京都大学教育研究振興財団助成事業
成　果　報　告　書

2019年　5月　31日

公益財団法人京都大学教育研究振興財団
会長　藤　　洋作　様

所属部局　化学研究所

職　　名　助教

氏　　名　Romain Blanc-Mathieu

助成の種類	**30 年度　・　研究活動推進助成**	
申請時の科研費研究課題名	珪藻類の多様化を支える進化メカニズムの解明	
上記以外で助成金を充当した研究内容	Not applicable	
助成金充当に関わる共同研究者	(所属・職名・氏名) Not applicable	
発表学会文献等	(この研究成果を発表した学会・文献等) None	
成果の概要	**研究内容・研究成果・今後の見通しなどについて、簡略に、A4版・和文で作成し、添付して下さい。(タイトルは「成果の概要／報告者名」)**	
会計報告	交付を受けた助成金額	1,000,000 円
	使用した助成金額	1,000,000 円
	返納すべき助成金額	0 円
	助成金の使途内訳	費目 / 金額
		RNA sequencing (March 2019) / 25,332 JPY
		Business trip Fukui (March 2019) / 18,000 JPY
		Business trip + conference Lyon (France) (2-11 July 2019) / 300,000 JPY (estimate)
		Conference Yamanashi (Japan) (10-13 September 2019) / 100,000 JPY (estimate)
		Business Trip + Conference Aussois (France) (6-12 October 2019) / 300,000 JPY (estimate)
		Sequencing of diatoms + diverse (books, etc) / 256,668 JPY (estimate)
当財団の助成について	(今回の助成に対する感想、今後の助成に望むこと等お書き下さい。助成事業の参考にさせていただきます。	

珪藻類の多様化を支える進化メカニズムの解明

Romain Blanc-Mathieu

Summary of research aim and results:

Diatoms are unicellular phytoplankton that contribute to 20% of the global primary production; they have colonized all marine and freshwater habitats and diversified into thousands of species. Investigation of their evolutionary success and prediction of their fate in response to the ongoing global environmental changes is critical to understand the sustainability of our environment. In this perspective we engaged in comparative population genomic of diatoms and their sister group Parmales. The use of Parmales genomic data gives us a strong advantage to infer what features (genes, genome organization, ploidy level, etc) were present in the diatoms-Parmales last common ancestor and which ones have been derived in Diatoms. This can give us hint as to what functional innovation (e.g. urea cycle) or phenotypic characteristics (e.g. ploidy change) may be responsible for the success of diatoms.

As a part of a collaborative work with the Parmales genome group led by Dr. Kuwata, I performed an *in silico* analysis to infer the ploidy level of the reference *Triparma laevis* (Parmales) strain (NIES-2565) whose cells are armed with silicate plates and deprived of flagella. Our results reveal an extremely low amount of segregating genetic variation within the culture of the reference *Triparma laevis* strain. This result itself is somewhat inconclusive (absence of evidence is not evidence of absence) as it is compatible with either Parmales armed cells being haploid or nearly-fully homozygous polyploid. Hence my collaborators performed additional workbench experiments to measure the relative amount of DNA in Parmales cells compared to other species whose genome size and ploidy level is known. Result revealed a DNA amount twice larger than the size of the reference genome assembly (40Mb) of *Triparma laevis*, thereby indicating that these cells are diploid.

This suggests that the ancestor of Diatoms and Parmales was already an organism alternating between a diploid and a haploid stage. Diatoms are known diplontic organisms (i.e. most of the life cycle is diploid). In the case of Parmales some cultures are made of naked flagellated cells (e.g. *Triparma pacifica* and this culture is haploid) and hence may be haplontic (spend most of their life cycle as haploids) while others (e.g. *Triparma laevis*) are made of armed cells and hence are though to be diplontic. Parsimony would suggest that the diatoms ancestor was diplontic but a more carful mapping of the ploidy phase onto the Parmales phylogeny would be required to confirm such a hypothesis. This result will be valued as paragraph in a manuscript in preparation for a peer reviewed journal reporting the genome characteristics of *Triparma laevis* (led by Dr. kuwata).

