氏　　名	飯塚 朋代
学位(専攻分野)	博士(理学)
学 位 記 番 号	総研大甲第 2118 号
学位授与の日付	2019 年 9 月 27 日
学位授与の要件	生命科学研究科 遺伝学専攻 学位規則第6条第1項該当
学 位 論 文 題 目	The evolutionary study of genomic changes associated with morphological evolution of septal pore cap in Agaricomycotina
論 文 審 査 委 員	主　査　教授 斎藤 成也 教授 明石 裕 教授 北野 潤 教授 宮城島 進也 助教 野澤 昌文 首都大学東京理学研究科

（様式３）

博士論文の要旨

氏　　名　飯塚　朋代

論文題目　The evolutionary study of genomic changes associated with morphological evolution of septal pore cap in Agaricomycotina

Morphological characters change in various directions during species divergence. These differences have often played ecological roles e.g. performing a physiological function. Furthermore, similar phenotypes sometimes appear independently through the evolutionary process, called convergent evolution. The molecular basis of these phenomena is important to understand phenotype-genotype relationships, adaptive evolution and measuring evolutionary repeatability.

One such example for typical morphological evolution occurs in the septal pore cap (SPC), which is involved in a plugging process of cell-like compartments in the major groups of filamentous fungi *Agaricomycotina*. SPC is located around the hole of cell-like compartments (pore), and was derived from the endoplasmic reticulum. SPC is classified into three morphological types: i.e., perforate, imperforate, and vesiculate types. Perforate SPC has many small holes (perforations) on their SPC. Imperforate SPC has a slightly flattened closed membranous structure. The vesiculate SPC consists of vesicles or tubules arranged in a hemisphere and surrounding the pore. Since perforations of perforate SPC allows passing mitochondria and actin filament, this fact suggests that the difference of SPC types contribute to the difference of the functional performance of SPC. Current integrated results of the species phylogeny in fungi and morphological characterization of SPC showed that vesiculate SPC is the most ancestral trait, and perforate SPC is the most neomorphic trait. Interestingly, perforate SPC emerged from imperforate SPC multiple times independently. This fact indicates that morphological convergent evolution had occurred on the perforate type lineages.

However, the genetic background of these evolutionary events remains unknown.

In this doctoral thesis, I therefore aim to clarify genomic changes associated with the morphological evolution of SPC using comparative genomics. I tackled three parts of the analysis; candidate genes extraction, analysis for understanding sequence evolution correlated with the morphological difference between vesiculate SPC and Imperforate SPC, and morphological convergence from Imperforate SPC to Perforate SPC. In order to detect candidate genes that correlated with the morphological convergent evolution of Perforate SPC, I conducted genome-wide surveys using the 12 fungal genomes in Chapter 2. I created an orthologous gene dataset and extracted the candidate gene from the dataset based on the results of the phylogenetic analysis of each ortholog. I found the candidate gene showed different branching pattern than the well-supported species tree. In particular, the candidate gene showed convergent amino acid substitutions at the same site between diverged species with perforate SPC. These results suggest the possibility of the involvement of the candidate gene to morphological convergence of perforate SPC. In chapter 3, in order to understand sequence evolution related to morphological evolution from imperforate SPC to perforate SPC, I verified how convergent amino acid substitutions occurred in the candidate *Rhizoctonia solani* (Rhiso) gene. The possible hypotheses are: 1, Rhiso and other perforate type species acquired the convergent substitutions independently, 2: Rhiso had acquired the same substitutions by e.g. horizontal gene transfer. To clarify which hypothesis is more possible, I conducted synteny analysis, determination of exon/intron structure and phylogenetic analysis of the candidate gene. The results provided no supporting evidence of hypothesis 2 from the genome sequences of Rhiso. Thus, these convergent substitutions appear to have occurred by independent sequence evolution. In vesiculate type species, the result of sequence similarity searches showed that they do not have the candidate gene in their genomes. The gene was also not detected from the other eukaryotes and all prokaryotes. Therefore, I presumed the

ancestor of the candidate gene in chapter 4. I searched a weak sequence homology to assess the ancestral gene of the candidate gene by using sequence motifs. To see the track of the candidate gene from the genome of outgroup species, synteny analysis was conducted. Based on these results, I discussed the origin of the candidate gene and its evolution.

In summary, I identified each genetic difference correlated with the morphological difference between vesiculate SPC and imperforate SPC, and morphological convergence from imperforate SPC to perforate SPC. Also, I clarified the process of these genetic changes. These results provide important advances for understanding the genetic basis of morphological evolution of SPC. These achievements contribute to uncovering the molecular mechanisms of convergent evolution and to finding a missing link between morphological evolution and sequence evolution in SPC. My results present the hints for deep understanding about genetic basis of morphological evolution and convergent evolution. In addition, this study provides an impetus for developments in fungal evolutionary biology and genomics.

Form8・Separate Sheet
（様式８・別紙）

Results of the doctoral thesis screening

博士論文審査結果

Name in Full
氏　　名　飯塚　朋代

Title
論文題目　The evolutionary study of genomic changes associated with morphological evolution of septal pore cap in Agaricomycotina

博士論文の出願者である飯塚朋代さんは、進化において形態の収斂進化が生じる分子基盤に興味を持ち、担子菌ハラタケ亜門(Agaricomycotina)の菌類の一部に見られる微小形態である septal pore cap（SPC；隔壁孔キャップ）をモデルとして研究をおこなった。SPC の３形態（vesiculate, imperforate, perforate）には、収斂進化現象のあることが知られており、それに着目して、SPC の形態変化と関連してゲノムに生じる分子進化現象を探索した。まず真菌類 12 種のゲノム配列を解析し、各オーソログ（順系相同遺伝子）の系統樹解析から、SPC の形態進化にかかわったと考えられる候補遺伝子 *spc33* を選択した。このタンパク質コード遺伝子では、perforate 型 SPC を持つ種で同一のアミノ酸置換が生じていた。*Rhizoctonia solani* 種を中心とした分子系統解析の結果、これらの置換は水平遺伝子移行の結果ではなく、平行置換の結果だと推定された。つぎに *spc33* の出現時期とその祖先遺伝子を、シンテニー解析と相同性検索から検討した。その結果、vesiculate 型には *spc33* が存在しない一方、Ser/Thr タンパク質キナーゼ遺伝子が *spc33* の祖先遺伝子候補として浮かび上がった。この結果については、今後実験的な検証が必要となるが、ゲノム配列データの解析だけから、SPC という微小形態の進化にかかわった可能性のあるアミノ酸変化や遺伝子の出現を推定した一連の解析結果は、高く評価できる。

本研究は、分子進化学で研究があまり進んでいない形態レベルと分子レベルをつなげようとする分野において貴重な成果をあげた。学位論文は明快な英語で書かれており、また研究の一部はすでに英文国際誌に投稿されている。以上の理由により、審査委員会は、本論文が学位の授与に値すると判断した。