5. Young Faculty Members Support Program for New IROAST Major Research Area

No.	Name	Project Title
5-1	**Yusuke Inomata**	Development of Alkaline Copper Halide Based Highly Luminescent Circularly Polarized Luminescent Materials
5-2	**Ryo Nozu**	Comparative genomics reveals the mechanism of gonadal sex change in fishes
5-3	**Yoshihiro Sekine**	Development of Diamond Spin-Qubit Based on Graphene Oxide
5-4	**Ahmad Muhammad Sohail**	Selective hydrogen separation membranes based on two-dimensional carbon nanosheets: Toward a stable supply of hydrogen energy
5-5	**Yi-Lun Tsai**	Soil chemotactic signal perception and response of plant-parasitic nematodes
5-6	**Akira Ueda**	Development of novel highly processable super/high conducting materials composed of purely organic small molecules

No.5-1	Development of Alkaline Copper Halide Based Highly Luminescent Circularly Polarized Luminescent Materials		
Name	Yusuke Inomata		
Affiliation Contact	Faculty of Advanced Science and Technology Email: inomata@kumamoto-u.ac.jp	Title	Assistant Professor
Research Field	Novel Functional Materials		

1. Overview and achievements

Materials showing circularly polarized luminescence (CPL) has attracted much attention owning to their application to light source of liquid crystal display, dye materials for 3D display and security painting. Current CPL materials are organic, supramolecules and organic-inorganic hybrid -based materials. The CPL properties can be finely controlled and they show high luminescence. However, the drawbacks are their chemical stability and complicated synthetic process. Alkali copper halides are inorganic luminescent materials with high quantum yield. The salts are soluble to polar solvents and they can be easily crystalized from water or other polar solvents. In addition to the luminescent properties, alkali copper halides tend to possess chiral crystal structure such as $CsCuCl_3$ (Space groups: $P6_122$ or $P6_522$), $CsCuBr_3$ (Space group: $C222_1$), Cs_2CuBr_3 (Space group: $P2_12_12_1$). In this work, we try to enantioselectivity crystalize the alkali copper halides and their luminescent properties are investigated.

Among the alkali copper halides, we synthesized $CsCuCl_3$ that has right-handed ($P6_122$) and left-handed crystal structures. $CsCuCl_3$ was crystalized from water using $CuCl_2$ and CsCl as precursors and chiral organic acids as chiral additives. Red-brown single crystals were obtained and single crystal XRD measurements were conducted to determine the absolute structures. We found that right-handed and left-handed crystals were selectively synthesized depending on the chirality (L or D) of organic acids (***Figure 1***). Cu(I) is known as a luminescent center of alkali copper halides. Therefore, $CsCuCl_3$ was partially reduced by $NaBH_4$ to reduce Cu(II) to Cu(I). The reduced $CsCuCl_3$ powders showed bright green luminescence. We further analyze the CPL properties of the partially reduced $CsCuCl_3$ and increase the stability.

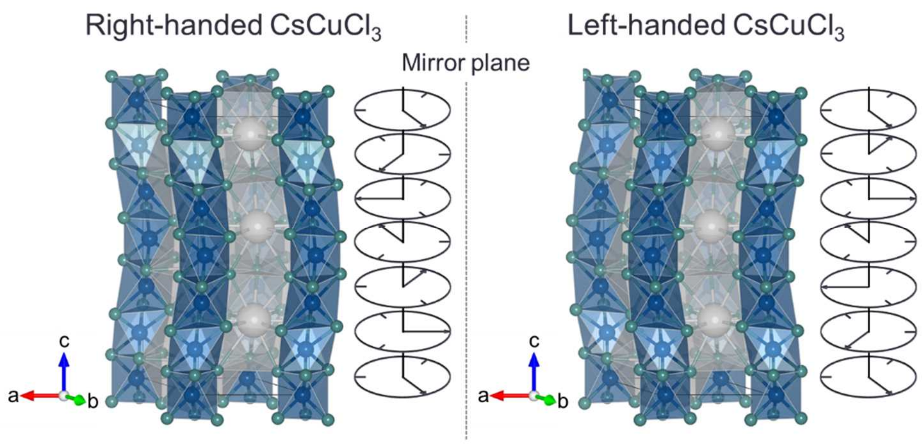


Figure 1. Crystal structures of right-handed ($P6_122$) and left-handed ($P6_222$) $CsCuCl_3$.

