Technology to Make and Analyze Ribozyme Mutants

Development of a new method in ribozyme research

Research background

DNA base information is copied to RNA, protein is synthesized based on the information of the RNA, and the protein catalyzes various chemical reactions in vivo as an enzyme. In the early 1980s, an RNA enzyme (ribozyme) acting as a catalyst was discovered, and it was revealed that RNA is involved in both genetic information and biochemical reactions. Later, it has been known that ribozymes exist in various organisms including vertebrates, but many of their roles and functions have been still unclear.

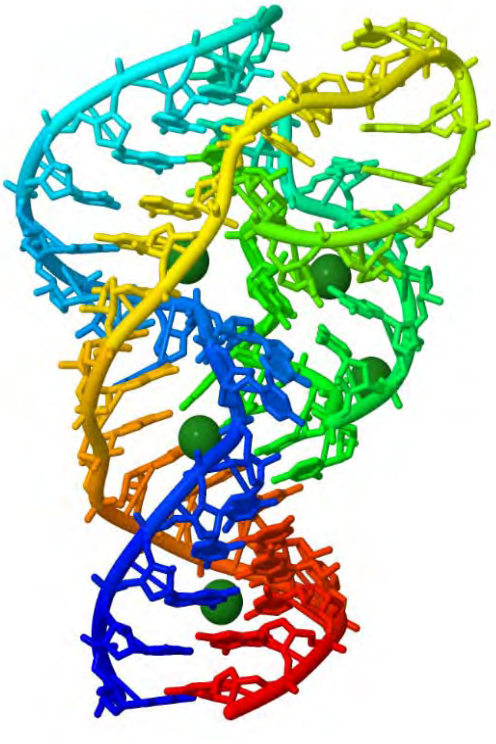
When studying the structure and properties of ribozymes, researchers usually make a mutant in which a specific base of a ribozyme is replaced with another base, and examine the change in functional aspects, etc., by the mutation. A ‘single’ mutant is a ribozyme that differs in only one base from the original ribozyme, and a ‘double’ mutant differs in two bases from the original ribozyme. For example, even for the small ribozyme made of 48 bases, there are 10,296 single and double mutants in total, and the conventional techniques allow only to make about several tens of variants at most in one experiment. Therefor, researchers must select arbitrary parts of the ribozyme as mutation targets, thus potentially overlooking other parts of the ribozyme that may be important for its function.

Research

In OIST, using a high-performance DNA sequence analyzer, researchers made mutants for specific ribozymes comprehensively, and developed a new method of verifying those mechanisms. By using this new method, all single base and double base mutants in "twister type ribozyme" found in rice genome were made and their catalytic activities were measured, whereby the base important for the activity of the ribozyme has been identified.

Moreover, since a significant fraction of the mutants retained detectable ribozyme activity, the ribozyme is highly robust against mutations.

Deeper understanding of ribozymes could allow the technical development to control gene expression in living cells and viruses applying the knowledge, and in the future it is expected to lead to progress in gene therapy and regenerative medicine. (Published in Angewandte Chemie magazine)



3D structure of the “twister ribozyme”

Keywords

RNA enzyme (ribozyme) , DNA, RNA

Applications/Future development

- Control of the gene expression by a ribozyme
- Application to gene therapy or regenerative medicine

Opportunity for joint research and technology transfer

- Currently accepting contacts from companies interested in joint research and licensing of this technology

Introduction of the research unit

Nucleic Acid Chemistry and Engineering Unit

Unit leader : Yohei Yokobayashi Associate Professor

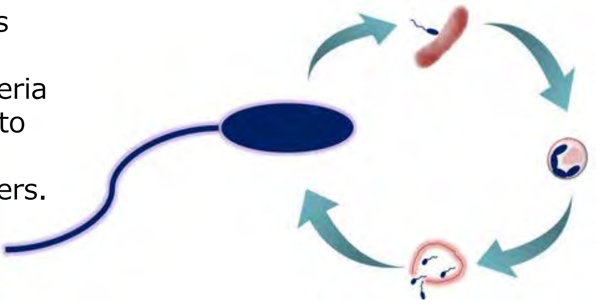
Nucleic acids DNA and RNA are fundamental building blocks of life. These biomolecules display remarkable chemical functions such as information storage, catalysis, and molecular recognition. The Nucleic Acid Chemistry and Engineering Unit’s goal is to harness the versatile chemistry of nucleic acids to design and engineer functional nucleic acids (DNA, RNA, and their synthetic analogs) that operate in test tubes, devices, and living cells.

