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## Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

**Phylogeny and evolution of mycophagy in Drosophilidae**  
(キノコ食ショウジョウバエの系統関係と食性の進化)

Drosophilid flies feed on various substances such as fermenting fruits, mushrooms, flowers, leaves, and so on, and most of the fungus-feeding species are included in the *Zygothrica* genus group and the *quinaria* species group of the subgenus *Drosophila*. Despite numerous phylogenetic studies on the family Drosophilidae, the positions of these two groups have been still equivocal. Corroborating the phylogenetic status of these two groups is important for understanding the evolution of mushroom feeding (mycophagy) in the family. In addition, mushrooms produce a defensive chemical compound,  $\alpha$ -amanitin, to protect themselves from fungus-feeding insects. A key adaptation for these insects is the ability to cope with the toxicity of their hosts. Several fungus-feeding species from the subgenus *Drosophila*, especially the *quinaria* group, are well known to tolerate this fungal toxin. However, it is unknown whether or not species of the *Zygothrica* genus group and other related species have such tolerance. In these contexts, this study focuses on the phylogeny and evolution of mycophagy and the  $\alpha$ -amanitin tolerance in Drosophilidae. The results are described in three chapters:

Chapter 1 focuses on the phylogeny and evolution of mycophagy in the *Zygothrica* genus group. Up to now, neither the phylogenetic position of the *Zygothrica* genus group nor the relationships within it have been resolved. Therefore, a phylogenetic analysis was conducted by assembling a DNA-sequence dataset of 24 genes from 92 species, including 42 species of the *Zygothrica* genus group mainly from the Palearctic and Oriental regions. In addition, the evolution of mycophagy in Drosophilidae is inferred by reconstructing ancestral food habits on the resulting phylogenetic tree. The resulting tree shows that the *Zygothrica* genus group is monophyletic and placed as the sister to the genus *Dichaetophora*, and that the clade *Zygothrica* genus group + *Dichaetophora* is sister to the *Siphlodora* + *Idiomylia/Scaptomyza* clade. Within the *Zygothrica* genus group, the genera

*Mycodrosophila* and *Paramycodrosophila* are both recognized as monophyletic, while neither the genus *Zygothrica* nor *Hirtodrosophila* is monophyletic. The ancestral reconstruction of food habits shows that fungus-feeding habits have been gained independently in two lineages. The most recent common ancestor (MRCA) of the subgenus *Drosophila* is estimated to have acquired mycophagy by expanding its ancestral feeding niche on fermenting fruits to decayed fungi, while the MRCA of the *Zygothrica* genus group may have shifted its niche from fruits to fungi as a specialist probably preferring fresh fruiting bodies.

Chapter 2 addresses a possible effect of nucleotide composition bias on the phylogenetic reconstruction of the *quinaria* species group of the subgenus *Drosophila*. In a recent phylogenetic study, the coalescent-based analysis of 43 genes has shown that the *quinaria* group is not monophyletic, while the concatenated-based analysis has shown the monophyly of it. A possible cause of incongruence may be the difference in GC content at the third codon sites among lineages, since such compositional bias is known to adversely affect the reconstruction of phylogenetic trees. In this chapter, species trees are constructed by the concatenation- and coalescent-based methods, excluding the 3rd codon sites of the nuclear genes from the dataset of previous study. All the resulting trees represented the monophyly of the *quinaria* species group, suggesting that the nucleotide compositional bias should be taken into account in phylogenetic reconstruction.

Chapter 3 focuses on the alpha-amanitin tolerance of Drosophilidae. Previous studies suggest the tightly correlated evolution of this tolerance with mycophagy. To test the hypothesis, the  $\alpha$ -amanitin tolerance of adult flies is compared among 16 drosophilid species including mycophagous and non-mycophagous ones. The results are as follows: all the tested mycophagous species are tolerant of this lethal toxin, while the non-mycophagous species cannot survive on a diet containing  $\alpha$ -amanitin with two exceptions, cactus-feeding *Drosophila hydei* and fruit-feeding *Drosophila sternopleuralis*. This suggests that the  $\alpha$ -amanitin tolerance may have evolved through multiple pathways of food-habit diversification in the family Drosophilidae.