2. Prospect for further research collaboration with other university/institution

N/A

3. Application plan of KAKENHI and other external grants

・Grant-in-Aid for Early-Career Scientists

・LOTTE foundation research grant

No.5-2	Comparative genomics reveals the mechanism of gonadal sex change in fishes		
Name	Ryo Nozu		
Affiliation Contact	Faculty of Advanced Sciences and Technology Email: rnozu@kumamoto-u.ac.jp	Title	Project Assistant Professor
Research Field	Fisheries biology		

1. Overview and achievements

It is well known that some teleost fish can functionally change sex (i.e., switch gametogenesis) during their lives. These fish are referred to as sequential hermaphroditic and/or sex-changing fish. Interestingly, recent studies have shown that not only sex-changing fish, but also gonads of gonochoristic fish (i.e., determined sex does not change) are potentially capable of functional sex-change throughout their lifetime. However, in gonochoristic fish, sex-change is considered to never occur under the natural environment (condition). Thus, it is still unknown what is responsible for the expression or not of sex-change ability in fish. Here, I thought that a genome-wide comparison between sex-changing and gonochoristic fish would be a useful approach to reveal this question.

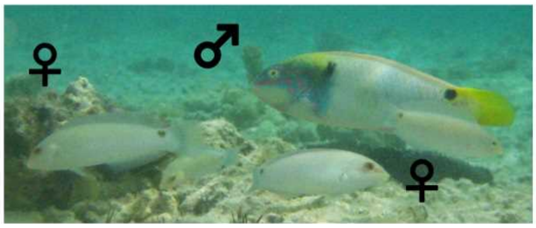


Fig. 1. Three-spot wrasse (*H. trimaculatus*) is a protogynous species. Females change sex to males under certain environmental condition.

The goal of this research is to determine the genome sequence of the protogynous wrasse (three-spot wrasse, *Halichoeres trimaculatus*, (Fig. 1) and compare it to that of a gonochoristic fish in order to understand the mechanism that enable the expression of sex-change ability. I have two specific approaches for genome-wide comparisons. (i) Search for genes specific to sex-changing and/or gonochoristic fish, and (ii) Comprehensive analysis of transcriptional regulatory regions of sex-related genes common to both sex-changing and gonochoristic fish. In this year, I focused on assembling the draft genome of protogynous three-spot wrasse.

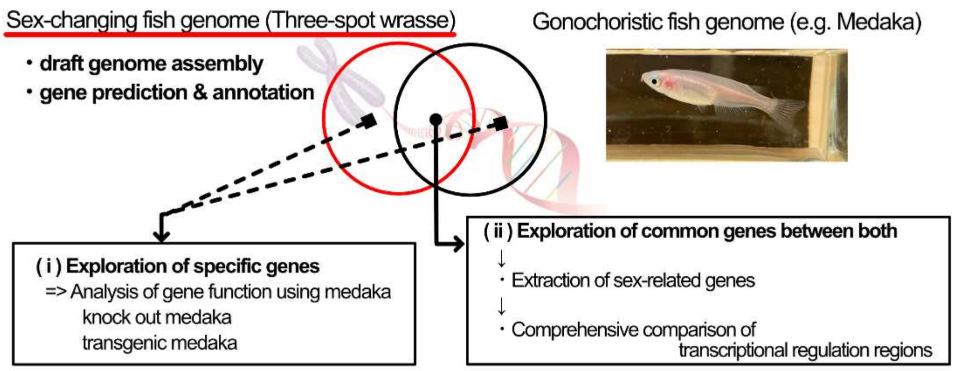


Fig. 2. Schematic of research strategy.