<Related research theme>

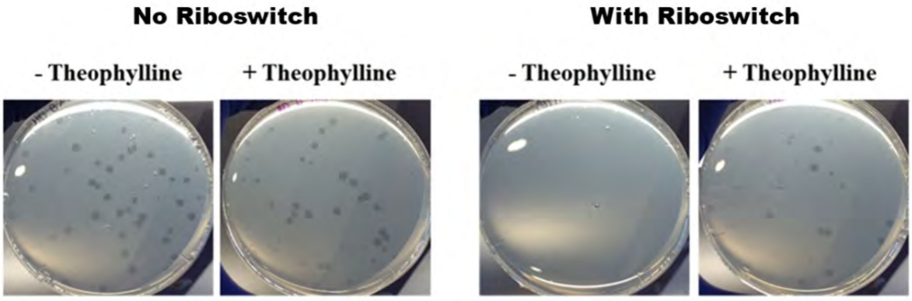
Research of predatory bacteria towards development of new antibiotics

While bacterial resistance to antibiotics increases and it becomes difficult to treat infectious diseases such as tuberculosis and gonorrhea etc., attentions have gathered for the predatory bacteria called “living antibiotics“. Bdellovibrio bacteriovorus is harmless to humans yet lethal to its prey “Gram-negative bacteria” which includes baddies such as E. coli, Salmonella, Legionella, and others. Currently researchers are developing genetic manipulation techniques to control the timing and extent of predation of Budellovibrio bacteriovorus.

(Published in ACS Synthetic Biology issued by American Chemical Society)



Bacterial predation by *B. bacteriovorus*



Petri dishes containing *B. bacteriovorus* and its prey *E. coli*.

Research unit website : https://groups.oist.jp/naceu

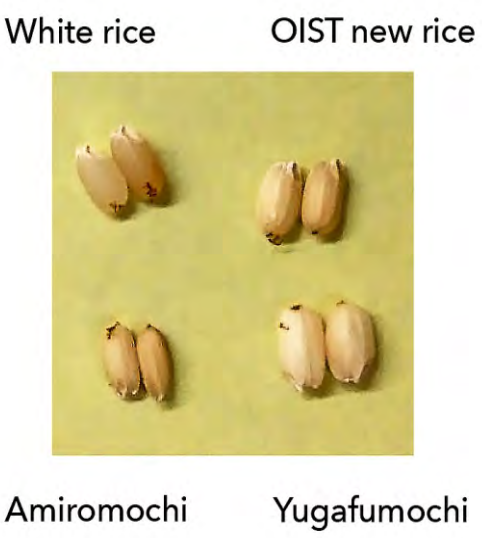
Resistant-Starch Okinawan Rice

Resistant-starch Okinawan rice aiming at prevention of lifestyle-related diseases

Research background

In recent years in Okinawa Prefecture, the obesity rate for both men and women, lifestyle-related disease prevention of the prefecture's population is recognized as and urgent issue. Although carbohydrates such as rice are decomposed into glucose in the process of digestion and used as a major energy source, excessive glucose causes lifestyle-related diseases such as obesity and diabetes.

The rice strain rich in starch which is hardly degraded to glucose is called a resistant-starch rice, and "Amiromochi" was developed at Kyushu University about 30 years ago as such. Amiromochi has a harder texture and inferior taste compared to conventional rice, and the yield when cultivating in Okinawa is reduced to about half compared with Honshu due to the influence of climate and others.



