Diversity and evolution of symbiotic protist communites in termites especially focused on genus *Reticulitermes*

by

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Abstract

The symbiotic protist communities of termites and wood-feeding *Cryptotercus* cockroaches are characterized by strong host specificity of its species composition. Cluster analysis based on the similarity of symbiont generic composition and a phylogeny comparison between the hosts and the symbionts revealed that cospeciation or cocladogenesis was the main determinant of the symbiont composition. Similarly, in *Reticulitermes* hosts in Japan, patterns of the symbiont communities reflected cospeciation and geohistorical events. Host hybridization induced symbiont community mixing and resulted in asymmetrical inheritance of protists from parent species. Hybridization or cannibalistic attack between different host lineages and following community mixing probably affected the evolution of the symbiotic protist communities.

Keywords: protist, symbiosis, community, cospeciation

Introduction

Lower termites and wood-feeding *Cryptocercus* cockroaches have symbiotic protist communities in their hindguts. The protist symbionts belong to two phyla Parabasalia and Preaxostyla and six orders Trichomonadea, Tritrichomonadea, Trichonymphea, Cristamonadea, and Spirotrichonymphea and Oxymonadea, respectively (Adl *et al*. 2012, Cepicka et al. 2010). Cellulases produced by the symbionts are essential for the host termites to digest their food, while the symbionts also depend on their hosts for food and an anaerobic habitat (Honigberg 1970, Inoue *et al*. 2000, Brune and Ohkuma 2011). The number of symbiont species in a host's hindgut range from one (several termite genera in the Rhinotermitidae) to 26 (*Cryptocercus punctulatus*) (Kitade 2004). The species composition of a symbiont community is usually specific to a host species (Kirby 1934, Honigberg 1970, Ohkuma and Brune 2010), probably reflecting the transmission mode of the symbionts. The anaerobic symbionts are transmitted between individuals in a colony through proctodeal trophallaxis, and a newly founded colony should follow the symbiont faunae of the king and queen (Inoue *et al*. 2000). Obligate

mutualism between the termites and protists suggests long-time cospeciation (cocladogenesis) that should be reflected in the pattern of symbiont communities.

Diversity and similarity of protist communities and cospeciation between hosts and symbionts

Based on data from a checklist by Yamin (1979), taxonomic papers, and direct observation of 12 termite species, we evaluated the similarity of symbiont genetic composition between host genera to understand the overall patterns of symbiont community similarity (Kitade 2004). UPGMA cluster analysis based on Jaccard's Similarity revealed a clear tendency that symbiont composition of host genera belonging to the same family or monophyletic family groups were similar. This trend was particularly remarkable in the clades of Kalotermitidae and (Rhinotermitidae + Serritermitidae). These results suggest that host phylogeny is a main determinant for the pattern of symbiont composition. Intriguingly, the genus *Reticulitermes* (Rhinotermitidae) had a fauna closely similar to *Hodotermopsis* (Termiopsidae), suggesting the possibility of horizontal transfer of symbionts between ancestors of these lineages (Kitade 2004, Lo et al. 2011). The strong effect of host phylogeny in structuring protist community composition and traces of symbiont horizontal transfer were also suggested by the recent exhaustive investigation of protist SSUrDNA using pyrosequencing (Tai et al. 2015). Molecular phylogenetic analysis of Rhinotermitid hosts and the symbiont genus *Pseudotrichonympha* (Noda et al. 2007) inferred almost identical topology for hosts and symbionts, indicating vertical transmission of *Pseudotrichonympha* and cospeciation.

Diversity and similarity of protist communities of *Reticulitermes* spp. in Japan

Reticulitermes in the Japan Archipelago are the most intensively studied termites for diversity and similarity of symbiotic protist communities. Field investigations revealed species-specificity for the 15 species of protist from six species of *Reticulitermes* (Kitade & Matsumoto 1993). Intercolonial variation (beta-diversity) of the symbiont community is low. UPGMA cluster analysis based on the similarity of symbiont composition divided host species into four groups, which corresponded to the geographical distribution of hosts and the host phylogeny inferred from mitochondrial genes. The largest difference in the fauna corresponded to the Tokara Strait, the biogeographical boundary between the Paleoarctic and the Oriental regions. These results support the strong effects of the host phylogeny and a causal paleogeological event on the symbiont communities.

Community mixing caused by the host hybridization

Hybridization of the different host species followed by symbiont community mixing is a likely mechanism for the symbiont horizontal transfer. However, the process(es) that resulted in the re-organizing process of the symbiont communities after the community mixing is totally unknown. We artificially produced hybrid colonies using *Retculitermes speratus* and *R. kanmonensis* alates and observed the prostist species compositions of the members of the hybrid colonies. Hybridization resulted in symbiont community mixing between the king and queen and their mixed-type symbiont faunae were initially transferred to the offspring. However, after 700 days members of most colonies had symbiont communities closely similar to *R. speratus*. These results show that the final protist communities that resulted from the original mixing were not a random assemblage. The community structure of the parent host termites were stable, probably reflecting coadaptaion. The symbiont transmission mode in the community re-organization process was strongly asymmetric. Horizontal transfer with strongly asymmetrical symbiont succession must have occurred in the ancestors of *Reticulitermes* and *Hodotermopsis*, which resulted in *Hodotermopsis*-like symbiont communities in the *Reticulitermes*.

Conclusion

The similarity in patterns of the symbiotic protist faunae and phylogeny congruence between hosts and symbionts indicated that current symbiont species composition has been strongly structured by cospeciation. Hybridization or cannibalistic attack between host lineages followed by symbiont community mixing is regarded as an occasional but important determinant in symbiont community evolution. Protist community mixing and asymmetrical symbiont inheritance have possibly functioned as a mechanism by which host termites obtain novel ligno-cellulose degradation systems.

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