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研究課題名(和文) Elucidation of the mutation rate as driver of insect endosymbiont genome evolution

研究課題名(英文) Elucidation of the mutation rate as driver of insect endosymbiont genome evolution

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研究成果の概要(和文)：我々は448のプラタバクテリウム(ゴキブリの細菌内共生体ゲノム解析を行い、うち300以上を単一コンティグにアセンブリした。また、その他のゲノムは断片化され、複数のコンティグにアセンブリした。さらにこれらのゲノムを解析して、プラタバクテリウムにおける遺伝子喪失の背景を再構築し、遺伝子喪失の原因となるメカニズムの特定を試みた結果、突然変異率の増加が遺伝子喪失の主な原因であることを突き止めた。この研究成果は、Molecular Biology and Evolution (IF:10.7)とMolecular Phylogenetic. Evol. (IF:4.3)に2つの論文として掲載された。

研究成果の学術的意義や社会的意義

My project improved our understanding of genome reduction in bacterial endosymbionts. It led to two publications in scientific journals, including one publication in MBE, a recognized journal in the field. My project was scientifically relevant and improved our understanding of endosymbionts.

研究成果の概要(英文)：We sequenced 448 Blattabacterium genomes, including over 300 assembled in a single contig. Other genomes were fragmented and assembled in multiple contigs. We analyzed these genomes to study the evolution of Blattabacterium, a bacterial endosymbiont of cockroaches. We reconstructed the history of gene loss in Blattabacterium and attempted to identify the mechanisms responsible for gene loss. We identified enhanced mutation rate as the primary mechanism responsible for gene loss. Our work yielded two papers published in Molecular Biology and Evolution (IF: 10.7) and Molecular Phylogenetics and Evolution (IF: 4.3).

研究分野：Evolutionary Biology

キーワード：endosymbionts insect evolution genomics

### 1. 研究開始当初の背景

Most cockroaches host *Blattabacterium* in their fat body, a bacterial endosymbiont participating in the cockroach nutrition, recycling the nitrogen wastes of their host into amino acid, the building blocks of proteins. The association of cockroach and *Blattabacterium* is ancient and date back to the origin of cockroaches themselves. *Blattabacterium* has been vertically transmitted across generations of cockroaches for over 200 million years. The genomes of *Blattabacterium* are seven times smaller than that of *Escherichia coli* and keep on losing genes at a multi-million-year timescale. My project aimed to identify and model the mechanisms responsible for this slow and gradual genome erosion.

Before we initiated this research project, a total of 67 *Blattabacterium* genomes were already available. While this is a good number of genomes allowing to investigate how *Blattabacterium* genomes have evolved, this number was insufficient to study the process of genome reduction. Indeed, only a handful of reduced *Blattabacterium* genomes were available, all of which already missing many genes, preventing the reconstruction of the cascade of gene losses that initiated genome reduction in *Blattabacterium*. In addition, many questions remained unanswered with the analyses of the available genomes, justifying the sequencing of new *Blattabacterium* genomes.

### 2. 研究の目的

My project aimed to document how endosymbiont genomes evolve at timescale of hundreds of millions of years. While the interactions between endosymbionts and their hosts have been extensively studied, a comparative genomic approach hasn't been used yet to study endosymbiont genome evolution. Comparative genomic approaches provide a powerful tool to identify the factors responsible of genome evolution. In this project, we originally planned to sequence 246 genomes to answer the following questions: A. Why does mutation rate differ among *Blattabacterium* strains? B. Is increased mutation rate linked to low GC-content? C. Is there correlated gene loss as predicted by the domino effect theory? D. Can we predict the likelihood of a gene to be lost? My project was the first to attempt to infer the sequence and timing of gene loss, how fast it occurred, and whether there are patterns of convergence in one endosymbiont lineage.

### 3. 研究の方法

We used shotgun sequencing of cockroach fat body tissues to reconstruct *Blattabacterium* genomes. *Blattabacterium* grow in specialized cells inside the fat bodies of cockroaches. DNA of cockroach fat body was extracted with standard DNA isolation kits and sequenced with the Novaseq platform to generate paired-end short reads. We generated between 2 and 20 Gigabases of short read data for each cockroach sample studied in this project. These reads were assembled and the *Blattabacterium* genomes were identified in the assemblies. The genomes of *Blattabacterium* are compact and mostly composed of genes. They can generally be assembled with short reads only. Assembled *Blattabacterium* genomes were annotated, and their genes were classified in orthologous groups. We carried out phylogenetic analyses on these orthologous groups to reconstruct a robust phylogenetic tree of *Blattabacterium*. We reconstructed the gene loss history of *Blattabacterium* genomes. We estimated GC-content for the first, second, and third codon positions, gene length, dN, dS, and dN/dS. We correlated these parameters with the loss probability of each gene. Finally, we estimated the mutational spectra of closely related strains of *Blattabacterium*. These analyses allowed us to answer questions A to D, with the final goal of improving our understanding of the evolution of *Blattabacterium*. My project aimed to use *Blattabacterium* as a model to study genome reduction in endosymbionts.