Grain of rice

Research

In the OIST prefecture-commissioned project “Nutraceutical Okinawan Rice Development to Prevent and Improve Lifestyle-related Diseases" (a sectoral outstanding performance award (in R&D and new technology sector) of The Food Action Nippon Award 2015) , researchers bred Amiromochi with a local variety “Yugafumochi" suitable for the climate of Okinawa, and developed a new resistant-starch rice variety that can obtain high yields even in subtropical climates. Due to efficient selection by molecular analysis and genome analysis using the next-generation genome sequencer and shortening of the vegetation period by an artificial climate chamber, field experiments are also initiated in less than three years from the start of the project.

The field test site of the new strain of resistant-starch rice:
The field test started in 2015.

Furthermore, in collaboration with the University of the Ryukyus School of Medicine, Osaka Prefecture University, Ishikawa Prefectural University etc., benefit assessment of the new rice strain is underway. Suppression of increase in blood glucose level, decrease in cholesterol level and decrease in lipid accumulation in the liver were verified in the experimental evaluation using mice, and clinical trials in humans are also conducted jointly with the University of the Ryukyus Hospital.

The new strain of resistant-starch rice can be applied to various foods, and the development of processed food products using rice flour has also started. While lifestyle-related diseases like type 2 diabetes have become life-threatening issues not only in Okinawa Prefecture but around the world, the resistant-starch rice has become a highly desirable disease prevention tool that can be applied to a weight loss method without dietary restriction.

Keywords

Gene, Epigenetics, Next-generation genome sequencer, Breeding development

Applications/Future development

- Market creation of the resistant-starch rice by health food development and others using the resistant-starch rice.

Opportunity for joint research and technology

- Food processing companies: develop processed food products using the resistant-starch rice.
- Medical related companies: conduct clinical trials for the prevention of lifestyle-related diseases such as diabetes.

Patent information
Varieties registration application pending

Introduction of the research unit

Plant Epigenetics Unit

Unit Leader : Hidetoshi Saze Assistant Professor

Genes dictate many aspects of how living things look and act, but genes are also controlled. Epigenetics, is the study of mechanisms that determine whether a gene is active or not, and thus whether it has any effect on an organism. The Plant Epigenetics Unit studies epigenetic regulation in Arabidopsis and rice. It is also improving traits of rice crops by applying genomic information obtained by high-throughput sequencing technology.

<Related research theme>

Elucidation of the mechanism for suppressing the influence of transposable elements

Approximately 98% of the human genome is made of “junk DNA” which does not have genetic information, and much of junk DNA originates from a gene group called transposable elements or “jumping genes”. The transposable elements jump around and insert themselves randomly in other parts of the genome, and these insertions are often deleterious because they could alter gene expression in a way that induces genetic disease. While defense mechanisms are known to stop other molecules from binding to the intruded transposable elements and stop the transcription of a different sequence into mRNA, it is newly found that transcription of normal mRNA progresses by joining the protein named IBM 2 to the transposable elements in the experiments using Arabidopsis. (Published in "Nature Communications")

