Biogeography of a defensive symbiosis

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Mutualistic microorganisms play important roles in nutrition, reproduction and defense of many insects, yet the factors contributing to their maintenance and dispersal remain unknown in most cases. Theory suggests that collaboration can be maintained by repeated interaction of the same partners (partner fidelity) or by selective discrimination against non-cooperative partners (partner choice). In the defensive mutualism between solitary beewolf wasps and their antibiotic-producing *Streptomyces* bacteria, partner choice by host control of vertical symbiont transmission reinforces partner fidelity and has helped to maintain this highly specific association since it originated in the late Cretaceous. However, co-phylogenetic and biogeographic analyses suggest that there has also been considerable horizontal transmission of the symbionts. While the beewolves clearly have a paleotropic or palearctic origin, with later colonization of the nearctic and neotropics via Beringia and the Aves ridge, respectively, the bacteria show only weak geographical clustering, implying global dispersal or vicariance within the confines of an otherwise apparently exclusive symbiotic relationship. We discuss several hypotheses that may explain these patterns. Future studies investigating the occurrence of beewolf symbionts in the environment could yield broadly applicable insights into the relative impact of animal-vectored and free-living dispersal on the distribution of microorganisms in nature.

Symbiotic associations between multicellular organisms and bacteria are ubiquitous, and they frequently underlie evolutionary innovations.[1,2] Growing recognition of the ecological importance of these associations motivates research on an increasing range of eukaryote-associated microbial communities in plants and animals including humans.[3,4] However, the accumulating knowledge about the identities of symbiotic partners is not matched by a corresponding understanding of how such associations emerge, how they are maintained over evolutionary timescales, and how they are affected by dispersal and colonization of new habitats.

Partner Choice and Fidelity in Symbioses

A well-established theoretical framework for the evolution of cooperative interactions contrasts "partner fidelity" and "partner choice" as the most important mechanisms promoting and maintaining cooperation.[5,6] Previous studies have empirically shown that partner choice via host sanctions or differential rewards can stabilize cooperation in environmentally transmitted symbioses like mycorrhizal fungi or nitrogen-fixing rhizobia of plants,[7,8] while the specialized intracellular symbioses of insects are generally assumed to be stabilized by partner fidelity.[9] It remains unknown, however, which factors contribute to the maintenance and specificity of the vast majority of symbiotic associations in animals that involve facultative microbial associates, which contribute significantly to the ecological success of insects[10] as well as many other organisms.[1]

The Beewolf Symbiosis

Beewolves are solitary digger wasps of the genera *Philanthus*, *Trachypus*, and

Philanthinus (Hymenoptera, Crabronidae) that hunt other Hymenoptera and provision them as prey for their developing offspring in subterranean brood cells. Female beewolves cultivate symbiotic bacteria in specialized gland reservoirs in the antennae and secrete them into the brood cells prior to oviposition.[11-14] The larvae later transfer the symbionts to the cocoon silk,[15] where they provide protection during subsequent development–which is often not completed until the following year[16-18]–by producing a mixture of at least 9 different antimicrobial substances.[19] Recent phylogenetic analyses indicated that the symbionts are descendants of soil-dwelling streptomycetes that were acquired by the insects at least 68 million years ago (**Fig. 1**).[20] The external route of vertical symbiont transmission from mothers to daughters resulted in host-symbiont co-diversification, but also

allowed for horizontal exchange of symbionts among hosts. Artificial infection of beewolf females with opportunistic soil bacteria revealed that these bacteria can grow in the antennal reservoirs but are not transmitted to the offspring, providing strong evidence for partner choice via host control over symbiont transmission.[20] We previously discussed the importance of partner choice for the long-term stability of the beewolf-*Streptomyces* mutualism[20] and will focus here on the implications of its biogeographic history.

