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Symbiosis: Did bacteria bias the beetle big bang?

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The massive species richness of certain taxonomic groups has long enchanted evolutionary biologists, but even within such groups there are biases in cladogenesis. A study of Metazoa's greatest radiation — the beetles — points to metabolic symbioses with bacteria as a possible driver of enhanced diversification in herbivorous clades.

The tree of life is profoundly asymmetric, pervaded by exceptional radiations of certain clades that evolution has seemingly favored. Of the ~1.8 million described lifeforms, ~1 million are insects, and approximately 400,000 of these are beetles (Coleoptera). The manifest diversity of beetles was an early obsession of Charles Darwin, Alfred Russel Wallace and Henry Walter Bates[1] and continues to inspire evolutionists interested in the forces that shape Earth's biodiversity. The question of why, precisely, our planet is home to so many beetle species is alluring, not because beetles are charismatic (though they are), but because the answer is not obvious. Scratch the surface of this problem and you'll encounter one of the great challenges of historical inference. In this issue of *Current Biology*, García-Lozano and colleagues[2] provide fresh insight into this problem by identifying a new potential driver of Coleoptera's extraordinary radiation.

Beetle diversity probably hinges at least in part on the modified morphology of these insects. Beetles possess elytra: hardened, shield-like forewings that cover the delicate, flight-capable hindwings[3]. Elytra give beetles an ecological edge. By protecting the wings, these structures allow beetles access to virtually all parts of terrestrial ecosystems, unlocking niches that are closed off to other insect taxa with unprotected wings[4,5]. Beetles can live in deep soil, under bark, inside the internal parts of plants (and affixed to their surfaces), as well as in bird nests, mammal dung, mammal fur, fungi, social insect colonies, and even submerged coral reefs. Beetles can specialize in ways that other insects can't. Moreover, because most beetles can fly, they can disperse, colonizing new areas — an ability that may synergize with elytra to enhance speciation. Scale these factors by the group's age — crown beetles date to the late Permian[6,7] — and it is conceivable how these insects became so speciose.

Yet, look more closely at beetle diversity and you'll notice that Coleoptera is an order with a striking disparity of richness — a sprawling amalgam of haves and have-nots. At the most recent count, there are 200 extant beetle families[8], but just nine of these house 10,000 species or more. This small handful of families collectively accounts for nearly 75% of living beetle diversity; without them, beetles would only be the fourth largest insect order. At the same time, a huge number of far less numerically impressive beetle families exist: 140 families contain fewer than 1,000 species, together accounting for around only 5% of beetle diversity. Many of these families are ancient groups, being far older than the 'big nine' but having failed to accrue anywhere near as many lineages. If species richness is so dramatically uneven across the beetle phylogeny, elytra cannot be the whole story. Additional forces must have biased diversification to certain parts of the tree.

Twenty-five years ago, a hypothesis was proposed that connected beetle diversification to the rise of flowering plants (angiosperms). In a landmark study, Brian Farrell resolved a branch of the beetle phylogeny named Phytophaga, a huge clade comprising ~140,000 mostly herbivorous species[9]. Phytophaga includes three of the big nine families: 'true' weevils (Curculionidae; ~51,000 species), leaf beetles (Chrysomelidae; ~40,000 species), longhorn beetles (Cerambycidae; ~35,000 species), as well as several smaller families. Mapping feeding mode onto the phylogenetic tree, and contrasting the species richness of sister taxa, a pattern emerged: lineages that began feeding on angiosperms underwent major radiations as these plants rose to dominate terrestrial ecosystems in the Early Cretaceous and Cenozoic. Conversely, phytophagan groups that remained on cycads and gymnosperms — more ancient but now species-poor plant taxa — failed to radiate and are themselves species-poor today.

The angiosperm co-diversification model is attractive, and probably correct, but there are limits to its explanatory power[10]. For one thing, it cannot account for the vast size of some non-herbivorous groups. The largest family, rove beetles (Staphylinidae), for example, is made up of 66,464 mostly predatory species, a remarkable radiation that evidently requires a different explanation[11]. But another problem is that, even within Phytophaga, phylogenetic tree shape is profoundly unbalanced. Not all angiosperm-feeding clades have been equally successful. This pattern encapsulates the central problem of explaining beetle diversification, which is its fractal nature. Species richness is biased to a few 'megadiverse' clades, within which diversification is further biased, and on this pattern goes.