Research unit website : https://groups.oist.jp/peu

Biomolecule Patterning Technology in Microfluidic Devices

Big hope for disease diagnostic applications brought by nanoscale new technology

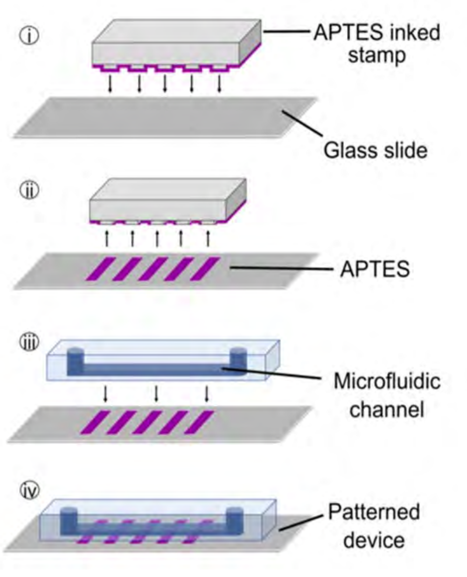
Research background

The specific substances which increase or decrease in vivo by being affected with disease are called biomarkers, and used as indicators of the status of disease and degree of cure. Disease diagnostic device kits which can measure the concentration of disease biomarkers within a patient's biological sample simply, fast and in low cost are required in clinical sites, and the kits with built-in microfluidic devices have been in use in recent years. When body fluids, such as blood and urine, are put into the diagnostic device, a biomarker in the body fluid is captured by a bio-receptor (protein) which is placed in advance on the sensor surface of the device, and the concentration of the biomarker can be calculated from the fluorescence intensity by adding a fluorescent dye that binds to the captured biomarker.

Although bio-receptor molecules can be transcribed and placed on the substrate surface of the device by microcontact printing (μCP) technology, when applying this technology to the nanoscale for DNA molecules and the like, deformation of the stamp shape and damage of the bio receptor occur and affect the diagnostic result.

Research

In OIST, researchers have developed a micro/nano printing technology as a new procedure to create a pattern of a bio-receptor in a microfluidic device. As a procedure, first, a water-soluble ink (APTES: silicon and oxygen compound) that captures the bio-receptor is transferred to the substrate, and then the bio-receptor is chemically linked to the pattern of APTES molecules formed. By using this technique, it is possible to avoid problems such as reduction in the activity of bio-receptor molecules in nanoscale patterning, and to produce a high-resolution bioassay (bioassay) system with long shelf-life at high speed and at low cost. In addition, one stamp allows to produce a substrate on which different types of bio-receptor molecules are arranged and this feature is expected to contribute to diagnosing complex diseases such as cancer that relies on tests by many biomarkers at the same time. (Published in "Analyst" issued by the Royal Institute of Chemistry)



A micro/nano printing technology:
(i) Applying the APTES solution to the stamp surface, (ii) pressing the stamp surface to transfer the APTES molecules onto the glass substrate, (iii) combining the microfluidic structure with the patterned glass surface, (iv) attaching bioreceptors to the pattern of the APTES molecules in each flow path, and then a sensor element such as a test kit is completed.

Keywords

Bioassay, Surface patterning, Microcontact printer, Nanocontact printer

Applications/Future development

- Nanoscale DNA assays, protein assays
- Development of a microfluidic integrated device by combined with a business-use microcontact printer etc.
- Low volume applications
- Establishment of point-of-care diagnostic method

Opportunity for joint research and technology transfer

- Licensing to diagnostic medical equipment makers etc.

Patent protection

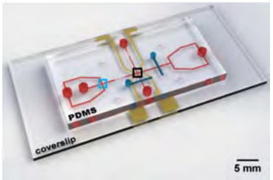
PCT/JP2017/003621「MICRO- AND NANOCONTACT PRINTING WITH AMINOSILANES: PATTERNING SURFACES OF MICROFLUIDIC DEVICES FOR MULTI- PLEXED BIOASSAYS」 (US : provisional 62/290,067)

Introduction of the research unit

Micro/Bio/Nanofluidics Unit

Unit leader : Amy Shen Professor

The Micro/Bio/Nanofluidics Unit focusses on using complex fluids and complex flows to create objects with morphology and structure tailored precisely for applications in biotechnology, nanotechnology, and energy. The unit employs lab-on-a-chip platforms with analytical capacity to study the physics of flow, the transport of mass, momentum, and energy, and reactive processes at nano- and micron length scales. Novel device designs have the potential to significantly enhance understanding of single-cell behavior, development biology, and neuroscience. These strategies can be used to address challenges in drug screening and the development of bio- and chemical-sensors for disease, security, and environmental monitoring.



Microfluidic device : performing mixed separation and the chemical reaction of various solutions in the flow path.