Using a NucleoBond AXG column, long genomic DNA with a peak of >30 Kb was extracted from muscle tissue of an adult female (Fig. 3). This was used to acquire high precision long read

(HiFi) data for one SMRT cell by Sequel II sequencer (PacBio). About 32 Gb of HiFi data consisting of about 1.9 million DNA sequences (average length 17 Kb) were assembled by the hifiasm program, which converged to 108 contigs. These contigs showed a maximum length of 43 Mb, N50 length of 35 Mb and total base length of approximately 850 Mb (Table 1). Evaluation of the completeness of the draft genome assembly using the BUSCO program showed a detection rate of more than 98% for highly conserved orthologs (of 3,354 genes) across vertebrata, resulting in a highly comprehensive and continuous assembly of the whole genome sequence for this species.

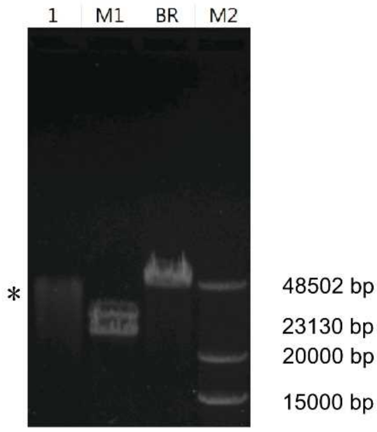


Fig. 3. Quality check of extracted genomic DNA by pulsed-field gel electrophoresis. Lane 1: sample; BR, M1 & M2: DNA markers. * indicates a band of the sample.

Table 1. The stats of draft genome assembly of the three-spot wrasse.

	Stats
Number of contigs	108
Max length (bp)	43,064,949
N50	35,407,678
Total length (bp)	849,850,474

2. Significance of the research and progress status

To investigate the mechanism of gonadal sex change using fish as a model, and the findings will provide new fundamental information on sexual plasticity not only in fish but also in vertebrates. The genome sequence of the protogynous fish will provide important molecular information for the future sex change and sexual plasticity research area.

Using the Hi-C analysis, I plan to perform chromosome-level scaffolding in the future to complete the whole genome information with a high degree of completeness. In addition, gene prediction and annotation will be performed and then, genome-wide comparisons will be conducted with gonochoristic fish.

3. Prospect for further research collaboration with other university/institution

This research is already underway in collaboration with Professor Shigehiro Kuraku (RIKEN BDR, National Institute of Genetics), who has expertise in genomic DNA extraction methods and advanced information analysis techniques. I plan to continue collaboration with RIKEN BDR, which has extensive experience in chromosome-level scaffolding using Hi-C data.

4. Application plan of KAKENHI and other external grants.

I am planning to apply for the FY2023 Grant-in-Aid for Scientific Research (C) on the theme of brushing up the sex-changing fish genome information developed in the present project and elucidating the sex-changing mechanism using a genome-wide approach.

No.5-3	Development of Diamond Spin-Qubit Based on Graphene Oxide		
Name	Yoshihiro Sekine		
Affiliation Contact	Priority Organization for Innovation and Excellence Email: sekine@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Material Science		

1. Overview and achievements

What brings about the creation of innovation in science and technology is basic and applied research that brings about new scientific and technological concepts. Carbon materials are attracting attention as materials that bring innovation in various fields, and diamond, in particular, is expected to have various functionalities due to the characteristics of doping and surface modification of the sp^3 carbon. These functionalities of diamond can be combined to create even better materials, and thus have great potential as materials that bring about innovation. As innovation in energy devices and catalysts as truly useful materials is eagerly awaited, there is a growing interest in research on the functional exploration of diamond and the development of materials.

The defects (NV centers) in nitrogen-doped diamond, also known as spin-Qubit, have great potential in quantum computation. The key to functional diamond synthesis is how to synthesize carbon materials containing reasonably hetero-doped elements, but the suitably synthetic strategies are not well established.