Here is where García-Lozano and colleagues' study provides insight — by identifying a possible cause of cladogenetic bias within Phytophaga[2]. To feed on plants, beetles need enzymatic assistance to break down cell

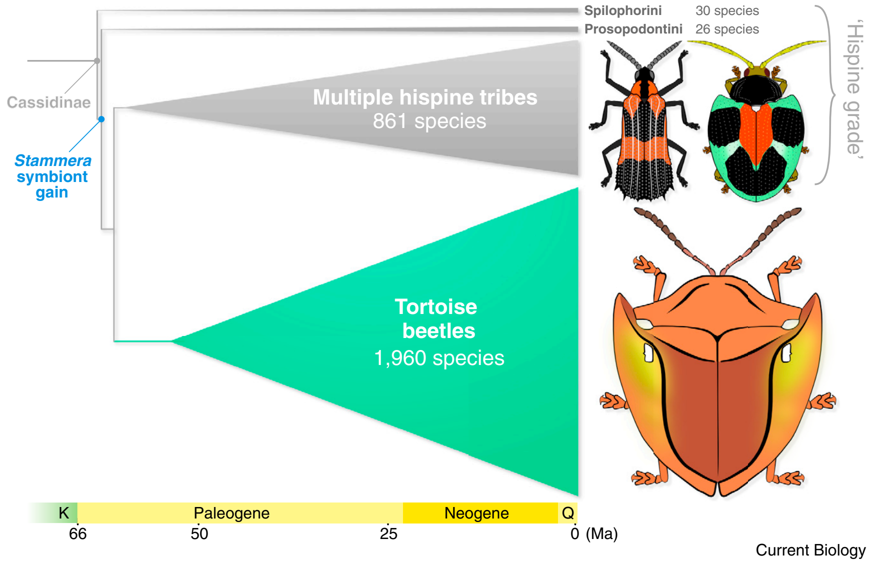

Figure 1. Phylogeny of cassidine leaf beetles (adapted from[12]).
Cassidinae is split into monophyletic tortoise beetles and paraphyletic hispine beetles. *Stammera* acquisition, 62.5 million years ago (Ma), is indicated. Clade sizes are proportional to number of extant species obtained from[20].

walls, which are composed of a polysaccharide matrix of cellulose, hemicellulose and pectin. Early in phytophagan evolution, horizontal gene transfer (HGT) from fungi and bacteria supplied these beetles with plant cell wall-degrading enzymes (PCWDEs), such as hemicellulases and pectinases[12–14]. Genomically equipped to digest plant tissue, phytophagans were primed to exploit the diversifying angiosperms. But certain phytophagans are now known to have gone a step further: they acquired bacterial endosymbionts to do the metabolic work for them[15]. In 1936, the German zoologist Hans-Jürgen Stammer detected microorganisms in the foregut of leaf beetles of the genus *Cassida*, colloquially known as 'tortoise beetles' because of the shell-like dorsum under which these beetles retract their appendages[16]. Eighty years later, the microorganisms were discovered to be a novel bacterial endosymbiont, named *Stammera*, with a tiny genome encoding PCWDEs[17].

Building on this foundational work, García-Lozano and colleagues[2] have extended studies of *Stammera* beyond *Cassida* to the whole Cassidinae subfamily, a clade encompassing more than 6,000 species. Cassidinae includes both the monophyletic tortoise beetles and a paraphyletic group known as 'hispine beetles' (Figure 1). Importantly, tortoise beetles and hispines together comprise three dozen tribes that vary in diversity over three orders of magnitude, from a single species to ~1,000 in the largest tribe, Cassidini. In the new study[2], the authors attempted to connect these disparities in species richness to the relative efficacy of plant cell wall digestion by HGT-acquired versus symbiont-encoded PCWDEs. Performing metagenome sequencing of 55 cassidines spanning 13 tribes, the authors assembled *Stammera* genomes from all beetle species except the five members of the earliest-branching lineage — the small hispine tribe Spilophorini, which diverged from the rest of the subfamily in the Early Paleocene, ~62 million years ago (Figure 1). Acquisition of *Stammera* occurred a mere half a million years later in the stem lineage leading to other cassidines. Subsequent co-cladogenesis of hosts and symbionts occurred as Cassidinae diversified into thousands of species.