<Related research theme>

Revealing polymeric fluids behavior at the microscopic scale

Researchers do not yet fully understand how micro fluids – particularly complex ones of biological origins - behave at micro scales in microchannels. Although the time (relaxation time) for the polymer in a dilute solution to return to its original shape after it being deformed by the flow is an important physical quantity characterizing the molecular motion of the polymer in the solution, current techniques for the relaxation time measurement of the small amount and the dilute solution were insufficient for it. By designing and developing a new microfluidic device, it became possible to measure the relaxation time of such a solution and characterize the dilute polymeric fluids. (Published in "Journal of Rheology" published by the American Physical Society)

Research unit website : https://groups.oist.jp/mbnu

Okinawa Marine Science Center

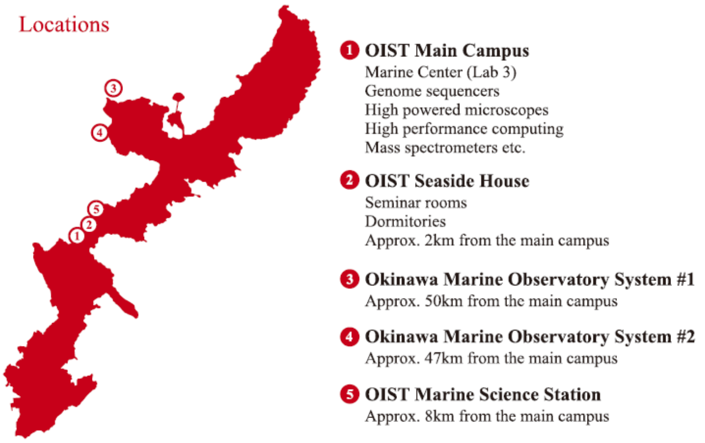
Revealing the effects of the ocean currents on marine organisms which live in hydrothermal vents and coral reefs around Okinawa

Research background

The Okinawa Marine Science Center (OMSC) is a Research Confederation hosted by OIST. It is comprised of members (OMSC members) consisting of researchers and students who come from universities and research institutions in Okinawa. OMSC members will benefit from having access to the outstanding array of research facilities and equipment available at OIST.

Goal of OMSC

In Okinawa there are unique marine environments such as coral reefs, deep-sea hydrothermal vents, the Black Current (Kuroshio), and mangroves. The goal of OMSC is to establish a Center of Excellence in Marine Science by taking advantage of the world-class research resources (facility, equipment and human resources) at OIST.


Locations

❶ OIST Main Campus
Marine Center (Lab 3)
Genome sequencers
High powered microscopes
High performance computing
Mass spectrometers etc.

❷ OIST Seaside House
Seminar rooms
Dormitories
Approx. 2km from the main campus

❸ Okinawa Marine Observatory System #1
Approx. 50km from the main campus

❹ Okinawa Marine Observatory System #2
Approx. 47km from the main campus

❺ OIST Marine Science Station
Approx. 8km from the main campus

OIST Marine Science Station (Marine station)

The onshore marine facility at Seragaki Port, located near Onna-son campus of OIST, will accommodate researchers who need to collect marine species locally and to maintain them alive for prolonged periods. This facility will serve both OIST and non-OIST scientists, enabling controlled experiments on coral responses to changing physical, biological and chemical conditions.

Okinawa Marine Observatory System

To enable real-time and continuous monitoring of Okinawan coral reef ecosystems, two cable-type ocean-observing systems have been installed. Physical, biological, and chemical variables are monitored and underwater cameras designed for plankton monitoring have been installed to capture temporal variations. Water samples are collected several times every month and stored in order to determine the accuracy of underwater sensors and process samples in the laboratory. Collaborators are permitted to bring their own sensors and OIST will connect them and carry out maintenance. Data storage is available, thereby allowing OIST to be an excellent testing site for ocean observing instruments.

Research

1) Investigation on the effects of global warming and ocean acidification to coral reefs ecosystems
2) Exploring the origin of life on Earth by studying deep-sea creatures living in hydrothermal vents

Current projects

Development of a deep-sea observation system enabling continuous real-time monitoring of coral reefs near Okinawa

Investigation of the hydrothermal vent areas using the research vessels of private companies and other research institutions

Kevwords
Real-time marine environment forecasting system, Okinawa coral reef,
Population variation of marine ecosystem

Applications/Future development

- Design of highly effective plans for protecting native species living around the hydrothermal vents at seabed-resource development (deep-sea drilling work that retrieves metals from the ocean floor).
- Greater efforts to protect the ecosystem from the impact of seabed-resource development with offshore drilling

Opportunity for joint research and technology transfer

- Domestic and foreign researchers can participate in OMSC as a technology core of marine research.
- Professional advice and collaborative research on the investigation of Okinawa's unique marine environment is available.