In this research, I have investigated the preparation of the suitable candidate of the spin-qubit materials based on the doped diamond. The doped diamond could be prepared by following strategies; A) the preparation of the doped graphene oxide and their reduction materials, B) High-temperature and pressure method yielded in the diamond.

A) This study focused on the preparation of the diamond by graphene oxide through high-temperature and high-pressure method.(Fig.1a)[1] In addition, another atom doped reduced-graphene oxide was prepared by graphene oxide through chemical doping and thermal reduction.

B) High-temperature and pressure method gave diamond-like materials from doped reduced graphene oxide, which was confirmed by XPS, raman spectra and temperature-dependent magnetic properties measurements. As a result, we have succeeded in synthesizing doped diamond-like materials. Next step is to investigate and characterize their relaxation times for evaluating spin-qubit functionalities.

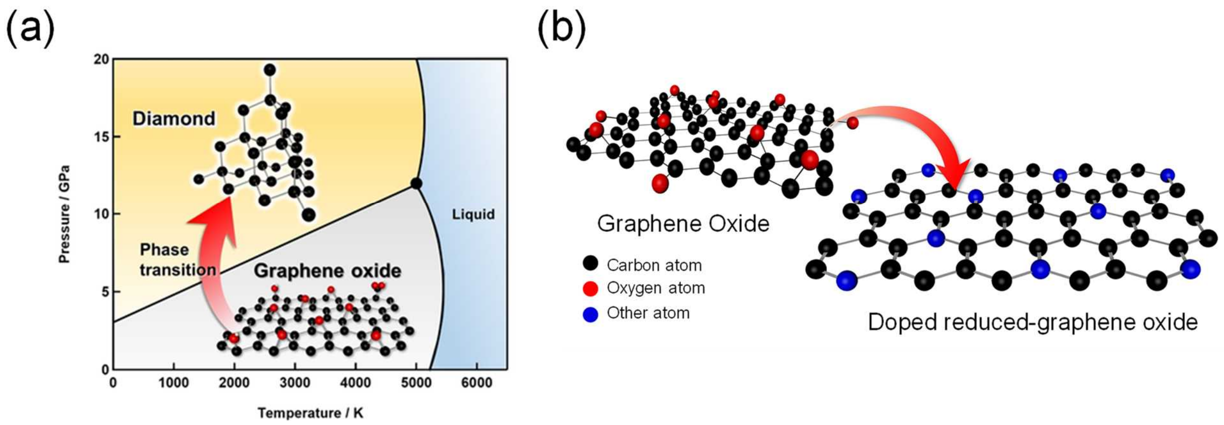


Fig1. (a) Phase diagram of carbon materials. (b) Schematic illustration of transformation from graphene oxide to doped reduced graphene oxide.

[1] M. Fukuda, Md. S. Islam, Y. Sekine, T. Shinmei, L. F. Lindoy, S. Hayami, *ChemistrySelect*, 6(14), 3399-3402 (2021).

2. Significance of the research and progress status

The significance of this research is to systematically prepare doped diamond materials (Fig. 2). The doped diamonds have potential to exhibit spin-qubit, but there is difficulty that how to prepare it and control the number and amount of dopant. This research could provide systematically controlled doping ratio and atoms, through systematic synthesis of doped graphene oxide. This research has potential to investigate the functionality of spin qubit and doping information of doped reduced graphene oxide and doped diamond materials.

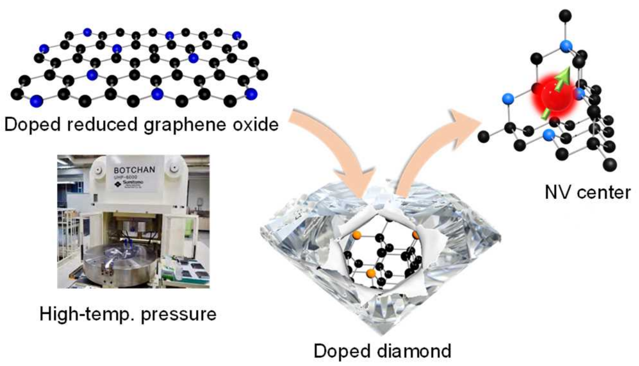


Fig. 2 The preparation scheme of NV center in doped diamond from doped reduced graphene oxide.