Biogeography of Beewolves

The reconstruction of the beewolf phylogeny and a calibration based on the fossil record allows for proposing hypotheses on the biogeographic history of these solitary wasps and their symbiotic association
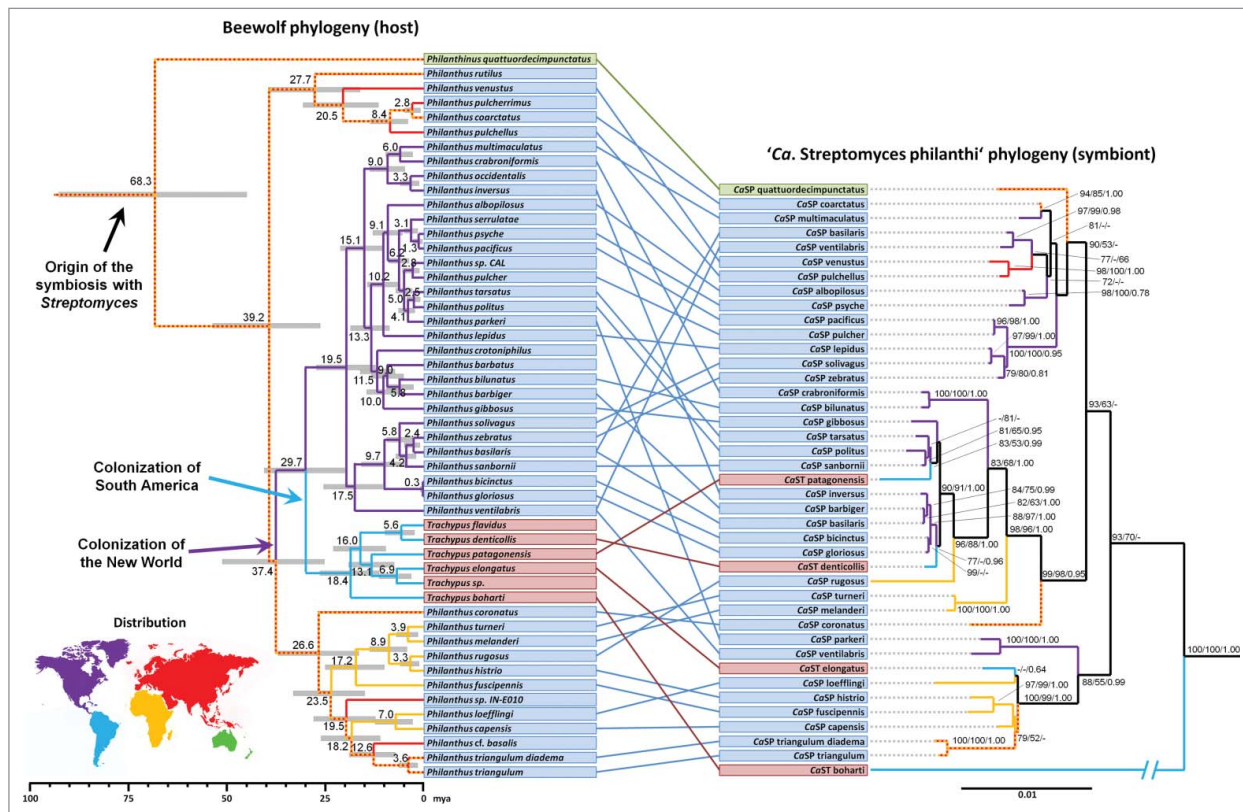
with *Streptomyces*. However, as the fossil record underlying the dating analyses is rather sparse (2 Philanthini, one Cercerini, and one Bembicinae fossil),[20] age estimates are characterized by broad confidence intervals, so the exact timing of biogeographic events remains speculative. Nevertheless, the phylogeny of Philanthinae reveals Eurasia or Africa as the probable origin of beewolves, because *Philanthinus* and the oldest nodes in *Philanthus* all have palearctic or paleotropical distributions (**Fig. 1**). The radiation of beewolves likely followed those of angiosperms and their most important pollinators, the bees, as adult beewolves feed on the nectar of angiosperms and predominantly use bees as larval provisions.[21] Bees originated during the early to middle Cretaceous in the southern hemisphere (Gondwana), most likely in Africa,[22] and the majority of extant *Philanthus* species

Figure 1. Biogeography of the beewolf-*Streptomyces* symbiosis. Node ages in the host phylogeny (left) are shown in million years ago (mya) with 95% highest posterior density (HPD) interval bars. Values at the nodes of the symbiont phylogeny (right) are local support values from the FastTree analysis (GTR model), bootstrap values from PHYML, and Bayesian posteriors, respectively. Branches are color-coded according to the geographic distribution of the host species (see world map, hatched yellow and red branches indicate occurrence in Africa and/or Eurasia). Colored boxes around host and symbiont names denote host genera (green = *Philanthinus*, blue = *Philanthus*, red = *Trachypus*). Host-symbiont associations are shown by connecting lines. Modified from ref. 20.

are African (77 of 137), so an African origin of beewolves seems likely. Interestingly, the South Indian *Philanthus* species (*Philanthus pulcherrimus, Philanthus* sp. IN-E010, and *Philanthus* cf. *basalis*) are interspersed among Asian and African taxa in the phylogeny, suggesting that the Indian subcontinent was colonized from both Africa and Asia.