Introduction of the research unit

Marine Biophysics Unit

Unit leader : Satoshi Mitarai Assistant professor

In the Marine Biophysics Unit, researchers are investigating the effects of the ocean currents on marine organisms which live in hydrothermal vents and coral reefs around Okinawa. Using a drift buoy, population genetics, computer modeling, a wave power driven remote control probe, and a physical ocean observation method, this unit is mapping the Kuroshio Current, tracking the distribution of larvae of deep-sea creatures, searching for starfish sources that damage corals, and monitoring the occurrence of plankton.

<Related research theme>

Quantification of larva dispersal of deep-sea organisms living in hydrothermal vent areas

Deep below the ocean’s surface, there are hydrothermal vent fields, or submarine hot springs that can reach temperatures of up to 400 °C. There are inhabitants specific to hydrothermal venting zones such as Gandalfus yunohana and Rimicaris kairei. They survive using various chemical substances emitted from the hydrothermal vents. Recently, OIST researchers and collaborators have computed the dispersal of larvae from these hydrothermal vent ecosystems to understand and safeguard the animals found there. (The results are reported in the Academy Bulletin of the United States (PNAS))

Research unit website : https://groups.oist.jp/mbu

Decoding Whole Genome of Okinawa Mozuku

Aimed at mozuku seaweed cultivation and its industrial use

Research background

99% or more of domestically cultivated mozuku is produced in Okinawa Prefecture and the 90% or more of it is Okinawa mozuku. In Okinawa, mass-farming of mozuku was successfully achieved and has become one of their most important aquatic products. In 2006, the production volume of Okinawa mozuku was about 20,000 tons annually, and the market size based on the shipping price reached about 5 billion yen. However, in 2010 the production volume fell below 10,000 tons, after that it has been changing while fluctuating between 10,000 tons and 20,000 tons. It is supposed that the main cause of the fluctuation is due to the effects of weather such as insufficient sunshine in winter. It is an urgent task to stabilize the production of Okinawa mozuku.

Mozuku farmed in the sea
(Izena island)

Moreover, brown algae including Okinawa mozuku constitute a seaweed bed which is a living place for marine creatures, and it is the most important element of the coastal ecosystem. Getting to know more about brown alga containing mozuku leads also to protecting the diversity of the creatures living in seaweed beds.

Research

OIST, the Okinawa Prefectural Fisheries and Ocean Technology Center (Itoman-shi, Okinawa) and other institutions succeeded for the first time in decoding the whole genome (all genetic information) of Okinawawa mozuku, which is actively cultivated in Okinawa.

Okinawa mozuku with vinegar

A genome, in which all genetic information is packed, is indispensable for understanding the living thing. However, genomic information on algae including brown algae such as Okinawa mozuku is only slightly decoded. Therefore, as a first step, the research team worked on the genomic analysis of Okinawa mozuku, which revealed that the genome of Okinawa mozuku is smaller than that of other brown algae and has fewer genes. In addition, the research team has revealed that a part of the genes considered to be involved in the synthesis of polysaccharide "fucoidan", which is contained much in the unique slime of brown algae and is also reported for health functionality, is fused to the genes of Okinawa mozuku.

The biggest challenge at Okinawa’s mozuku farming sites is to stabilize production, and the instability in production is thought to be mostly caused by environmental factors. In the Okinawa Prefectural Fisheries and Ocean Technology Center which is a joint research partner of this research, from this point forward, researchers are planning to work on hybrid technology of mozuku and develop varieties that can cope with various environments. All the genomic information on mozuku decoded this time is expected to be effective and helpful to objectively judge the hybrid strains.