3. Prospect for further research collaboration with other university/institution

From this research, I have succeeded in the preparation of the target materials consisting of total isolated spin unit. The preparation of doped diamond materials from reduced graphene oxide were conducted with Dr. T. Shinmei in Ehime university. Now we are collaborating with Prof. Sato in Osaka city university, who is the specialist of the pulse-ESR measurements. The pulse-ESR measurement is one of the strong tools to investigate their relaxation time of spin.

4. Application plan of KAKENHI and other external grants.

My current Kakenhi will end in the end of March, 2023. Therefore, I have plan to apply the Kakenhi in this year.

No.5-4	Selective hydrogen separation membranes based on two-dimensional carbon nanosheets: Toward a stable supply of hydrogen energy		
Name	Ahmad Muhammad Sohail		
Affiliation Contact	Faculty of Advanced Science and Technology Email: sohail@kumamoto-u.ac.jp	Title	Research Assistant Professor
Research Field	Materials Chemistry and Chemical Engineering		

1. Overview and achievements

Membranes are important in gas separation applications due to their high energy efficiency, low operational costs, and durability. However, the traditional systems for the gas generation/separation technology in the industry are energy-intensive and environmentally unfriendly. In contrast, the fast-growing field of membranes technology offers new strategies for sustainable gas separation, providing modularity, scalability, compactness, and high energy efficiency. In this context, graphene membranes have emerged as promising alternatives for gas separation application due to their atomic thickness enabling ultrahigh presence but they suffer from low gas selectivity. In this direction, I aimed to develop such graphene-based membrane that has high permeability and selectivity toward hydrogen separations from mixed gases.

Currently, Hydrogen (H_2) energy is considered one of the preeminent alternatives to fossil fuels due to its natural abundance, zero pollutant transpiration, and high energy capacity. In the H_2 production process, specifically, methane reforming reaction, there are several byproducts e.g., N_2, CO, CO_2, and CH_4 which cause detrimental influences on the energy contents and usage of H_2. However, the tradeoff between permeability and selectivity is a crucial challenge, which is also difficult to adjust during the separation process.

In FY2021, I focused on the controlled synthesis of graphene oxide, which should have both electron (sp2 domain of graphene oxide) and proton conductivity (sp3 domain of graphene oxide). The H_2 permeation mechanism in mixed conducting graphene oxide membrane can be seen in the figure right side.

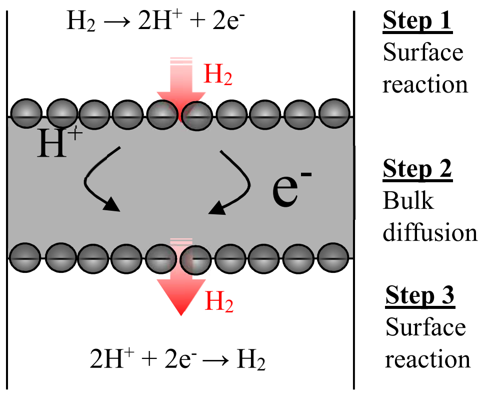
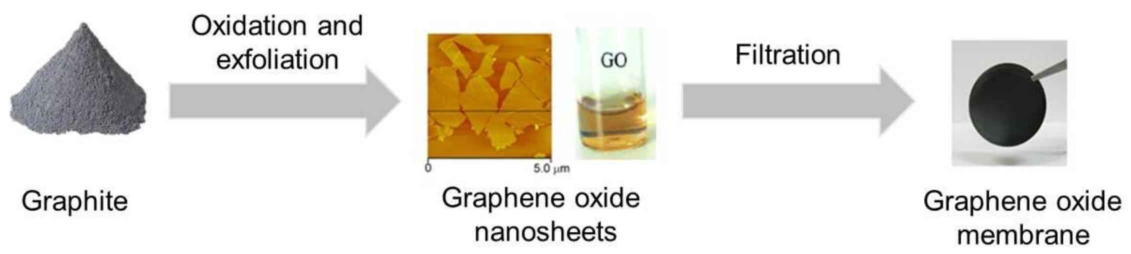


Fig. H_2 permeation mechanism in GO.

