Abstract of Doctoral Dissertation

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Molecular evolutionary studies on major histocompatibility complex and amylase genes in Eurasian badgers, genus *Meles* (Mammalia, Carnivora, Mustelidae)
(ユーラシアアナグマ *Meles* 属における主要組織適合遺伝子複合体とアミラーゼ遺伝子に関する分子進化学的研究)

Duplicated genes in genome play important roles in biological activities in organisms. In the present study, major histocompatibility complex (MHC) genes and amylase genes known as the duplicated genes were investigated in four related species of Eurasian badgers (European badger *Meles meles*, Southwest Asian badger *M. canescenes*, Asian badger *M. leucurus* and Japanese badger *M. anakuma*), genus *Meles*. Through the investigation, interesting characteristics on the genes were newly found as follows.

The MHC genes encode proteins that play an indispensable role in the adaptive immune system and autoimmunity of vertebrates, to defense against diverse spectrum of pathogens. To further understand the variation and evolution of MHC genes, including both class I (MHCI) and class II genes in the four Eurasian badger species, the most polymorphic region exon 2 of MHC class II *DRB* gene, exons 2 and 3 and intervening intron 2 of MHCI gene were analysed. As a result, 60 MHC *DRB* and 64 MHCI alleles were isolated from 28 and 25 badger individuals, respectively. Of the obtained alleles, four alleles of MHC *DRB* and nine of MHCI were putative pseudogenes with insertions and deletions (indels) or stop codons. Both exons 2 of MHC *DRB* and MHCI, but not exon 3 of MHCI, accumulated extensive nonsynonymous than synonymous substitutions at the antigen binding sites (ABSs), indicating historical positive selection. Both recombination and codon based positive selection analyses showed signatures of recombination and positive selection in both MHC *DRB* and MHCI genes in these Eurasian badgers, implying that both recombination and selection more likely act on the driving and maintaining of polymorphism in MHC gene in genus *Meles*. Molecular phylogenetic analyses for exons 2 of MHC *DRB* and exons 2 and 3 and intron 2 of MHCI indicated trans-species polymorphisms at different taxonomic scales, transgressing family-, genus- and species-level splits, which are indirect evidence for long term balancing selection. The smaller number of shared alleles and higher number of pseudogenes were observed in MHCI genes compared to MHC *DRB* gene. It is consistent with the faster evolutionary rate of typical MHC class I loci than that of MHC class II. With various degrees of MHCI divergence, varied number of nonclassical genes and pseudogenes are generated from their counterparts — classical genes.

The amylase genes encode digestive enzyme amylases, which are important in catalysis of starch into maltose in the oral, stomach, and intestine of mammals. To get insight into the copy number variation of amylase genes and its relationship to diet, partial exon 4 of amylase gene was amplified in the real time quantitative PCR system. The results showed that only Asian badgers among the four Eurasian badgers studied were found to have amylase copy numbers between one and four. Within Asian badgers, the copy numbers are variable among local populations. This suggests that the Asian badger is better adapted to a diet rich in starch and/or glycogen than the other badger species. Because Eurasian badgers are basically omnivorous but favor the animal diet, the amylase gene copy number variation could be related to the seasonal dearth of animal food in the range of Asian badgers, on which more vegetal diet is enforced. This in turn would lead to more efficient digestion of dietary starch, which could be achieved by increasing the copy number of the pancreatic amylase gene.

The above achievements were presented in three different Chapters I, II, and III. Chapter I mainly included the results and interpretations of the MHC class II genes in the four Eurasian badgers, which published in Biological Journal of The Linnean Society in October, 2017 (https://doi.org/10.1093/biolinnean/blx077). While Chapter II comprised of the results and interpretations of the MHC class I genes in the four Eurasian badgers, of which the data are accepted by Heredity for publication. Lastly, Chapter III presented the results and interpretations of the amylase gene copy number variation in the four Eurasian badgers and related taxa in family Mustelid.