1版

様　式　C－１９、F－１９－１、Z－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　６ 月 １７ 日現在

機関番号：３８００５
研究種目：研究活動スタート支援
研究期間：2020～2021
課題番号：２０K２２６７２
研究課題名（和文）The evolutionary origin of symbiotic mosaics in mealybugs

研究課題名（英文）The evolutionary origin of symbiotic mosaics in mealybugs

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交付決定額（研究期間全体）：（直接経費）　2,200,000円

研究成果の概要（和文）：研究対象であるコナカイガラムシについて、15以上のメタゲノムシークエンスを行った。そのメタゲノムから共生細菌のゲノムを抽出し比較ゲノミクスおよび系統発生学の手法を用いて解析を行った。宿主昆虫のドラフトゲノム（全ゲノムの概要）からは、宿主の特異的な代謝経路と目的の遺伝子に着目して解析をおこなった。共生細菌が宿主内のどこに存在するかを共焦点顕微鏡観察により特定した。本プロジェクトによりコナカイガラムシ科における共生の進化を完全に再構築することが可能となった

研究成果の学術的意義や社会的意義
Two publications (one in Current Biology and one in Molecular Biology and Evolution) were published and two more are in preparation. The results are significant for our understanding of the evolution of complex symbioses such as mitochondria during the origin of the eukaryotic cell.

研究成果の概要（英文）：Over 15 metagenomes of mealybugs were sequenced, genomes of symbiotic bacteria were extracted, and analyzed with comparative genomics and phylogenomics methods. Draft genomes of the insect hosts were analyzed for specific pathways and genes of interest. Confocal microscopy was used to localize the symbionts. The project allowed us to fully reconstruct the evolution of symbioses within Pseudococcidae.

研究分野：evolutionary cell biology

キーワード：symbiosis　evolution　cell　mealybugs　insects　bacteria　genomics　microscopy

１．研究開始当初の背景

Although most sap-feeding insects house symbiotic bacteria inside their cells, two of the most extreme examples of highly mosaic and organelle-like insect symbioses are strikingly found in mealybugs (Coccoidea: Pseudococidae). First, all Pseudococcinae mealybugs harbor a tripartite nested (bacterium-within-bacterium-within-insect cell) symbiotic arrangement functioning as an interdependent metabolic patchwork with the innermost bacterium being repeatedly replaced. Second, one lineage of Phenacoccinae mealybugs harbors a 'symbiotic collage' where two unrelated bacteria (and their genomes) likely merged into a single organism.

Why do these incredibly mosaic ('crazy') symbioses become fixed in mealybugs more often than in other sap-feeding insects? To answer this question, one has to first establish the starting point of these symbioses, i.e. to sample deep-branching lineages of mealybugs (Phenacoccinae and Rhizoecinae). Unfortunately, these mealybugs have been so far mostly neglected due to their relatively 'standard' single-symbiont arrangement, so there is only a single symbiont genome (and no host genome) available for them. Proper sampling of these phylogenetically important lineages is therefore crucial to tackle the evolutionary and cellular mechanisms behind the symbiotic mosaics in mealybugs.

２．研究の目的

The purpose of the project was therefore to investigate highly mosaic symbiotic systems of mealybugs (Coccoidea: Pseudococcidae) with diverse sequencing and imaging data. These data can then be combined to infer the most likely scenarios that lead to the origin of mosaic mealybug symbioses and reveal potential implications for the origin of cellular organelles.

３．研究の方法

Insect samples available in our unit's collections were used for DNA extractions using the MasterPure DNA kit (Lucigene) and PCR-free DNA library preparations were prepared with the NEBNext Ultra II kit for Illumina (NEB) and Oxford Nanopore 1D Ligation kits. Libraries were barcoded and sequenced on the NovaSeq6000 (Illumina) and MinION (Oxford Nanopore) sequencers. Genome assemblies were binned with a custom pipeline developed in my group specifically for insect-symbiont metagenomes (unpublished). Genomes of obligate symbionts (Bacteroidetes, Gammaproteobacteria, Betaproteobacteria) were closed into circular-mapping molecules, annotated, and used for comparative genomics, metabolic pathway reconstructions, and phylogenomics. Pseudogenes were analyzed with Pseudofinder software newly developed in my group specifically for endosymbiont genomes (Syberg-Olsen et al.; Molecular Biology and Evolution, in press.). Confocal microscopy (FISH) with diverse probes was used to localize the newly detected symbionts in their insect hosts.

