures information on the responses of different species within a community to environmental change, and therefore higher response diversity should confer stability to ecosystems and their processes. There has been little empirical work on response diversity to date, so I present some methods development which should catalyse future work on the topic, and introduce the global response diversity network: a collection of international scientists interesting in coordinating research on response diversity. Through such coordinated efforts, I hope that within a few years the field of response diversity will have blossomed to include empirical demonstrations of response diversity's role in mechanistically driving ecological stability. Tohoku University - OIST 3rd Joint Workshop on Biodiversity: From Genes and Species to Ecosystem Services and Resilience

Poster Session

17:00~18:00, October 24, 2022 Venue: Sakura Hall, Tohoku University

Lightning Talk

Characterization of glycoprotein hormones and receptor pathway in reproductive development of scallop, *Mizuhopecten yessoensis*

Wenbin GU¹, Shenelle Pamela Bairan¹ LIM, Kazue Nagasawa¹, Makoto Osada¹

¹Tohoku University, Graduate School of Agricultural Science

Glycoprotein hormones (GPHs) are secreted from the pituitary gland under the stimulation of gonadotropin releasing hormone (GnRH) and are important hormones that control reproduction in vertebrates. However, with the absent of pituitary gland in bivalve species, the existence of GPHs and their roles are still in mystery. In this study, the Fshrlike receptor (myFshr-l) were identified in yesso scallop Mizuhopecten yessoensis. Conserved 7 transmembrane domain and leucine-rich repeat domain were found in myFshr-l. Tissue distribution analysis and in-situ hybridization assay revealed that it showed high expression in gonad tissues and was localized in spermatogonia and oocyte, suggesting its potential roles in gonad development. Further, 5 potential binding ligands were predicted by protein database screening based on GPHs conserved domains. 5 predicted ligands, named as GPA2, GPB5, Bura1, Bura2 and Burß, all showed expression in both ganglion (CPG and VG) tissues. Western blot assay showed that GPA2 and GPB5 can form both homodimer and heterodimer structure, and luciferase reporter assay revealed that only heterodimer structure GPA2+GPB5 can activate myFshr-l and further activate cAMP-response element. This research suggested the existence of GPHs-GPCR signaling pathway in bivalve and its potential function in their reproductive development.

A theory of hybrid speciation by sexual selection Kotaro Kagawa¹

¹ Graduate School of Life Sciences, Tohoku University

Species boundary is often maintained by interspecific differentiation in mate preference of female and sexually selected displays of male. However, the mechanism for the incipient speciation generating such species pairs with distinct mating trait phenotypes remains unclear. Here, based on evolutionary simulations, I propose that hybridization between distantly related evolutionary lineages can promote the formation of a new species with a novel combination of mate preference and sexual display. The simulation considered hybridization between allopatrically diverged parental lineages that share similar mating trait phenotypes. When mating traits are polygenically controlled, recombination of genomes of the parental lineages could generate hybrids with various novel mating trait phenotypes. This disturbed the matching between female mate preference and male sexual display in the hybrid population, which sometimes promoted co-evolution of mate preference and sexual display towards a new direction: a hybrid population could reach an alternative evolutionary stable combination of mating trait phenotypes that has not been occupied by the parental lineages. Therefore, hybridization could form a new reproductively isolated species with novel mating trait phenotypes. These theoretical results may offer plausible explanations for puzzling cases of non-ecological hybrid speciation and sexual radiation.

Lightning Talk

Active microbiome of squid Sepioteuthis lessoniana

Lucia Zifcakova¹, Zdenek Lajbner¹, Giovanni Massucci¹, Jonathan Miller¹

¹ Physics and Biology Unit, Okinawa Institute of Science and Technology

The objective of our study was to describe the active microbiome of squid *Sepioteuthis lessoniana* from different body parts for the first time ever.

We have extracted fourteen RNA samples from different tissues of one female and one male animal of "Shiro ika. We have also sampled brain and muscle tissue from "Kuwa ika" from *S.lessoniana* species complex. RNA was extracted with in house protocol combined with Zymo kit for RNA extraction. Ribosomal RNA was removed prior sequencing with Illumina paired-end 2x250. Obtained reads were processed by MG-RAST and normalized to same sum per sample prior statistical analyses. Differences between groups (sex and body_parts) were tested by one-way Anova and Post-hoc Turkey HSD test.

The most abundant bacterial phyla across the samples were Proteobacteria. *Rhodococcus erythropolis* (Proteobacteria) was present in all samples in large quantities, which is not surprising, since it was isolated from local sea water. In two samples of female skin, *Vibrio harveyi* and *Vibrio parahaemolyticus* (Proteobacteria) were the most abundant species, making up 63% of all bacterial species there. These species are opportunistic pathogens of sea animals.

Abundance of parasitic or commensalistic bacteria belonging to Tenericutes phylum was significantly higher in male than female squid. Male brain was different from female one, skin samples were different from brain and from reproductory tract, when comes to abundance of Proteobacteria.

Whether *S.lessoniana* has a specific microbiome or not remains to be answered in the future due to sample limitation of our study.

Acclimation dynamics of coral-dinoflagellate symbiosis in situ

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Physiological plasticity allows an organism to persist in dynamic environmental conditions. For corals, regulation of the endosymbiotic photosystem is the central process involved in maintaining a stable coral-dinoflagellate symbiosis. The collapse of this relationship leads to coral bleaching and mortality. While coral bleaching and symbiont shuffling are deemed rapid adaptive mechanisms in corals, reports focus on environmental extremes rather than natural seasonal fluctuations. To identify the primary mode of seasonal acclimation, biodiversity, cell density, and photophysiology of the Symbiodiniaceae assemblage were quantified for twenty colonies across five reef flats through seasonal change. Additionally, ten coral colonies were divided and transplanted across four coral gardens. Transplanted colonies were monitored for disease, bleaching, predation, and mortality. Under naturally variable environmental conditions, Symbiodiniaceae assemblages display site and season-specific photophysiological acclimation while maintaining a stable biodiversity and cell density. Transplanted colonies experienced an initial drop in cell density after transplantation, but stabilized within four weeks, converging upon the acclimation patterns of their source colonies. Therefore, Symbiodiniaceae actively acclimate their photosystem under natural environmental fluctuation to maintain a stable coral symbiosis.