Keywords

Okinawan mozuku, Genome decoding, Bioinformatics, Brown algae, Fucoidan

Applications/Future development

- Hybrid technology development of mozuku
- Cultivation technology development for mozuku seaweeds
- Development and improvement of new varieties by selection of strains containing many functional ingredients such as "fucoidan"
- Elucidation of the evolution process of whole brown algae

Opportunity for joint research and technology transfer

- Using genome information, it is expected to improve added value to mozuku seaweeds and in order to achieve this, joint research with private companies will be required.
- As mentioned above, researchers are planning to develop varieties for stable mozuku production and expect a transfer of this technology in the future.

Introduction of the research unit

Marine Genomics Unit

Unit leader : Noriyuki Sato Professor

Exploring the genomes of marine organisms will help in clarifying their large-scale evolutional strategy and relationships among organisms in ecosystems. The Marine Genomics Unit has a technology to search large genomes at high speed and became the first laboratory in the world to decode gene sequences of coral, zooxanthellae, and molluscs. Moreover, in this unit, the research team has discovered evidence that there exists a common ancestor connecting humans and starfish.

<Related research theme>

Finding the Dicyemida's proper position on the animal phylogenetic tree

By utilizing the next generation type sequencer, the researchers revealed that Dicyemida is a member of the spiral animal group (spiral cleavage animal) and was born from their common ancestor, and also that it is the closest relation to Orthonectida which is another marine parasite phylum within the Spiralia clade. (This paper is published in Zoological Letters)

Genome sequencing of crown-of-thorns starfish (Acanthaster planci)

In collaboration with Australian researchers, using crown-of-thorns starfish collected at two places, Okinawa and the Great Barrier Reef, Australia, the genome of crown-of-thorns starfish which is a predator of corals was sequenced, and the candidate proteins that seem to be used by crown-of-thorns starfish for communication specific to species were successfully identified. (The results of this research are published in the science magazine "Nature" in the UK)

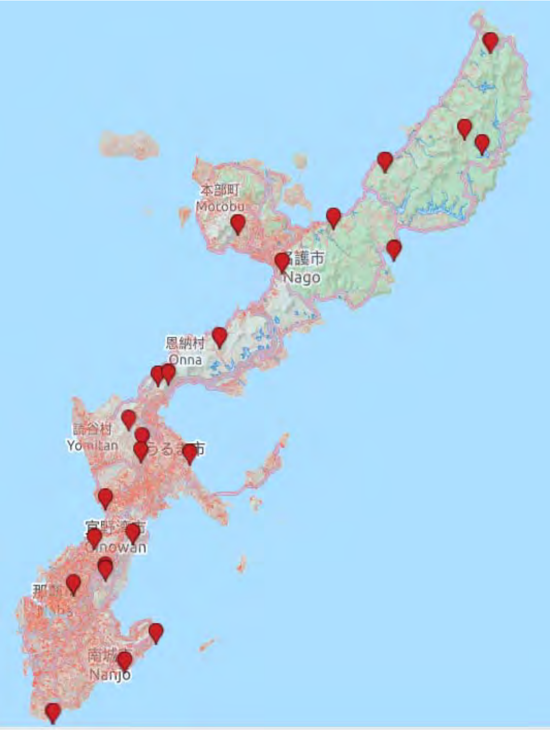
Research unit website : https://groups.oist.jp/mgu

OKinawa Environmental Observation Network/OKEON

Monitoring the environmental changes of Okinawa and revealing their impact

Research background

It is a globally important issue to monitor the environmental changes such as climate change, the devastation of habitats of animals and plants, intrusion and spread of invasive species, and then to understand the impact on the future. OIST has established the OKinawa Environmental Observation Network (OKEON), and is working on building the Okinawa terrestrial environmental monitoring system and a collaborative user network involving researchers at OIST, collaborators of various institutions, museums and high schools across Okinawa, and the local governments etc., under "OKEON Churaumi Project".



Locations for investigation:
24 monitoring sites covering the north to the south of the main island collect arthropods, record weather data, and monitor the distributional and appearance of mammals and birds throughout the year.

