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Two pathways of the host immune response to microsporidian parasites and microsporidiosis co-infections

Microsporidiosis is one of the most serious threats to global beekeeping. It is caused by obligate intracellular parasites belonging to the *Nosema* genus. These fungi infect the epithelial cells of bees' midguts, resulting in significant economic losses for the global beekeeping industry. Infection with *Nosema ceranae* results in a shortened lifespan for worker bees of up to one-third, weakening bee colonies, reducing honey production and impairing the pollination capacity of cultivated plants. Despite the growing importance of this issue in the context of the decline of the global bee population, the immunological response mechanisms of different insect species to microsporidioses and the evolutionary aspects of parasite-host interactions remain poorly understood.

The project's primary goal is to thoroughly investigate the physiological, biochemical and molecular responses of different species to microsporidioses by comparing the reactions of honeybees (*Apis mellifera* L.), natural co-evolving hosts, with those of the greater wax moth (*Galleria mellonella* L.), an accidental host of this parasite. The research will focus on analysing evolutionary adaptations in parasite-host interactions, identifying biochemical markers that enable the early diagnosis of infections and determining the impact of microsporidiosis on the cognitive functions of bees, particularly memory, which forms the basis of hygienic behaviours in the colony.

The study employs a comparative experimental design involving *A. mellifera* and *G. mellonella* infected with the same microsporidian. Both larval and adult stages are examined through systematic sampling on days 6, 12, 18, and 24 post-infection. The methodology combines several cutting-edge analytical approaches, including molecular analysis via quantitative polymerase chain reaction (qPCR) and transcriptome sequencing; high-resolution magnetic resonance imaging (MRI) for anatomical changes; biophoton emission analysis; gut microbiota sequencing; bee memory studies using the proboscis extension response (PER); and volatile compound profiling with electronic nose technology.

The research addresses the question of why social insects rely on colony-wide hygienic behaviours rather than on the immunity of individual bees. The study hypothesises that microsporidiosis may impair the cognitive functions of bees, particularly their spatial and social memory. This would weaken their colonial immunity system, which is based on collective pathogen recognition and elimination.

The project's innovative comparative research approach enables direct comparisons to be made between the responses of the same parasite species in natural and accidental hosts. This will facilitate the identification of evolutionary adaptations in parasite-host interactions. The integration of advanced analytical techniques, including genomics, medical imaging, and chemometric analysis, constitutes a comprehensive methodological approach that has not previously been applied in microsporidiosis research.

Expected outcomes include identifying new diagnostic biomarkers for early microsporidiosis detection, understanding the evolutionary mechanisms of parasite-host co-evolution, understanding the relationships between cognitive functions and colonial immunity, and developing practical diagnostic tools for beekeeping. These results will advance evolutionary science, parasitology, and insect immunobiology while providing practical solutions for safeguarding bee health and promoting sustainable beekeeping practices.