Assessing impact of aquaculture on fish communities

using environmental DNA

Naoma Motomatsu

Graduate School of Life Sciences, Tohoku University

In recent years, the decline in biodiversity due to the increasing scale of use of the natural environment by human activities has been a major issue. Aquaculture, the primary human activity involves the stocking of large numbers of specific species in a small area, which may collapse the balance of coastal ecosystems and cause a decline in diversity. However, it is not well understood how the effect of aquacultures on the diversity of coastal communities varies with the intensity and the species cultured. The Sanriku coast has more than 20 bays within its 600 km coastline, so that the local fish communities established in each bay can be assumed to share a regional species pool. In addition, the amount of production and species cultured are different among bays. This study aimed to clarify the effects of aquaculture on the diversity of coastal fish communities. We investigated the number of fish species and the production and species and the production and species and the production of aquaculture. Environmental DNA(eDNA) was used to estimate fish community diversity. Preliminary results will be presented in the presentation. (196 words)

The cosmopolitan appendicularian *Oikopleura dioica* reveals hidden genetic diversity around the globe

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Appendicularian tunicates are some of the most abundant mesozooplankton organisms with key roles in marine trophic webs and global carbon flux. Like most appendicularians with cosmopolitan distributions, Oikopleura dioica Fol, 1872 is considered a single species worldwide based on morphological features that distinguish them from other appendicularians. Despite their abundance however, there are still only \sim 70 described appendicularian species, compared with over 2,800 ascidian tunicates. Here we perform a molecular phylogenetic, morphological, and reproductive assessment of O. dioica specimens collected from the Ryukyu Archipelago, mainland Japan, and Europe. The specimens are morphologically very similar, with only detailed examination of the oikoplastic epithelium and quantitative measurements revealing minor distinguishing characteristics. Phylogenetic analyses of the ribosomal gene loci and mitochondrial cytochrome oxidase I (COI) gene strongly indicate that they form three separate genetic clades despite their morphological similarities. Finally, in vitro crosses between the Ryukyu and mainland Japanese specimens show total prezygotic reproductive isolation. Our results reveal that the current taxonomic O. dioica classification likely hides multiple cryptic species, highlighting the genetic diversity and complexity of their population structures. Cryptic organisms are often hidden under a single species name because their morphological similarities make them difficult to disinguish and their correct identification is fundamental to understanding Earth's biodiversity. O. dioica is an attractive model to understand how morphological conservation can be maintained despite pronounced genetic divergence.

Diverse viral immediate-early genes for genetic engineering, Application in Mollusca

Jeongwoong Yoon, Wen-Bin Gu, Mizuki Konuma, Mutsuko Kobayashi, Hayato Yokoi, Makoto Osada, Kazue Nagasawa

Graduate School of Agricultural Science, Tohoku University

Strong promoters are often discovered from viral genes, such as CMV IE (mammals), opIE2 (insects) and CaMV 35S (plants) promoters, because of their nature relying on the host cell machinery to replicate themselves. In particular, viral immediate early genes, a set of genes expressed in the host cell in the absence of other viral gene products, can possess highly active promoter sequences which can be co-opted for genetic engineering. There has been no success in strong transgene expression in molluscan cells for decades, and we reasoned that a promoter from genes in oyster-infectious virus could be a breakthrough. We first established a promoter activity assay system using Yesso scallop primary cells and then evaluated activities of putative immediate-early gene promoters in ostried herpesvirus-1 (OsHV-1). As a result of screening, we discovered a highly functional promoter in molluscan cells from *oshv117* gene, termed 'OsHV-1 IE promoter.' The OsHV-1 IE promoter enabled strong GFP expression in molluscan cells, and thus a robust gene overexpression system, for the first time in molluscs. Our work provides a recent example of co-opting diverse viral genes for genetic engineering, which can be reproduced in other non-model systems.



Intrinsic and extrinsic drivers of species diversity: the case of the planktonic tunicate *Oikopleura dioica*

Charles Plessy and the Luscombe laboratory (group authorship)

Genomics and Regulatory Systems Unit, Okinawa Institute of Science and Technology Graduate University

Larvaceans are planktonic tunicates that graze smaller organisms by filter feeding through a special apparatus that they secrete, called the house. Larger than the animal, the house is essential in allowing continuous feeding and a fast life cycle. Discarded houses are an important component of the marine snow that feeds other animals or sediments on the sea bed. For this reason, larvaceans play an important role in the carbon cycle.

The *Oikopleura* genus of larvacean comprises species adapted to different environments such as shore or open sea. Among them, *O. dioica* was discovered in the XIXth as the only dioceous (non-hermaphrodite) tunicate species, and was later found to be present near the coastline of most seas of the world. The morphology of *O. dioica* is very conserved, especially in the region of the body that secretes the house, where the number and arrangement of specialized cells is totally fixed. However, our recent studies have demonstrated that there exists multiple cryptic species of *O. dioica* that diverged multiple million years ago.

We found that structural variations in O. dioica genomes are very frequent, even in single individuals, and reaches extents that are predicted to compromise cross-over between parental alleles, thus raising the question that intrinsic genomic instability could drive a continuous speciation process in *O. dioica*. Nevertheless, as *O. Dioica* is a planktonic species, we expect that environmental and geophysical processes such as sea currents to exert a strong influence in shaping the population structure.

To study the distribution of *O. dioica* and other planktonic species in more detail, we are developing an environmental DNA (eDNA) sequencing approach based on long sequence reads bridging ribosomal RNA regions highly conserved at the genus level with regions polymorphic below the species level, in order to acquire taxonomic information at a high dynamic range. Therefore, in this presentation I will show that by combining genomic and environmental sampling approaches, we can study the interplay between the intrinsic and extrinsic drivers of species diversity.

