博士論文要約（Summary）

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タイトル	The cuticle ultrastructure of Aphelenchoididae nematodes

キーワード（**morphology**）　（**natural history**）　（**transmission electron microscope**）

Chapter 1: General Introduction

The nematode cuticle structure is extremely variable, not only among different taxa but also between sexes and across the developmental stages within a species. Therefore, the cuticle, flexible exoskeleton, appears to be an important factor for the great capacities of nematodes to adapt to various environments. However, we seldom understand what the cuticle structural differences mean. In addition, we do not know whether each cuticle structure really reflects life history of each species or not. This is mainly because the relationship between the cuticle structure and the biological trait is unclear. The purpose of the present study is to obtain fundamental information about cuticle ultrastructure in Aphelenchoididae species and to clarify the relationship between cuticle structures and physiological/ecological traits in detail. First, I examined cuticle structure of two inbred strains of *B. xylophilus,* differing in virulence and oxidative stress tolerance (Chapter 2). Next, I examined the body cuticle structure of several Aphelenchoididae species, and compared the structures between species different in an association manner with their host/carrier insects (Chapter 3), and different in feeding habitats (Chapter 4). In Chapter 5, I studied not only cuticle structure but also body wall muscle structure and compared the structures between species/sexes that seem to be different in mobility. Finally, I discuss about the structural changes associated with nematode’s evolution (Chapter 6).

Chapter 2: Tolerance to oxidative stress of inbred strains of the pine wood nematode, *Bursaphelenchus xylophilus*, differing in terms of virulence

The virulence of *Bursaphelenchus xylophilus*, commonly known as the pine wood nematode, varies greatly among different populations. Two inbred strains, called P3 and P9, were recently established via repeated full-sib mating. They exhibited remarkable differences in pathogenicity-related traits. Although their propagation did not differ when cultured on fungal lawns, P9 reproduced better in host seedlings and exhibited higher virulence. In the present study, I hypothesised that the difference in virulence was associated with tolerance to oxidative stress, and examined this tolerance and the cuticular ultrastructure. P9 survived better under hydrogen peroxide (H_2O_2)-stressed conditions than did P3. In addition, P9 had a thicker cuticle than P3, suggesting that the difference in tolerance was due not only to a physiological feature, such as H_2O_2-degrading ability, but also to a physical factor (cuticle thickness).

Chapter 3: Cuticle structures of insect-phoretic and parasitic stages of Parasitaphelenchinae nematodes

I examined the body cuticle ultrastructures of phoretic and parasitic stages of the parasitaphelenchid nematodes *Bursaphelenchus xylophilus*, *B. conicaudatus*, *B. luxuriosae*, *B. rainulfi*; an unidentified *Bursaphelenchus* species, and an unidentified *Parasitaphelenchus* species. Nematode body cuticles usually consist of three zones, a cortical zone, a median zone, and a basal zone. The phoretic stages of *Bursaphelenchus* spp., isolated from the tracheal systems of longhorn beetles or the elytra of bark beetles, have a thick and radially striated basal zone. In contrast, the parasitic stage of *Parasitaphelenchus costati*, isolated from bark beetle hemocoel, has no radial striations in the basal zone. This difference probably reflects the peculiar ecological characteristics of the phoretic stage. A well-developed basal radially striated zone, composed of very closely linked proteins, is the zone closest to the body wall muscle. Therefore, the striation is necessary for the phoretic species to be able to seek, enter, and depart from host/carrier insects, but is not essential for internal parasites in parasitaphelenchid nematodes. Phylogenetic relationships inferred from near-full-length small subunit ribosomal RNA sequences suggest that the cuticle structures of parasitic species have apomorphic characters, e.g., lack of striation in the basal zone, concurrent with the evolution of insect parasitism from a phoretic life history.

Chapter 4: The relationship between cuticle structure and ecological trait in Aphelenchoididae nematodes

In the present study, I focused on the Aphelenchoididae family, which is a highly divergent group including free-living fungal feeders, plant parasites, insect parasites and predators. I observed the cuticle structure of several aphelenchoidid nematodes by transmission electron microscope, and compared their structural differences in relation to the life histories and phylogenetic relationships in an evolutionary context. As results, the cuticle structure of five mycophagous species (*B. xylophilus, B. conicaudatus, B. luxuriosae, B. rainulfi* and *Aphelenchoides xylocopae*) were largely similar, *i.e.*, their cuticle consisted of four parts: a triple-layered epicuticle, electron-lucent cortical and median zones, and a radially striated basal zone. On the other hand, three predator species (*Ektaphelenchoides spondylis*, *Ektaphelenchus* sp. and *Seinura caverna*) had different cuticle structure, i.e., they had an osmophilic median zone. In addition, the phylogenetic analysis showed that the emergence of the osmophilic zone was concurrent with the evolution of predatory species from mycophagous species. Furthermore, *Peraphelenchus orientalis*, an obligate insect parasite derived from predatory nematode species, had no osmophilic median zone, indicating that the zone disappeared with the loss of predatism. These results suggest the osmophilic median zone is essential for the predacious life history, *e.g.*, avoiding cannibalism.

Chapter 5: Sexual dimorphism in cuticle and body wall muscle in free-living mycophagous nematodes

Sexual dimorphism in motility-related traits is widespread among animals, including several species of Nematoda. However, no study has examined motility-related structural components and compared them between sexes. I examined the motility-related components in four species: *B. conicaudatus, B. rainulfi*, *B. doui*, and *P. costati*. I observed the cuticular structure, measured the amount of cuticle and body-wall muscles and estimated their relationship to the body diameter or the total cross-sectional area. Although no structural differences were observed in the muscle, the relevant muscle area of *B. doui* and *P. costati* was significantly smaller in females than in males. This difference was greatest in *P. costati*. In all but *B. doui*, the relative cuticle thickness was significantly smaller in females than in males. Furthermore, only *P. costati* females had no striated basal zones in their cuticles; these are thought to be cross-linked proteins that provide strength to nematode cuticle during body movement. These results indicate that sexual dimorphism in motility-related structural components is present in *P. costati* and that females invest less energy in the components than do males.

Chapter 6: General Discussion

To understand the relationship between cuticle structure and biological trait, I observed cuticle structures of various Aphelenchodidae nematode species and associated the structures with their physiological/ecological traits. I showed that difference in the thickness of cortical and median zones between virulent and avirulent strains of *B. xylophilus* seems to reflect chemical resistance, which suggests the importance of the thickness of the zones (Chapter 2). In addition, I demonstrated that cuticular structure had dramatically changed with the evolution of specific ecological traits in the family of Aphelenchoidae (Chapters 3, 4 and 5). The structural changes observed in this study, i.e., changes of radial striation in the basal zone and appearance of the osmophilic median zone, appear to reflect the functional modifications of cuticle. The radial striation in the basal zone seems to play a role in active nematode movement; it gives body bending strength. On the other hand, the osmophilic median zone seems to play a role in avoiding cannibalism; it may give body puncture resistance. As I showed in the present study, aphelenchoidid nematodes change and optimize their cuticular structures depending on their habitats and life histories. Further nematode cuticle structural studies combining morphological, molecular biological and genetic approaches would reveal functions of cuticle structures and structural changes associated with their functional changes in more detail.