Four additional *Triparma laevis* strains were isolated from water samples collected by Dr. Kuwata in the sea of Okhotsk (TL4 and TL3) and Oyashio (TL5, TL6, NIES-2565). Total DNA for these strains was sequenced. I used these sequence data to call polymorphisms along the reference genome of NIES25-65. Polymorphisms analysis revealed that among the five strains of *Triparma laevis*, only 2 distinct lineages exist: NIES-2565, TL3, TL6 were genetically identical over the full genome and TL4 and TL5 were identical. This near absence of genetic diversity between strains isolated from different oceanic regions suggests that the *Triparma laevis* is evolving clonally or with very limited gene exchange. This is a rather surprising result that will deserve deeper investigation and would probably be of interest for the plankton scientific community.

In this fiscal year we started to investigate genetic diversity for *Triparma laevis* by using a larger sample size (we already generated sequence data for two additional strains and will add more). Clonal evolution is expected to less efficiently purge deleterious mutations and prevents the spread of beneficial mutations. The dynamic of transposable elements is also known to be affected by the level of recombination in a genome, although the direction of the

effect is still matter of debate. We will therefore investigate the state of these genomic features (deleterious mutations and transposable element dynamic), which are usually associated with clonal evolution, in *Triparma laevis*.
In parallel we will continue our effort to obtain the genome sequence of the diatom *T. nordenskioeldii.* We could not generate axenic strain yet so we decided to use transcript sequencing to have a first insight into to functional potential of this species. RNA extraction and sequencing was performed using the Global Unit grant and was successful. We assembled the sequence data into 26,368 contigs from which 19,904 proteins were predicted. Careful gene content analysis will be performed latter this year and this transcriptomes can be used a reference for future plan population genomics study. We will next engage in a comparative genomics analysis of *T. nordenskioeldii* with other diatom genome sequences to pinpoint interesting genomics features and relate it to its peculiar ecology and evolutionary history.

Planned usage of grant:

The amounts listed below are estimates that may change when booking.

I/ Travel expenses to participate the annual conference of the Japanese Society of Microbial Ecology to be help in Yamanashi from 10 to 13th September 2019. Results obtained for this project will be presented as a talk or poster.

Registration: 10,000 JPY
Train/bus (return): 50,000 JPY
Hotel (4 nights): 40,000 JPY
Total 1: 100,000 JPY

II/ Travel expenses for a trip to Europe to present my results obtained with this grant in a conference and to collaborators from ~23rd September to -11th October 2019 as follow:

1) Participate to the "evolutionary biology meeting in Marseille" (France) from 24 to 27 Septembre:
Registration: 55,000 JPY
Hotel (4 nights): 40,000 JPY
2) Meet with Dr. Gwenael Piganeau in Banyuls-sur-Mer (France) to discuss my results and potential collaboration on the population genomics of Parmales and Diatoms algae from 28 September to 5th October.
Train from Marseille to Banyuls-sur-Mer: 7,000 JPY
Hotel (7 nights): 70,000 JPY
3) Participate to a collaborative meeting of the Tara Oceans project to be held in Barcelona (Spain) between 6th to 10th October (exact dates are not fixed yet) to meet collaborators working on Diatoms and related algae and discuss my results.
Train or Bus from Banyuls-sur-Mer to Barcelone: 6,000 JPY
Hotel (5 nights): 50,000 JPY

Flight from Marseille to Osaka and from Barcelone to Osaka: 150,000 JPY.
Daily allowance for a total of 16 days: 40,000 JPY

Total 2: 418,000 JPY

Total for travel expenses: 518,000 JPY

Usage of the rest of the grants will be used to buy consumable and equipment.