Preparation of domoic acid and kainic acid analogues using the cyclases, and evaluation of their toxicities

Motomi Yamada¹, Yukari Maeno¹, Yuichi Kotaki², Ryuta Terada³, Masafumi Hidaka¹, Yuta Kudo^{1,4}, Yuko Cho¹, Keiichi Konoki¹, Mari Yotsu-Yamashita¹

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Domoic acid (DA, 1) and kainic acid (KA, 2) are the natural kainoids, which are potent agonists of ionotropic glutamate receptors in the central nervous system. The chemical syntheses of DA, KA and their derivatives requires considerable effort to establish a pyrrolidine



ring containing three contiguous stereocenters. Recently, biosynthetic cyclases for DA (DabC)¹⁾ and KA $(KabC)^{2)}$ were identified. DabC cyclizes the linear precursor of isodomoic acid A (IA, **3**) to IA, a bioactive DA analogue. KabC expressed in *Escherichia coli, in vivo* was reported to produce KA in gram-scale²⁾. In this study, we developed a bioconversion system to obtain DA and KA analogues from linear substrates prepared by simple chemical synthesis, using DabC and KabC expressed in *E. coli, in vivo*³⁾. Three IA analogues with various substitutions at the C7'-geranyl terminus were prepared (Table 1): two minor natural analogues, 7'-methyl-IA (**4**) and 7'-hydroxy-IA (**5**), and one new unnatural analogue, 3'-hydroxy-KA was prepared (Scheme 1). The toxicities of these analogues in mice were evaluated by intracerebroventricular injection. The results suggest that 7'-carbonyl group in the side chain of IA and 3'-methyl group in KA are crucial for their toxicity.



References

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Lightning Talk

Predation drives the morphological diversity of ant mandibles

Julian Katzke¹, Evan P. Economo¹

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Radiating into thousands of species, ants evolved to perform vastly different behaviors and their range of body plans is disparate. Mandibles are the primary tools that ants rely on to interact with the world, as they use them in social, environmental, and dietary interactions. However, we lack understanding of the factors that promoted their notable morphological diversification. Here, I use a comparative approach to show how diet relates to shape diversity. I quantify shape with geometric morphometric methods from μ CT models of more than 200 genera. Four archetypical mandible shapes best summarize morphological diversity. Elongated forms are often associated with specialized predatory function. Across the phylogeny, they occur by modifying different subregions of the mandible and thus increase the overall diversity of mandible shapes. Even in species-poor clades, extreme shapes occur. I further aim to use 3D anatomical and phylogenetic data to identify the most plausible evolutionary scenarios for mandible diversification and link these to the evolution of mandible musculature and head shapes. Ultimately, I want to ask how diverse functions and shapes in different body parts map towards each other to result in complex morphological diversity patterns.

Evolutionary relationship between gall inducing insect and host plant

Kanako Bessho-Uehara¹ ¹ Laboratory of Evolutionary Genomics, Graduate School of Life Sciences, Tohoku University

Abstract:

Insect galls show big morphological diversity, although composed of host plant tissues, their development might be largely controlled by insect derived factors. However, it has not been well discussed whether this is due to the plant organ preference of the insect, i.e., whether the insect lays eggs on different organs and the plant responds differently, resulting in different shapes (due to the plant) or whether different factors are used to induce different shapes even if the eggs are laid on the same organ (due to the insect). I found that each order of insects has a different organ preference when laying eggs, with Coleoptera and Lepidoptera inducing galls mainly on stems, Hymenoptera on leaves and stems, and Diptera on almost all plant organs. In addition, RNA-seq results on material from Cuscuta campestris and Smicronyx madaranus (weevil, Coleoptera) showed increasing expression of the flower bud induction genes in the early stage of gall. Higher expression of flower bud induction genes has also been reported in the insect galls induced by insects belonging to other orders. This suggests that the response of the plant side is partly common in all organs of the plant, and that the various insect gall shapes are due to differences in inducing factors from the insect.

Exploring the genomic correlates of the convergent evolution of an ultrafast biomechanical innovation in ants

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Convergent evolution has produced numerous stunning adaptations across the tree of life. Yet, we have just begun to understand the molecular architecture of complex convergent phenotypes. In our work, we aim to explore the genomic and transcriptomic landscapes associated with the convergent evolution of an ultrafast biomechanical innovation - the poweramplified trap-jaw mandibles in *Strumigenys* ants. To capture their exceptionally fast prey items, these ants produce extremely fast mandible closure, generating the fastest acceleration of any resettable movement in the animal kingdom. Recent work from our Unit showed that trap-jaw mandibles and their associated morphological, behavioral, and neurological adaptations have evolved independently in different parts of the world. Here, we exploit this "natural experiment" of replicated evolution and ask whether similar genes and pathways are involved during the convergent evolution of the "trap-jaw phenotype", or whether there are multiple routes at the molecular level to arrive at similar phenotypes? In my poster, I will describe my ongoing work on this system, where I combine comparative genomics and transcriptomics to explore the molecular mechanisms underlying phenotypic convergence. I will provide some preliminary insights into the reference-quality genomes of Strumigenys we have sequenced so far and outline our approach going forward.

DNA barcoding of freshwater zooplankton in Japan

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DNA (meta)barcoding is a method for identifying organisms using short, standardized fragments of genomic DNA. Although it is useful (e.g., eDNA metabarcoding in ANEMONE), it requires the development of a specific reference sequence library for sample application. Unlike fish, such "barcode libraries" are currently unavailable for Japanese freshwater zooplankton, even though they play an important ecological role by linking primary producers to planktivorous fish. Therefore, I started collecting zooplankton DNA barcodes from various fresh waters throughout the four main islands (Honshu, Hokkaido, Kyushu, Shikoku). At first, I focused on the Folmer fragment of mtCOI, which is often used for zooplankton metabarcoding outside Japan, and obtained sequences from planktonic crustaceans (copepods and cladocerans). Then, I performed a mock community (defined mixture of planktonic crustacean species) experiment to check whether the gene region is usable for the metabarcoding of Japanese planktonic crustaceans. Results suggested that the gene region is not useful very much because copepods were strongly under-represented relative to cladocerans.

Lightning Talk

Global diversity of coral symbionts

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Corals associate with several bacterial and protists endosymbionts, in different interactions such as mutualistic or pathogenic. We sampled sibling hexacoral species of the genus *Palythoa* as a model system to examine diversity of symbionts of the same coral hosts across global regions with different seawater conditions. Molecular analyses showed that coral samples from Atlantic and Indo-Pacific have different bacterial and Symbiodiniaceae compositions. On the other hand, the same generalist lineage of corallicolids was found associated with corals from the Atlantic and Indo-Pacific oceans, while another three lineages were found associated with corals from distinct locations. Additionally, stress-tolerant symbionts (e.g., *Durusdium*) were found associated with corals from the Red Sea and Iotorishima Island, Japan (warmest seawater temperature and low pH seawater conditions, respectively). The next step of this project is to use population genomic analyses to understand how coral's phylogeography is related to symbionts diversity.