４．研究成果

Over 15 Pseudococcidae metagenomes were sequenced in my group during the project. Sampling of the lineages was phylogeny-informed, so we managed to acquire data from all the targeted lineages (Rhizoecinae, Rastrococcinae, Phenacoccinae, Pseudococcinae). Five of the sampled Rhizoecinae and Rastrococcinae species were found to house Bacteroidetes symbionts from several different lineages. Unexpected findings were fungal symbionts in two mealybug species. The remaining species were all confirmed to house betaproteobacterial and gammaproteobacterial symbionts (most, but not all, known from previous studies). Comparative genomics revealed the metabolic contribution of the different symbionts for the host and phylogenomics (incl. dating with fossil calibration points) untangled the evolutionary history of mealybug symbioses (the main goal of the project; Figure 1). Two publications were already published in the frame of the project and two publications with the main results are in preparation (Choi & Husnik, in prep.).

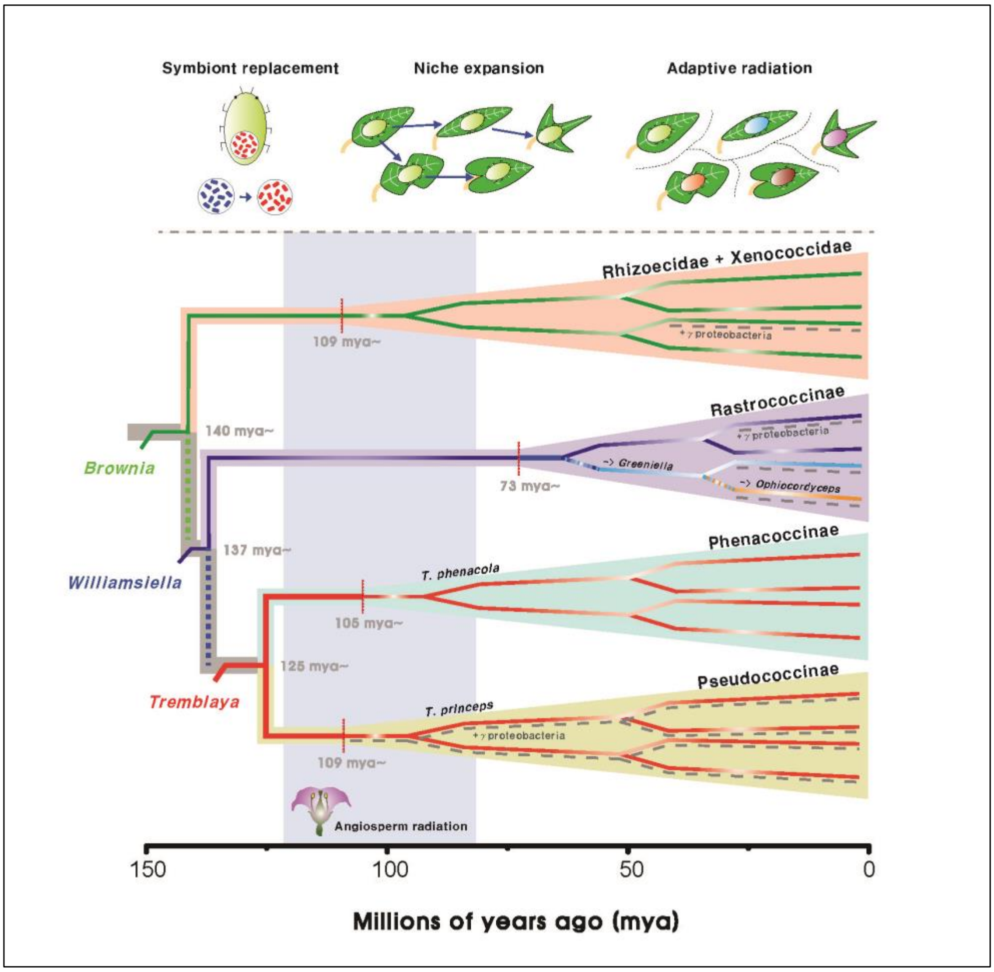

Figure 1. Evolutionary history of mealybug symbioses as reconstructed in the frame of the project. *Brownia*, *Williamsella*, and *Greeniella* are newly sequenced Bacteroidetes symbionts that were important missing links for understanding the origin of mosaic symbioses in mealybugs.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　0件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Husnik F, Tashyreva D, Boscaro V, George E, Lukes J, Keeling P	４．巻 in press
２．論文標題 Bacterial and archaeal symbioses with protists: functional and evolutionary comparisons with animal models	５．発行年 2021年
３．雑誌名 Current Biology	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　1件／うち国際学会　0件）

１．発表者名 Filip Husnik
２．発表標題 What can we learn about the origin of mitochondria and plastids from much younger endosymbioses?
３．学会等名 OIST-RIKEN Symposiun. Green and blue planet - How can ecological research shape our future?（招待講演）
４．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関