2. Significance of the research and progress status

Production, purification, storage, and transport of hydrogen gas are essential technologies to use H_2 as energy effectively. H_2 permeable membranes are important materials for separating and purifying hydrogen gas from a gas mixture.

In this project, FY 2021, graphene oxide was synthesized by a typical modified Tour's method (oxidation and exfoliation of the graphite). Further, the thin-film membranes have been developed with high electron and proton conductivity by controlling the physical properties such as surface oxygen functional groups. Finally, a free-standing membrane is readily obtained by stacking the nanosheets via filtration (Figure below).



Graphene oxide has the potential to replace conventional technologies for hydrogen separations. The figure on the right side shows the results, indicating that hydrogen selectively permeated via graphene oxide membrane while helium was not permeated. These results encourage us to move forward with this study.

This technology, which can purify the hydrogen selectively using graphene-based membrane will greatly contribute to the realization of a low carbon society.

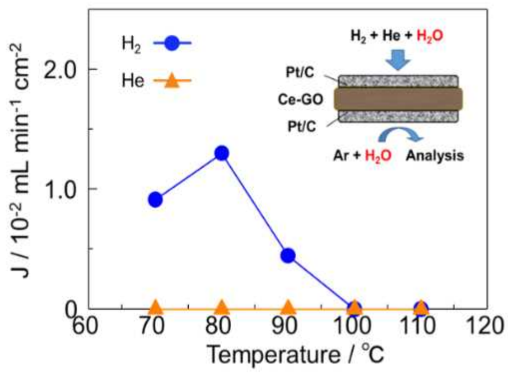


Fig. Preliminary results.

<u>FY2022, the below experiments are planned</u>

- ✓Characterization of the graphene oxide membrane
- ✓Further, enhance the permeability of the hydrogen gas
- ✓Submission of the manuscript.

3. Prospect for further research collaboration with other university/institution

The following Collaborative research is planned in FY2022

- ✓ Graphene oxide for enhanced photoelectrochemcial hydrogen evaluation with King Fahad University of Petroleum and Minerals, Saudi Arabia

4. Application plan of KAKENHI and other external grants.

Kakenhi, Grant-in-Aid for Early-Career Scientists

No.5-5	Soil chemotactic signal perception and response of plant-parasitic nematodes		
Name	Yi-Lun Tsai		
Affiliation Contact	Faculty of Advanced Science & Technology, International Research Center for Agricultural & Environmental Biology Email: tsai-yilun@kumamoto-u.ac.jp	Title	Assistant Professor
Research Field	Plant biology		

1. Overview and achievements

I am interested in exploring the inter-specific interactions between plants and soil organisms, by using the plant-parasitic root-knot nematodes (*Meloidogyne incognita*, RKN) as a model. RKN are soil-borne obligate parasites that infest plant roots and feed on plant cells in order to survive and propagate. During the infestation, RKN converts the plant host's cells into specialized feeding organs known as galls or root knots, by utilizing the host plant's own hormone signaling pathways.

RKN are hatched as free-living juveniles in the soil, and must seek out appropriate host plants to infect as soon as possible. It is now known that RKN juveniles utilize chemotaxis and follow gradients of chemicals secreted by the host plant to find potential hosts to infect. The identities of these RKN chemo-attractants thus play critical roles in regulating RKN infection efficiencies. Despite their importance, currently little is known about the chemical structures of compounds that can act as RKN chemo-attractants.