An AI system for identifying and enumerating freshwater zooplankton species

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Zooplankton is an important intermediate consumer in aquatic ecosystems and is sensitive to environmental change. Therefore, continuous zooplankton monitoring is essential to evaluate and conserve the aquatic environment. However, we need taxonomic knowledge and identification skill to monitor it reliably. Therefore, we are trying to develop an image-based Automatic Identification and Enumeration system (AIdES) for zooplankton. For these objects, we first developed a protocol for taking a single high-resolution composite image covering the whole aqueous sample containing individuals of various zooplankton species using an intelligent microscope. Using the protocol, we processed 67 samples from 18 lakes and reservoirs and collected a total of 17,167 individual images of 75 plankton taxa. Then, we trained an Automatic Identification Sub-system (AIS) developed based on the Convolutional Neural Network algorithm. After that, we tested the system's performance. It revealed that even for samples taken in different places from training data, the correct identification rate of the AIS was 83.1%. Furthermore, even for species with low identification accuracy, the AIS identified the high taxonomic classes with more than 90% accuracy. Although the development of the automatic enumeration sub-system is underway, the present results suggest that the AIdES will be an ideal system for monitoring zooplankton.

How natural selection drives the evolution and lifestyle innovation of coral symbiotic algae

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Dinoflagellate algae in the family Symbiodiniaceae, which are famous for their symbiosis with corals, have intracellular and extracellular symbiotic lifestyles with many non-photosynthetic organisms. Although it is shown that the symbiotic ability is widely maintained within this family, how their diverse lifestyles have evolved remain unclear, as symbiotic and free-living species are intermingled in each phylogenetic lineage. Here we show that, using six species in the genus Symbiodinium in Symbiodiniaceae, a number of gene families have been under natural selection in the process of lifestyle diversification. Single copy orthologs were extracted from all CDS regions of four symbiotic species and two free-living species and used for analysis. We identified candidate genes under positive natural selection by using the CodeML program of the PAML software and the aBSREL model of Hyphy software. As a result, 35 genes including starch synthetase SSY1, cholesterol transporter ABCA5, and sterol glycosyltransferase RAE1 were obtained as the candidate genes which have been evolving under positive selection relevant to the symbiotic lifestyles. Our data suggest that, in the symbiosis of Symbiodiniaceae, the changes in the metabolism of sugars and sterols, which are essential molecules delivered to the host from symbiont in oligotrophic tropical and subtropical oceans, may have promoted the diversification and led to the innovation of algal lifestyles.



Lightning Talk

Bobtail squid diversity and evolution

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Cephalopods are a morphologically diverse group of mollusks that inhabit a wide range of contrasting marine environments, from warm to cold and temperate waters, and show extensive diversity in mainland Japan and the Ryukyu Archipelago.

Among hundreds of living species of cephalopods, the bobtail squid, a lineage with rich diversity, is emerging as a model species to understand the molecular evolution of cephalopods' novel traits and complex behavior. They are small nocturnal cephalopods with light organs producing either autogenic or symbiotic luminesce, which makes them a model for studying symbiosis between bacteria and metazoans. Using transcriptome sequences and a reference genome, we reconstructed the evolutionary relationship of bobtail squid distributed in many ocean basins worldwide, producing an initial framework to understand the molecular signatures of morphological novelties in these animals. With the upcoming sequencing of many reference genomes from not only bobtail squid but also other cephalopods, we will begin to understand the molecular basis of the diverse morphological traits across cephalopods.



A new Okinawan species Euprymna brenneri



Light organ Host A. fisheri

Accesory nidamental gland Host bacteria consortium

Symbiotic organs of a bobtail squid

Classification of community indicators based on context-dependent dynamics: A case study of a coastal fish community

Yuri Otomo¹, Reiji Masuda², Yutaka Osada¹, Kazutaka Kawatsu1, Michio Kandoh1

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Various indicators, such as species richness, total abundance and diversity indices, have been proposed to capture the structure of ecological community. However, as it remains unclear how those indicators reflect a governing rule of community dynamics, we have no criteria for which indicators apply to understanding the response of communities to environmental changes. To resolve this issue, we focused on the similarity in the timing of dynamic changes among community indicators, measured by the prediction skill with an indicator's dynamics as supervised data to the other. Employing a nonlinear forecasting-based method to the time series of 10 community indicators of a marine fish community, we aimed to detect changes in their dynamics and classify them according to the similarity in the timing when dynamic changes occurred. As a result, the 10 indicators were classified into three super-groups: Group II (species richness) with the most robust dynamics to the environmental changes; Group II (species diversity and total abundance) showing an abrupt change in the middle of the monitoring period; Group III (species evenness) with highly variable dynamics. This suggests that we need a balanced selection of indicators from these three groups to appropriately capture the response of communities to environmental changes.



Fig1. Heatmaps showing the timing of dynamics changes of community indicators. Indicators with similar heatmaps are similar in the timing of dynamic changes.

Recovery process of rocky intertidal community after the 2011 off the Pacific coast of Tohoku Earthquake

Arisu Shikine

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Earthquakes can repeatedly cause drastic changes on community on the Pac ific coast. However, it is unclear how the communities respond to the earth quakes and how they maintain or change their structure due to the rarity of earthquakes. The rocky intertidal shore in the Sanriku coastline was attack ed by the tsunami and experienced the subsidence by 50 cm induced by the 20 11 off the Pacific coast of Tohoku Earthquake. This subsidence was a largescale disturbance for sessile organisms, because their community structure varies in tens centimeters vertically according to the gradient of inundati on time for the tides. The purpose of this research is to reveal the recove ry process of the rocky intertidal community after the 2011 off the Pacific coast of Tohoku Earthquake. We recorded the abundance of sessile species on the Sanriku coast from 2002 to 2021. First, I calculated pre-earthquake com munity structure by averaging abundance per species for 9 years before the earthquake as the baseline of recovery. Next, I estimated the dissimilarity between pre- and post-earthquake community structure for every year after t he earthquake. The recovery process of the community structure was evaluate d from the temporal change of the dissimilarity for 10 years after the eart hquake.