Research

In OKEON Churaumi Project, in order to understand how natural and anthropogenic factors affect the present and future of the natural environment of Okinawa, at the monitoring sites and other research sites spanned across the main island of Okinawa, the project members are conducting research utilizing the latest technology such as a gene analysis system, geographic information system, and database system that integrates and shares this information.

- **Arthropod Sampling** : Three flying insect traps (SLAM traps) are installed at 24 monitoring sites throughout the island and collected all year round. The current natural environment and its changes are analyzed and tracked using insects by studying insect faunas, their seasonal changes and annual changes at each investigated site.
- **Acoustic Monitoring** : By recording sound in the environment, the seasonal changes of the spread and activity of given species such as birds are found.
- **Ecological Genomics** : A new high-throughput technology is under development to analyze the genes of large numbers of samples collected. By using this, the biodiversity of Okinawa can be deeply analyzed to the gene level.
- **Camera Trapping** : Camera traps record the spread and activity of mammals and other animals. They provide the basic data for understanding the impact of the invasive species on the natural environment in central and south.
- **Physical Parameters** : Wind speed in a plot, wind direction, rainfall, air temperature, humidity, soil temperature, soil moisture, solar insolation, etc. are recorded all year round. The weather change in a plot is recorded thereby more precisely, and used for investigation of the seasonal change of a biological activity, etc.
- **GIS** : The current situation of land use and vegetation inside the main island of Okinawa are assesed. Furthermore, by tracking the transition from the past to the present, the temporal change of the terrestrial environment is clearly shown. It also helps predict environmental changes by simulation using this data.

Keywords

Environment monitoring, Gene analysis, GIS, Weather observation

Applications/Future development

Planning and launching of various projects using OKEON are advancing, and monitoring of fire ants has started as the second project, under the cooperation of Okinawa Prefecture and Incorporated Foundation Okinawa Environmental Science Center.

Opportunity for joint research and technology transfer

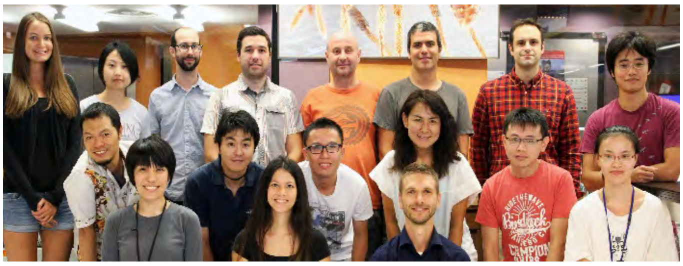
OKEON will contribute to the realization of a next-generation environmental symbiotic society through cooperation in a wide range from research to natural environment education with local universities, high schools, museums, and governments etc.

Introduction of the research unit

Biodiversity and Biocomplexity Unit

Unit leader : Evan Economo Assistant Professor

The Biodiversity and Biocomplexity Unit explores how ecological and evolutionary processes generate and sustain biodiversity. The unit integrates theoretical, field, and lab approaches to investigate how species evolve, move around, and adapt to their environments. Projects focus on the dynamics of ant communities in the Pacific islands, global diversity patterns of all ants, and macroevolution of the "hyperdiverse" ant genus *Pheidole*.



Research unit website : https://groups.oist.jp/bbu

Ecology and Evolution Unit

Unit leader : Alexander Mikheyev Associate Professor

Evolution is the unifying principle of life sciences. Recent technological advances have revolutionized the way it is studied, providing new insights into historical questions. The Ecology and Evolution Unit utilizes cutting-edge technology to address a wide range of research questions. The Unit's investigations have included coevolution of mutualists, landscape genetics of adaptation by herbivores to host plants, genomic changes in little fire ant castes that influence invasiveness, coevolution of leaf-cutting ants and their cultivated fungi, and proteomics of pit viper venoms. Future projects will employ massive sequencing of environmental samples and museum collections to link major themes in ecology and evolution.

Research unit website : https://groups.oist.jp/evolution