Previously I have found that RKN juveniles are attracted to seeds of the model plant Arabidopsis (*Arabidopsis thaliana*), in a seed coat mucilage-dependent fashion (Fig. 1)[1]. The seed coat of many flowering plants are known to synthesize a gelatinous substance known as mucilage, which absorbs water and expands when the seeds are wetted. Seed coat mucilage typically consist of cell wall carbohydrates such as hemicellulose and pectin, and this finding suggests that cell wall carbohydrates may be involved in RKN chemotaxis[2]. Indeed, later I discovered that the mucilage from flaxseed (*Linum usitatissimum*) also attracts RKN juveniles. Specifically, the flax mucilage rhamnogalacturonan-I with L-galactose sidechains were found to be essential for RKN attraction[3]. This suggests that RKN juveniles can not only perceive cell wall carbohydrates as chemo-attractants, but can distinguish molecular features such as hydroxyl groups and chiral centers on the attractant molecules as well.

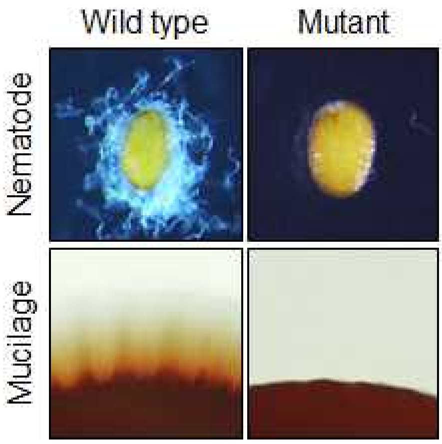


Fig. 1. RKN behavior toward Arabidopsis seeds (top panels), and Ruthenium red-stained mucilage of the corresponding seeds (bottom panels)

2. Significance of the research and progress status

Plant-parasitic nematodes are significant agricultural pests in many parts of the world, including Kyushu, Japan, and are known to cause formidable economic losses annually. Currently effective, long-term and environmentally-friendly strategies to protect crop plants from plant-parasitic nematodes remain lacking. Chemotaxis thus may be a viable approach to control nematode infections in agriculture, as chemo-attractants can be applied in fields to direct nematodes away from vulnerable crop plants[4]. The identification and characterization of RKN chemo-attractants may serve as the foundation to develop novel strategies to reduce RKN infections in agriculture.

Currently, I am interested to expand the characterization of inter-specific communication beyond plants and nematodes. The soil is a highly complex ecosystem that house many micro-organisms aside from nematodes. Many of these microbes preferentially occupy the soil region adjacent to the plant roots, or reside within the roots, in order to take advantages of the metabolites secreted by plants[4]. One important group of such microbes are the rhizobacteria that colonize roots of legume plants and form special organs call nodules, and provide the host plants with organic nitrogen. The rhizobacteria nodules and RKN galls are thematically similar in that both are induced novel organs that house micro-organisms in roots. Yet these organs have very different outcomes as rhizobacteria are functionally symbionts while RKN are considered parasites. I am interested in how these two micro-organisms affect each other when infecting the same host plant. Furthermore, I'd like to investigate how RKN and rhizobacteria root colonization affect the composition of other micro-organisms that inhabit the surface or within plant roots. Currently I am optimizing the experimental conditions using the model legume plant *Lotus japonicus*, which can be infected by both RKN and the compatible rhizobacterium *Mesorhizobium loti* (Fig. 2). In addition, I'm also optimizing the experimental condition to screen for soil microbes that colonize plant roots.

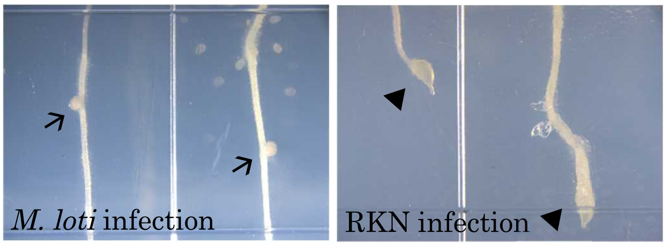


Fig. 2. *L. japonicus* infected with *M. loti* (left panel) and RKN (right panel). Arrows denote *M. loti* nodules, arrowheads denote RKN galls.