Using landscape genetics to understand the maintenance of a symbiotic metacommunity

Nonno Hasegawa, David Armitage

Okinawa Institute of Science and Technology Graduate University

Symbioses are abundant in the natural world and occur in many ways, from commensal, parasitic, mutual, to name a few. These relationships are further distinguished as facultative or obligate and symmetric or asymmetric. While single species studies are conducted frequently, species interactions interacting with landscape features are not well understood. Landscape genetic approaches can be used to infer how landscape features and anthropogenic introductions affect dispersal and gene flow, which then shapes the population structure. Here I present my PhD thesis projects, which involve pitcher plant – arthropod inquiline system to understand how metacommunities are maintained spatially and temporally.

Genomic measures relevant to the conservation of green turtles

Tomoko Hamabata^{1,2}, Satomi Kondo³, Kazunari Kameda⁴, Takuya Fukuoka^{5,6}, Katsufumi Sato⁵, Makoto Terauchi⁷, Hideki Noguch⁷, Masakado Kawata¹

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Marine turtles, iconic endangered animals, declined before early 1900's due to heavy exploitation by humans all over the world. It is, however, reported that many populations start to recover since 2000's. Potential resilience as well as conservation efforts is important for the population recovery. Here we examined genomic diversity of 28 green turtles in five regions from the North Pacific (Ogasawara, Western Pacific islands, Southeast Asia, Hawaii, and Eastern Pacific) by whole genome resequencing data. Diversity was variable among the populations, and the Northernmost Ogasawara population showed lowest diversity. However, few turtle had long runs of homozygosity (ROH) more than 2 Mbp that reflected the coalescence dozens of generations back in time, corresponding to the time period severe exploitation in Ogasawara. The historical demography reconstructed by the multiple sequentially Markovian coalescent (MSMC) suggested that the genetic diversity of Ogasawara population was gradually lost because of isolation after divergent from the ancestral population around one million years ago. The present results were unexpectedly inconsistent with the numbers of nesting females and hatchlings that suggest near population collapse in early 1900's in Ogasawara. The genetic diversity may have been preserved in juveniles and males that were not under the hunting pressure, contributing to the potential resilience.



Green turtle, Chelonia mydas

Screening for Nav inhibitors from the marine sponge *Halichondria okadai* and characterization of fatty acid modulation of Nav

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Voltage-gated sodium channels (Na_v) are closely associated with epilepsy, cardiac and skeletal muscle diseases, and neuropathic pain. This study aimed to identify Na_v inhibitors from the marine sponge *Halichondria okadai* using mouse neuroblastoma Neuro-2A cells (N2A), which primarily express the specific Na_v subtype Na_v1.7 using the whole-cell patch-clamp recordings. As the result, we isolated arachidonic acid (AA, 1) only from the hexane extract of *H. okadai* (Figure 1).



Figure 1. Halichondria okadai.

The fatty acid-mediated modulation of Na_v in N2A was then investigated in detail using two ω -6 polyunsaturated fatty acids (PUFAs) including AA (1) and two ω -3 PUFAs for the first time (**Figure 2**). Steady-state analyses, resting state block, use-dependent block, and recovery time from the inactivated state were examined in the presence and absence of these PUFAs, suggesting that distributed binding to the resting and the inactivated states of Na_v would be significant for inhibition of Na_v , which presumably depends on the active structure of each PUFA.





Tohoku University - OIST 3rd Joint Workshop on Biodiversity: From Genes and Species to Ecosystem Services and Resilience

Talk Session 4

10:00~11:50, October 25, 2022 Venue: TOKYO ELECTRON House of Creativity 3F, Lecture Theater, Katahira Campus, Tohoku University

Eco-evolutionary dynamics for understanding biodiversity patterns and processes

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Adaptive evolution is ecology in action. Selection pressures in natural systems, therefore, cannot be understood without accounting for the often-complex demography, ecology, and ecosystem embedding of populations. Conversely, predicting changes in natural systems exposed to strong anthropogenic impacts requires accounting for the prospect of rapid contemporary evolution. This presentation provides an overview of some approaches linking ecological and evolutionary dynamics, including adaptive speciation, harvest-induced evolution, and evolutionary community assembly.

Evolution and functional ecology of solar-powered jellyfishes and their cassiosomes

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In warm coastal waters around the world, snorkelers and sea kayakers can sp ot large groups of Cassiopea jellyfish (Cnidaria, Scyphozoa) pulsing rhythm ically, upside down on the seafloor of mangroves, lagoons and artificial ma rine waterways. These upside-down jellyfish and kin (order Rhizostomeae) ho st single-cell algae (Symbiodiniaceae) in their cells, and derive nutrition largely from photosynthates. Conversely, when heterotrophic feeding is requ ired, unlike other jellyfishes, upside-down jellyfish lack a central mouth but, possess instead numerous small pores from which they release a mucus w eb to trap and ingest zooplankton prey items. Unsuspecting bathers in the v icinity of this mucus meet with irritating stings unless properly covered w ith protective clothing. This phenomenon was dubbed "stinging water sensat ion", but its cause remained unknown until our group discovered self-prope lling stinging structures, we called cassiosomes (Fig 1), within the snarin g clouds of mucus. As novel, autonomous cellular aggregates, cassiosomes co mprise an outer layer of nematocytes surrounding a core containing Symbiodi niaceae. Herein, we present our current understanding of the evolution of c assiosome production as a synapomorphy of the order Rhizostomeae (Fig 2), a nd discuss the efforts of our international collaborative network (Aquatic Symbiosis Project) to build genomic foundations to uncover molecular pathwa ys underlying co-evolution among photo-endosymbiotic algae and jellyfishes.