#### 4. 研究成果

We sequenced 448 *Blattabacterium* genomes, including over 300 assembled in a single contig. Other genomes were fragmented and assembled in multiple contigs. Therefore, we sequenced more genomes than originally planned in this project. We analyzed these genomes to answer questions A to D. We published two papers in *Molecular Biology and Evolution* (IF: 10.7) and *Molecular Phylogenetics and Evolution* (IF: 4.3). Of note, the analyses are still ongoing and will lead to additional papers in the coming years. The two papers published at the moment are:

Kinjo Y.\*, Lo N.\* (\*equal first authors), Villa-Martin P., Tokuda G., Pigolotti S. & Bourguignon T. (2021) Enhanced mutation rate, relaxed selection, and the 'domino effect' are associated with gene loss in *Blattabacterium*, a cockroach endosymbiont. *Molecular Biology and Evolution*, 38, 3820-3831. [Journal IF: 10.7; ranked 4/52 in Evolutionary Biology]

Che Y., Deng W., Li W., Zhang J., Kinjo Y., Tokuda G., Bourguignon T., Lo N. & Wang Z. (2022). Vicariance and dispersal events inferred from mitochondrial genomes and nuclear genes (18S, 28S) shaped global *Cryptocercus* distributions. *Molecular Phylogenetic and Evolution* 166, 107318. [Journal IF: 4.1; ranked 11/52 in Evolutionary Biology]

5. 主な発表論文等

〔雑誌論文〕 計2件（うち査読付論文 2件/うち国際共著 2件/うちオープンアクセス 2件）

|   |                               |
|---|-------------------------------|
| 1. 著者名<br>Che Yanli, Deng Wenbo, Li Weijun, Zhang Jiawei, Kinjo Yukihiro, Tokuda Gaku, Bourguignon Thomas, Lo Nathan, Wang Zongqing                         | 4. 巻<br>166                   |
| 2. 論文標題<br>Vicariance and dispersal events inferred from mitochondrial genomes and nuclear genes (18S, 28S) shaped global <i>Cryptocercus</i> distributions | 5. 発行年<br>2022年               |
| 3. 雑誌名<br>Molecular Phylogenetics and Evolution   | 6. 最初と最後の頁<br>107318 ~ 107318 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1016/j.ympev.2021.107318  | 査読の有無<br>有                    |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である）   | 国際共著<br>該当する                  |

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| 1. 著者名<br>Kinjo Yukihiro, Lo Nathan, Martin Paula Villa, Tokuda Gaku, Pigolotti Simone, Bourguignon Thomas   | 4. 巻<br>38                |
| 2. 論文標題<br>Enhanced Mutation Rate, Relaxed Selection, and the “Domino Effect” are associated with Gene Loss in <i>Blattabacterium</i> , A Cockroach Endosymbiont | 5. 発行年<br>2021年           |
| 3. 雑誌名<br>Molecular Biology and Evolution  | 6. 最初と最後の頁<br>3820 ~ 3831 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1093/molbev/msab159  | 査読の有無<br>有                |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である）  | 国際共著<br>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）

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| 1. 発表者名<br>Thomas Bourguignon  |
| 2. 発表標題<br>Genome evolution of the cockroach endosymbiont, <i>Blattabacterium cuenoti</i>                              |
| 3. 学会等名<br>Seminar in Ecology and Evolution of the Institute of Biology of the Freie Universitat Berlin, Germany（招待講演） |
| 4. 発表年<br>2021年  |

〔図書〕 計0件

〔産業財産権〕

〔その他〕

This project yielded two papers published in recognized journals. We have not published all the genomes we sequenced yet and are currently working on additional papers. I expect to publish an additional three papers in the coming years. Many of the genomes we produced in this project will be published in these papers.

6. 研究組織

|  | 氏名<br>(ローマ字氏名)<br>(研究者番号) | 所属研究機関・部局・職<br>(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 |
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