After spreading across the paleotropics and palearctic, beewolves colonized the Americas about 37 mya (95% CI: 25–51 mya). As the De Geer and Thulean bridges broke up around 63 and 56 mya, respectively,[23] colonization via Beringia during the warm climate of the late Eocene seems most likely, which has previously been suggested for other insect taxa (e.g. aphids).[24] The colonization of South America may have occurred via the Aves ridge or island arc (∼34 mya), which existed roughly around the time of the estimated split between the South American *Trachypus* and the North American *Philanthus* clade (about 30 mya, 95% CI: 19–41 mya, see **Fig. 1**).[25] A plausible scenario assumes a single colonization event with a subsequent radiation of the *Trachypus* clade in South America. However, the current range of *Trachypus* extends north to southern Texas, and although it is possible that the northern species have arisen since the emergence of the Panamanian land bridge, the phylogenetic relationships of these taxa have yet to be investigated and they may date from an earlier time.

Horizontal Transmission and Dispersal of the Symbionts

Despite a monophyletic origin of the beewolf symbiont clade, it shows many discrepancies with the beewolf phylogeny, indicating frequent horizontal transfer of symbiont lineages among host species.[20] Such transfer could conceivably occur through interspecific predation or nest reuse, or by infection from an environmental reservoir of symbiont spores,[20] all of which require the co-occurrence of a host and its horizontally acquired symbiont. Surprisingly, the symbiont strains show only a moderate degree of clustering according to their hosts' geographical distribution (**Fig. 1**). This pattern is unlikely

to be explained by poor resolution or phylogenetic errors, because the phylogeny is based on the sequences of 5 different genes[20] and was recently corroborated by an independent analysis using genome-wide AFLP markers.[26]

Four mutually non-exclusive hypotheses may explain the occurrence of closely related symbionts in geographically widely separated host taxa: (i) The host taxa colonizing new geographical areas may have carried a mixture of symbionts, which were subsequently exchanged among hosts, and individual symbiont strains were subsequently lost in different host lineages. Although possible, this scenario seems unlikely, as recent analyses suggest a high degree of homogeneity in symbiont populations within individual beewolves.[26] Furthermore, given the ancient separation of Old and New World species, biogeographic patterns would still be expected in the symbiont phylogeny under this scenario. (ii) The symbionts may be dispersed via wind or water over large distances (even across continents and oceans) and infect novel hosts. Although recent studies provide increasing evidence for dispersal limitation and biogeographic patterns in microorganisms,[27] some microorganisms indeed appear to be globally dispersed,[28] and dormancy can be expected to facilitate long-range dispersal and successful colonization of new habitats.[29] In fact, trans-oceanic dispersal events of microorganisms in dust clouds have been well documented, particularly from Africa to the Americas.[30-32] Previous studies have shown that the beewolf symbionts undergo morphological differentiation on the beewolf cocoon and thereby survive inhospitable conditions as dormant cells for at least 9 months, and possibly much longer.[17] (iii) Along similar lines, horizontal infection could occur from 'seed banks'[29] of ancient symbiont spores in the environment, thereby obscuring apparent biogeographic patterns among supposedly extant symbiont lineages. (iv) It is conceivable that the symbionts are only facultatively associated with beewolves and occur as free-living bacteria in the environment, which would provide ample opportunities for horizontal transfer and at least partially obscure biogeographic patterns (although isolation

by distance would still be expected, if dispersal is limited).[27] Possibly, the symbiotic ancestors were already globally distributed, and subsequent vicariance events and host switches resulted in the diversification of bacterial strains and led to the observed phylogenetic patterns, with closely related symbiont strains occurring in geographically distant host taxa. The recent isolation of closely related *Streptomyces* strains from Moroccan soil[33] and from chilli pepper rhizosphere in Thailand[34] could provide evidence for the environmental occurrence of the beewolf symbionts, although the phylogenetic affiliation of these isolates with the symbiont clade is currently based on 16S rDNA only and needs confirmation by multilocus sequencing.

Conclusion

Beewolves and antibiotic-producing *Streptomyces* bacteria participate in a defensive symbiosis involving both vertical and horizontal symbiont transmission. The biogeographic history of beewolves is well defined, but that of the symbionts is only loosely concordant with it, suggesting global dispersal or vicariance. This pattern implies that the symbionts may occur in the environment. Finding them there could greatly advance our understanding of the biogeographic history of the ancient association between beewolves and *Streptomyces*, and thereby illuminate larger questions about the relative importance of animal-vectored and free-living dispersal in shaping the distribution of microorganisms in nature.

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