Fig 1.Single cassiosome (bar²50 μm) Source: Ames, Klompen et al, 2021 (Comms Biol)



Fig 2. Cassiosome emergence in Rhizostomeae

Branching morphogenesis of medusa tentacles in the jellyfish sp ecies *Cladonema pacificum*

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Branching of tissues/organs increases their epithelial surface area to maxi mize their function in association with their environment. In well-studied branching model animals, such as mammals and Drosophila, branching is known as a common mechanism to rely on the interaction between the epithelial cel 1 layer and its underlying mesoderm. However, it remains largely unknown ho w tissues/organs become branched in other animals, in particular, those mes oderm-free diploblastic animals. In this study, the jellyfish *Cladonema pac* ificum was used to investigate branch formation. It was found in C. pacific um medusa tentacles that new branches were successively created one after a nother at the proximal region of the main tentacle while the main tentacle grows. At the new branching sites, hydrozoan-specific pluripotent stem cell s, namely interstitial cells (I-cells), were repetitively accumulated. Duri ng branch elongation, the accumulated I-cells remained located at the tip o f the growing branches, while proliferating and leaving behind their differ entiating descendant cells. This study highlights an essential role for I-c ells in branching morphogenesis of a diploblastic animal. In addition, it i dentifies patterning through repeated applications of a single rule as a co nserved mechanism across the animal kingdom, a possible mechanism of which will be presented in this talk.

Cryptic species diversity of deep-sea dogfishes in Japan

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The north-western Pacific is one of the global 'hot spots' for shark biodiversity, but few empirical data are available on the region's cryptic deep-sea sharks, the mesopelagic dogfishes (family Squalidae). Taxonomic uncertainties and continued misidentifications of cryptic *Squalus* species in the Northwestern Pacific and Atlantic is an ongoing issue and has severely affected the quality of data available for these commercially exploited species. Using mitochondrial markers, we assessed species-level phylogenetic relationship and genetic divergence of regional congeners by comparing them with relevant *Squalus* dogfishes from the Pacific, the Atlantic and Mediterranean Sea. The reconstructed phylogeny showed hidden diversity with high local endemism within the genus *Squalus* including undescribed species from Japan. The morphological divergence among Japanese dogfishes was further examined and was largely consistent with the molecular separation.

Underestimated diversity and limited distribution ranges of dogfishes presents a serious challenge in terms of resilience to anthropogenic stressors and deep-sea shark conservation.

Does generality exist in biodiversity dynamics: a time-series analysis of dynamical similarity with ANEMONE dataset

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¹ Graduate School of Life Sciences, Tohoku University

Seeking a general rule behind the biodiversity pattern is a core challenge in ecology. Therefore, many studies have been accumulated to describe similarities/dissimilarities among ecological communities based on snapshot data of community structure (e.g., species composition and relative abundance). However, evidence remains to be validated for whether generality exists in biodiversity *dynamics* between different ecosystems due to the lack of analytical methods identifying other governing rule from noisy ecological time series.

Recently, I have developed a model-free, nonlinear time-series analysis called LMDr, which can accurately reconstruct the system's time evolution rule even under severe noises. Then, I applied the LMDr to the ANEMONE dataset, which consists of eDNA time series of hundreds of fish genera sampled from hundreds of monitoring sites around coastal areas in Japan, and measured the site-site similarities of biodiversity dynamics. The results demonstrated that the degree of dynamical similarity positively correlates with the other similarity indices, such as geographical and environmental ones. In particular, the similarities in community structure were the most important explanatory variables for the observed variance of dynamical similarity. That is, the ecological relationships among coexisting species control the local biodiversity dynamics, indicating the existence of the general ecological rule.

Microbiome diversity improves host functioning in a plant-microbe mutualism

David Armitage¹

¹ Integrative Community Ecology Unit, OIST

Most biodiversity-ecosystem functioning (BEF) theory has been developed and benchmarked in plant communities. In my talk, I ask whether BEF theory extends to microbial communities, whether it is dynamic through time, and — in cases where the microbial community is hostassociated — whether such 'ecosystem functions' include boosting the performance or fitness of the host organism. My examples draw from experimental results concerning the development and functioning of the microbial digestive community found in the fluid-filled cavities of carnivorous pitcher plant leaves. I will show that host-associated microbial communities undergo predictable successional dynamics that result in time-variant BEF relationships, increasing functional diversity, and increased nitrogen mineralization rates. In concert, these results suggest that microbial communities also assemble in ways that benefit their host organism and these benefits are, in part, diversity dependent. Tohoku University - OIST 3rd Joint Workshop on Biodiversity: From Genes and Species to Ecosystem Services and Resilience

Talk Session 5

13:00~15:10, October 25, 2022 Venue: TOKYO ELECTRON House of Creativity 3F, Lecture Theater, Katahira Campus, Tohoku University

Analyzing the Fossil Record to Reveal the Evolution of Marine Biodiversity

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Determining the drivers of divergence between species at the core of evolutionary biology; how these mechanisms scale up to diversification of major groups (clades) is at the core of macroevolution (ev olution at the species level and above). A full picture of diversification requires information from multiple areas (ecology, organismal biology, functional morphology, paleontology) at multiple scales (e.g. spatial, temporal, molecular, and taxonomic). Fishes can provide essential data on all these a spects. The 34,000 living lineages of fishes include everything from keystone species like parrotfis hes to classic radiations like cichlids and wrasses to developmental model systems like zebrafish an d medaka. The abundant fish fossil record stretches back 450 million years and includes the ancestor s of all living vertebrate clades. Here, I cover how we can synthesize of multiple lines of evidenc e, including trait, ecosystem and species-level data from the fossil record, to undercover of the or igins of fish biodiversity. First, I show how new analyses of global data for the earliest fishes ar e revealing the environmental and geographic origins of vertebrate biodiversity. Next, I show how qu antitative analyses of species-rich fossil fish clades are revealing unexpected roles for diet, habi tat choice, and even life histories in determining the fate of nascent radiations, particularly afte r mass extinction. These discoveries contribute to a new understanding of both the origins of modern fish biodiversity and the processes which determined its structure.

How phytoplankton diversity is maintained in a lake

Naoto Shinohara (Tohoku University)

The diversity that we observe in nature is surprising, given that theories and experiments have predicted that competitively superior species outcompete others. One of the mechanisms that allow multi-species coexistence is the trade-off among species in the degree of limitation by different resources (that is, different species are most limited by distinct resources). However, little evidence has been obtained regarding the contribution of the trade-offs to species coexistence and maintenance of diversity in natural ecosystems. In this talk, I propose a novel way to detect how each species is limited by nutrition levels by focusing on community dynamics. Using this approach, I show that eutrophication (increased levels of phosphorus) in a lake reduces phytoplankton species diversity by collapsing the trade-offs. The results suggest that trade-offs between large and small phytoplankton species are essential to the maintenance of species diversity in lake.