Within this phylogenetic framework, the authors traced the evolution of PCWDEs across symbiotic and non-symbiotic cassidines. The ancestral *Stammera* likely possessed three PCWDEs. One of these, named GH28, hydrolyzes the bonds between galacturonic acid units in pectin and is encoded by all *Stammera* genomes. Another enzyme is a pectinase that targets bonds between galacturonic acid and rhamnose; this enzyme has been irreversibly lost in some *Stammera* lineages. So too has the third, ancestrally encoded enzyme, a hemicellulase targeting glucuronic acid–xylose bonds within hemicellulose. In contrast, *Stammera* has also seemingly acquired novel enzymes: *Stammera* from the hispine tribe Arescini, for example, encode a unique pectinase and a hemicellulase. The latter enzyme, GH5, is predicted to target glucomannan, a form of hemicellulose abundant in monocotyledonous plants. García-Lozano and colleagues[2] speculated that GH5 may facilitate dietary specialization of some hispines on monocots such as grasses and palms. Comparing mannanase activity of gut extracts from cassidines possessing *Stammera* with or without GH5, the authors found that, indeed, only the former could efficiently depolymerize glucomannan[2].

In contrast to other cassidines, the earliest-branching, *Stammera*-less Spilophorini possess only the pectinase GH28, encoded by three copies within the beetle's genome, and one genomically encoded hemicellulase (which other cassidines also possess). Arguably, the presence of *Stammera* significantly expands the diversity of sugar–sugar bonds in pectin and hemicellulose that cassidines can potentially digest. The authors hypothesized that, by providing an expanded repertoire of PCWDEs, *Stammera* may have broadened the range of possible plant material that could fuel cassidine diets[2]. In so doing, the bacterium enabled cassidines to more readily undergo host shifts to new plants, increasing the likelihood of speciation. Analyzing relative diversification across the phylogenetic tree, the authors found *Stammera* acquisition coincided with an increase in lineage diversification and host plant utilization[2]. In contrast, the *Stammera*-less Spilomorphini remained species-poor and dietarily narrow (Figure 1).

A bacterially enhanced metabolism, then, might explain inflated species richness within this part of the phytophagan tree. One caveat with this inference should be mentioned, namely that nearly 23 cassidine tribes were not included in this study; also missing were taxa in which evolutionary loss of *Stammera* has been reported[18] (which is curious — have *Stammera* PCWDEs moved into the beetle's genome via HGT in these cases?). The species numbers that were used to compare clade sizes were taken from a study published in 2007[19]; since then, new species descriptions and taxonomic rearrangements have been reported. For example, in Spilophorini — the all-important, *Stammera*-lacking clade — the most recent Hispinae catalogue lists 70 species in four genera[20] instead of 30 species in two genera, as used by García-Lozano and colleagues. How robust the study's conclusions will prove to be in light of these discrepancies is as yet unclear.

Regardless of taxon sampling, the evolutionary scenario inferred by García-Lozano and colleagues[2] is provocative, and the lines of evidence gathered to support it are, together, compelling. Their findings have generated a hypothesis that should now be examined in other herbivorous beetle groups. If endosymbiotic bacteria drove diversification in cassidines, analogous scenarios may have transpired in other, symbiont-harboring beetle taxa, which are increasingly being detected[15]. García-Lozano and colleagues' study is an exceptional piece of integrative biology — one that identifies a putative historical molecular evolutionary contingency within a clade of beetles, provides functional support for it, and attempts to connect it to a macroevolutionary outcome millions of years later. Establishing the generality of the phenomenon of bacterially biased diversification will require comparative studies of equivalent depth across the beetle tree of life.

DECLARATION OF INTERESTS

The author declares no competing interests.

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