3. Prospect for further research collaboration with other university/institution

Currently we are working with Prof. Masayoshi Kawaguchi (National Institute for Basic Biology) on the propagation and handling of *L. japonicus* and *M. loti* experimental systems. In the future, pending on the direction of the research, we may rely on Prof. Kawaguchi to access *L. japonicus* and *M. loti* mutants with compromised root colonization. We also plan to collaborate with Dr. Bruno Favery (The Institut national de la recherche agronomique, France) and Prof. Carolina Escobar Lucas (University of Castilla-La Mancha, Spain) to access other species of plant-parasitic nematodes. Lastly, we are also open to collaboration with experts in microbiome genome sequencing and analysis.

4. Application plan of KAKENHI and other external grants

I am planning to apply for the Kakenhi Grant-in-Aid for Scientific Research (C) of FY2022. I am also open to contribute to joint grant applications where my research interests can coincide with broader topics.

[1]Tsai AY et al. (2019) Molecular Plant 12(1): 99-112
[2]Tsai AY et al. (2021) Plant Cell Physiol. pcab099
[3]Tsai AY et al. (2021) Science Advances 7(27): eabh4182
[4]Tsai AY et al. (2020) Frontiers in Plant Science 11: 1167

No.5-6	Development of novel highly processable super/high conducting materials composed of purely organic small molecules		
Name	Akira Ueda		
Affiliation Contact	Faculty of Advanced Science and Technology Email: aueda@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Chemistry, especially Physical Chemistry and Functional Materials Chemistry		

1. Overview and achievements

The development of superconducting or high-conducting materials is crucially important not only from the viewpoint of fundamental science but also from the viewpoint of practical applications for electronics. In particular, such materials composed of organic molecules are expected to have several unique features different from those of inorganic conductors, such as structural diversity of the component molecules and their aggregates and also high flexibility and processability of the materials themselves. However, this kind of highly processable high-conducting organic materials is limited to polymer-based ones; therefore, in this project, this researcher aims to develop a new type of highly processable super/high conducting materials composed of purely organic “small” molecules, to lead to a breakthrough in this research field and also to open a new possibility to create innovative science and technology for building a well-being society.

In FY2021, on the basis of his original molecular design strategy, this researcher has successfully developed three kinds of new conducting materials composed of purely organic small molecules and characterized them in the crystalline state as the first step. They are found to have unique structures different from those of the conventional organic conductors and to show sufficiently high electrical conductivity, which proves the novelty and originality of the present molecular design. A part of these results was reported in some academic conferences and now the research paper is in preparation (to be published in early 2022).

2. Significance of the research and progress status

The significance of this research is to challenge the establishment of a novel methodology for developing superconducting or high-conducting organic materials. The present practical superconductors are inorganic ones, such as Nb-Ti alloy and cuprates; in which there are some intrinsic problems, such as the rarity of the elements and the difficulty in the processing. On the other hand, organic materials are composed of the common elements and have some advantages against inorganics, such as light weight, flexible and highly processable, and thus are expected as promising candidates for the next-generation superconductors.

As described above, the research in FY2021 has successfully revealed that the molecular design strategy proposed by this researcher is promising to achieve the purpose of this project. The desired superconductivity and high processability are to be realized by further chemical modification in the near future.

3. Prospect for further research collaboration with other university/institution

This researcher has published more than 60 original papers in collaboration with a wide range of researchers in other universities/institutions including overseas ones (a recent example: *npj Quant. Mater.* **6**, 87, (2021)). Device fabrication and specialized physical measurements of the materials developed in this project will be performed in collaboration with them.

4. Application plan of KAKENHI and other external grants.

This researcher has received a JSPS KAKENHI (C) (FY2019–FY2022). On the basis of the above-mentioned research results, he will apply for JSPS KAKENHI (B) and also for PRESTO (Sakigake) and FOREST (Sohatsu) programs.