Breakdown of ant community seasonal patterns with land-cover change across an island-wide monitoring network in Okinawa

Jamie M. Kass¹, Takuma Yoshida¹, Masako Ogasawara¹, Mayuko Suwabe¹, Masashi Yoshimura¹, Francisco Hita Garcia¹, Georg Fischer¹, Kenneth L. Dudley¹, Ian Donohue², Evan P. Economo¹

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Insects are a major component of Earth's biodiversity and are responsible for ecosystem functions and services, but concerns have mounted about widespread population declines due to anthropogenic stressors. Less attention has been given to other potential changes in temporal dynamics that can compromise important functions and services. Here, we use data from an observation network covering the subtropical island of Okinawa, Japan to examine relationships between anthropogenic stress and the variability of communities over time, decomposing which aspects of variability explain any differences across a land-cover gradient. With high-frequency sampling over two years, we detected 91 species and >1.3 million individuals. We found that as forest cover increased, sites had higher temporal variability at both the functional (summed community) and compositional (individual species turnover) levels. Temporal decomposition indicated that this was linked to diminished seasonality and higher stochasticity with increasing developed cover. As seasonality of individual species in developed areas was lower than the same species in forested areas, site composition differences cannot be responsible for these patterns, and climate does not change much across the island. Our results show that the degradation of seasonality for insect communities should be a major concern alongside declines, even for subtropical biomes.

Origin and diversification of Ryukyuan citrus by hybridization and apomixis

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 ⁷ Chan-Zuckerberg BioHub, San Francisco, CA, USA.

Mandarin-type citrus, including oranges, grapefruit, and lemons, yield consumer-friendly small and peelable fruit. Since most cultivated mandarins can reproduce seeds of maternal clones from somatic tissue (apomixis), the desirable genotypes relating to commercially valuable phenotypes could be replicated. Mandarins are also widely hybridized with other citrus species. The Ryukyu Islands and mainland Japan are home to unique wild and cultivated citrus whose ancestry is poorly understood. In order to resolve the relationships and explore the nature, evolution, and biogeography of east Asian citrus, we sequenced the genome of 69 traditional, wild, and atypical citrus of the Ryukyus and southern mainland Japan, and analyzed these genomes together with previously sequenced wild and cultivated Chinese mandarins and other citrus. We identified a previously unrecognized wild citrus species native to the Ryukyu islands (Citrus ryukyuensis sp. nov.). We also found that previously described a single wild population of Chinese mandarins contained two subspecies. The discovery of these three ancestral lineages uncovered the taxonomic complexity of east Asian mandarins including tachibana, shiikuwasha, and other traditional Ryukyuan mandarin types. We also traced the origin of apomixis alleles to Chinese wild mandarins, which played a central role in citrus domestication via adaptive wild introgression.

Flatfish metamorphosis: molecular basis underlying unique morphogenesis leading to asymmetric body plan

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Flatfish, such as flounder, turbot and sole, show striking morphological asymmetry in terms of the eye-positioning and pigment pattern. Their larvae, however, develop in a symmetric body plan just same as ordinary fish. Then at metamorphosis, one eye migrates to the other side and pigment cells differentiate only in the ocular side, which lead to the left/right asymmetric body plan, representing one of the most diversified



vertebrates. In addition to such biological interest, some flatfish are important because of their market value. I will introduce our recent approach to understand the molecular basis of flatfish metamorphosis. We compared gene expression profile between ocular and blind side and identified genes expressed in left/right asymmetric manner, associated with the eye migration or ocular side specific pigmentation. We also examined the significance of retinoic acid (RA) for the asymmetric pigment cell differentiation, as the food intake of RA was implicated in the abnormal pigmentation in aquaculture. Gain-of-function and loss-of-function experiments showed that RA is critical for the ocular-side specific pigmentation. Together with functional analysis using experimental model fish, we hope to understand the underlying mechanisms of enigmatic metamorphosis.

What nematode genomes can teach us about phenotypic evolution

Asako Sugimoto

Laboratory of Developmental Dynamics, Graduate School of Life Sciences, Tohoku University

During the 20th century, only a limited number of model organisms were genetically accessible. However, recent advances in genome sequencing and gene manipulation technologies have made it possible to use a wide range of non-model organisms as subjects for genetic analysis. We have been using the model nematode *Caenorhabditis elegans* and its related species to elucidate how genomic changes alter the gene networks that control developmental programs by taking advantage of the wealth of gene-phenotype information accumulated in the long history of *C. elegans* studies. In this talk, we will present a comparative analysis of *C. elegans* and its recently identified sister species, *C. inopinata*, which was found in fig syconia on Ishigaki Island, Okinawa. The morphology, developmental processes, and behavior of *C. inopinata* differ significantly from those of *C. elegans*. Comparative genomic and gene manipulation analyses indicate that the activated transposable elements contribute to the acceleration of trait evolution in *C. inopinata*.

Tohoku University - OIST 3rd Joint Workshop on Biodiversity: From Genes and Species to Ecosystem Services and Resilience

Talk Session 6

15:25~17:35, October 25, 2022 Venue: TOKYO ELECTRON House of Creativity 3F, Lecture Theater, Katahira Campus, Tohoku University

The Sanriku Coast as a Marine Phylogeographic Research Field

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Off of the Sanriku Coast is one of the world's best fishing grounds. This blessed situation is due to the mixing of the Kuroshio Current from the south, the Oyashio Current from the north, and the Tsugaru Warm Current, a branch of the Tsushima Current. The flow of the Tsugaru Warm Current into the Pacific Ocean was caused by a rise in sea level after the last glacial period. This current transported the populations of marine organisms that had been isolated in the semi-enclosed Sea of Japan during the Pleistocene to the Pacific Ocean side, providing in secondary contact with genetically divergent Pacific Ocean populations around the Sanriku Coast. Hence, this coast should have unique landscape value not only as a blessed fishing ground, but also in phylogeographical studies testing allopatric speciation and/or evolutionary consequences of hybridization between divergent lineages in marine organisms. However, only a few studies have investigated the contact zone of different lineages in this area and studied the reproductive isolation or hybridization between the lineages. In this talk, I will introduce our recent phylogeographic studies including population genetic analyses in the contact zones of two lineages in the rocky intertidal goby and amphidromous freshwater shrimp.

Morphological adaptations associated with the evolution of forward jumping in ants

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Ants, one of the most successful groups on the planet, play an essential role in almost all terrestrial ecosystems. They evolved different locomotion patterns to be able to move on different surfaces, to exploit different habitats, and also to cope with adverse conditions. Some ants are specialized in running, such as desert ants, some are specialized for gliding, swimming, and many other locomotion types. One rare specialization in the evolution of worker ant locomotion is the ability to jump. Forward jumping behavior independently evolved within four distantly related ant genera The functional behaviors that propel them forward are known to vary across these groups, with some using middle legs only, some middle and hind legs, and at least one using the gaster to gain forward momentum. It is possible that changes to the size and architecture of locomotory muscles and/or the evolution of power amplification mechanisms could underlie these behaviors, but these are unknown. We investigate the morphological changes associated with jumping behavior by comparing changes to the locomotory musculature between jumping and non-jumping relatives, using 3D x-ray micro-CT and quantification of muscle volumes and architectures.

Possible link between brain size and flight mode in birds: Does soaring ease the energetic limitation of the brain?

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Elucidating determinants of interspecies variation in brain size has been a long-standing challenge in cognitive and evolutionary ecology. As the brain is an energetically expensive organ, energetic tradeoffs among organs are considered to play a key role in brain size evolution. This study examined the tradeoff between the brain and locomotion in birds by testing the relationship between brain size, flight modes with different energetic costs (flapping and soaring), and migratory behavior, using published data on the whole-brain mass of 2242 species. According to comparative analyses considering phylogeny and body mass, soarers, who can gain kinetic energy from wind shear or thermals and consequently save flight costs, have larger brains than flappers among migratory birds. Meanwhile, the brain size difference was not consistent in residents, and the size variation appeared much larger than that in migrants. In addition, the brain size of migratory birds was smaller than that of resident birds among flappers, whereas this property was not significant in soarers. Although further research is needed to draw a definitive conclusion, these findings provide further support for the energetic tradeoff of the brain with flight and migratory movements in birds and advance the idea that a locomotion mode with lower energetic cost could be a driver of encephalization during the evolution of the brain.

Horizontal gene transfer in evolution of an animal phylum

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An increasing number of studies show roles of horizontally transferred genes (HTGs) in evolution of not only prokaryotes and unicellular eukaryotes but also multicellular animals. However, little is known about how HTGs were incorporated in dynamic evo-devo processes of metazoans. Tunicates or urochordates are closest relatives of vertebrates but quite unique among metazoans, in which they can synthesize cellulose by themselves, a feature common in bacteria and plants but never found in other animals. Tunicate ancestor acquired this potential more than 500 MYA by horizontally transferred cellulose synthase gene (CesA) of bacterial origin, by which tunicates could produce a cellulose-containing outer cover that guards their soft body. The expression of CesA in larval epidermal cells and its function in cellulose production have been shown by a transposon-mediated mutant of CesA. In addition, tunicates are characterized by drastic metamorphosis of swimming larvae to form sessile juveniles, a feature not found in the two other chordate phyla, Cephalochordata and Vertebrata. Ascidians develop the papilla at the anterior-most part of larvae, which plays pivotal roles in the metamorphosis since papilla-cut larvae show its abnormality. We here present evidence for that a horizontally transferred CesA is expressed not only in Ciona larval epidermis but also in the papilla and likely involved in the metamorphosis. Although detail cellular and molecular mechanisms remain elusive, our study provides first insights on the role of HTG in the evolution of a chordate lineage that might be characterized as an animal taxon, Tunicata.



Substantial seasonal intrusion of marine fish species elucidated by environmental DNA metabarcoding in Yura River, Kyoto

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Abstract

Seasonal changes in fish biodiversity of the Yura River, the longest river in Kyoto Prefecture, were investigated using environmental DNA (eDNA). Metabarcoding with MiFish primer was conducted for the eDNA samples six times in surveys from March 2020 to March 2021 at 13 points from the coast to approximately 62 km upstream. In total eDNA of 219 fish species were detected. The top three species with the highest number of sequence reads were two species of mullet, *Mugil cephalus* and *Chelon haematocheilus*, and one species of goby *Tridentiger* sp. Two marine fish species, black sea bream *Acanthopagrus schlegelii* and Japanese sea bass *Lateolabrax japonicus*, were also frequently detected in eDNA. The number of detected species was the lowest in the dam, while the highest in the estuary. The saltwater wedge continued 21 km upstream from the river mouth in summer, but declined to 11 km in winter. The ratio of marine fish in each point detected was positively correlated with salinity at the sampling point (Spearman's rho = 0.92, p < 0.01). This indicates that marine fish could enter the river by utilizing the saltwater wedge. This study implies a substantial contribution of marine fishes for structuring the ecosystem of the river.

Dispersal's Role in Structuring Diversity at Hydrothermal Vents , a Model System for Studying Connectivity and Conservation.

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Connectivity among marine habitats is crucial for maintaining the structure and fu nction of biodiversity in ecosystems threatened by anthropogenic activity. The dis persal of planktonic larvae is the primary facilitator of connectivity among most marine ecosystems. However, dispersal's role in connectivity is unclear due to th e interacting effects of local environmental and biological filtering. Hydrotherma 1 vents present a natural laboratory to study the role dispersal plays in connecti vity, as an 'island-like' ecosystem that is truly discrete in space and connecte d via dispersal on relatively stable and predictable deep-ocean currents. Here we present a novel approach which uses empirical data of species' distributions and simulations of larval dispersal to explore connectivity among a network of hydroth ermal vents in the Northwest Pacific. This approach uses methods from graph theory to combine empirical observations and simulated data under paradigms of metacommun ity dynamics. The resultant model clarifies the interacting effect regional disper sal and local selection has on structuring diversity at hydrothermal vents current ly threatened by mining activity. This model can be adjusted to incorporate anthro pogenic impacts such as mining or global climate change to predict their effect on diversity at this vulnerable marine ecosystem.