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Beetle–Bacterial Symbioses: Endless Forms Most Functional

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Abstract

Beetles are hosts to a remarkable diversity of bacterial symbionts. In this article, we review the role of these partnerships in promoting beetle fitness following a surge of recent studies characterizing symbiont localization and function across the Coleoptera. Symbiont contributions range from the supplementation of essential nutrients and digestive or detoxifying enzymes to the production of bioactive compounds providing defense against natural enemies. Insights on this functional diversity highlight how symbiosis can expand the host's ecological niche, but also constrain its evolutionary potential by promoting specialization. As bacterial localization can differ within and between beetle clades, we discuss how it corresponds to the microbe's beneficial role and outline the molecular and behavioral mechanisms underlying symbiont translocation and transmission by its holometabolous host. In reviewing this literature, we emphasize how the study of symbiosis can inform our understanding of the phenotypic innovations behind the evolutionary success of beetles.

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1. INTRODUCTION

Beetles exhibit extraordinary morphological and physiological diversity and represent Earth's most speciose animal order (43, 87). As key players in nearly all terrestrial and freshwater ecosystems, the Coleoptera have radiated to occupy a multitude of ecological niches (9). This review outlines the role of bacterial symbioses in facilitating the ecological success of beetles. Like many other insect groups (36), beetles engage in associations with a diverse set of bacterial symbionts (**Table 1**). However, insights into how these microbes contribute to beetle fitness have lagged behind histological and morphological descriptions exploring the distribution of bacterial symbionts across this insect order. Molecular and analytical advances have enabled research into the mechanistic basis of beetle–bacterial partnerships, revealing a diverse array of beneficial roles impacting host development, reproduction, nutrition, and defense (4, 26, 46, 101, 110) (**Figure 1**, **Table 1**). This growing body of knowledge continues to highlight important correlations between beetle ecology and symbiosis, as well as the developmental and behavioral traits contributing to the persistence and regulation of bacterial populations within a holometabolous host.

We review eight decades of research on beetle–bacterial symbioses, tying seminal descriptions by Paul Buchner (21) and Hans-Jürgen Stammer (113, 114) to more recent studies exploring symbiont distribution, function, and transmission across this hyperdiverse order. We synthesize this growing literature to provide an overview of how symbioses evolved in conjunction with adaptations that are key to beetle radiations, including cuticle synthesis and the origin of herbivory. Varying in form, many of the partnerships outlined below correspondingly vary in function: from diverse bacterial communities inhabiting the digestive tract (14, 26, 108) to endosymbionts housed within specialized organs and cells (40, 56, 99, 101) (**Figure 2**). We additionally discuss how beetles translocate beneficial symbionts throughout development (82, 83, 120) in light of the challenges and opportunities posed by holometaboly (52). Finally, we detail how symbionts possessing markedly divergent phylogenetic histories converged in function across different beetle clades (4, 17, 40, 56, 99, 103). The pervasiveness of beetle–bacterial symbioses (**Figures 1 and 2**, **Table 1**), coupled with the wide array of recently discovered symbiont-encoded functional traits, reflects the important role that bacterial partners play in the adaptation and evolutionary plasticity of their beetle hosts.

2. FUNCTIONAL DIVERSITY OF BEETLE–BACTERIAL SYMBIOSES

2.1. Plant Biomass Digestion

Herbivory is closely tied to the evolutionary success of the Coleoptera (9, 43, 86, 87), with herbivorous taxa comprising nearly half of all beetle species. This diversity is reflected in rapid diversification rates that coincided with the Jurassic origins of flowering plants (87). Recent genomic efforts point to the integration of microbial digestive enzymes as a key innovation that allowed beetles to capitalize on the emergence and diversification of angiosperms as a novel ecological niche (72, 87). Microbial plant cell wall–degrading enzymes contribute to the efficient digestion of a plant diet rich in recalcitrant polysaccharides such as cellulose, hemicellulose, and pectin (22). These enzymes were acquired by beetles via horizontal gene transfer events (72, 87) or through symbiosis (24, 99, 101, 103). Mapping the distribution of enzymes encoded by the host versus those supplemented by symbiotic bacteria reveals a strikingly clear division of labor between beetles and their bacterial partners (72, 87, 99, 101, 103).

Bess beetles (Passalidae) are key players in forest decomposition and ecosystem turnover in North America, consuming more than four times their body weight in woody debris each day (25). Passalids deconstruct the complex plant polymers within their diet through contributions from their spatially structured gut microbiome, whose members perform complementary metabolic

Table 1 Bacterial symbiont diversity, localization, transmission, and function across the Coleoptera

Family	Subfamily	Beetle species	Symbiont taxon	Localization	Transmission mode	Function	Evidence for symbiont function	Key reference(s)
Bostrichidae	Lycinae	<i>Lycius brunneus</i>	<i>Candidatus</i> Shikimato-generans	Intracellular	Vertical	Possibly tyrosine precursor provisioning	Close phylogenetic relationship to tyrosine-provisioning <i>Shikimatogenerans</i>	40
Bostrichidae	Dinoderinae	Multiple species	<i>Candidatus</i> Shikimato-generans	Intracellular	Vertical	Possibly tyrosine precursor provisioning	Close phylogenetic relationship to tyrosine-provisioning <i>Shikimatogenerans</i>	40
Brentidae	Brentinae	Multiple species	<i>Candidatus</i> Nardonella	Unknown	Vertical	Possibly tyrosine precursor provisioning	Close phylogenetic relationship to tyrosine-provisioning Nardonella	126
Cerambycidae	Lamiinae	<i>Anoplophora glabripennis</i>	Gut bacteria	Extracellular	Unknown	Nitrogen fixation and recycling, amino acid supplementation	Isotope labeling, transcriptome	6, 7
Chrysomelidae	Donaciinae	Multiple species	<i>Candidatus</i> Macroplicolamutae	Intracellular and extracellular	Vertical	Amino acid and B vitamin supplementation, plant biomass digestion	Genome	99
Chrysomelidae	Cassidinae	Multiple species	<i>Candidatus</i> Stammera capleta	Extracellular	Vertical	Plant biomass digestion	In vivo bioassay, genome, transcriptome, enzymatic assays	101, 103
Chrysomelidae	Galerucinae	<i>Psyllodes chrysocephala</i>	<i>Pantoea</i>	Extracellular	Horizontal	Detoxification of plant secondary compounds	In vitro and in vivo bioassays, chemical analysis	108
Chrysomelidae	Chrysomelinae	<i>Leptinotarsa decemlineata</i>	Oral bacteria	Extracellular	Unknown	Inhibition of plant defenses	In vitro and in vivo bioassays, chemical analysis	28, 29
Curculionidae	Dryophthorinae	<i>Rhynchophorus ferrugineus</i>	Gut bacteria	Extracellular	Unknown	Defense against pathogenic bacteria (via host immune stimulation)	In vivo bioassay, transcriptome	91
Curculionidae	Scolytinae	<i>Dendroctonus frontalis</i>	<i>Streptomyces</i>	Extracellular	Unknown	Defense against antagonistic fungus of the beetle's cultivar	In vitro bioassay, chemical analysis	105
Curculionidae	Lixinae	<i>Conorhynchus pallambus</i>	Gut bacteria	Extracellular	Unknown	Nitrogen fixation	Metagenome	10
Curculionidae	Cryptorhynchinae	<i>Eusectes postfasciatus</i>	<i>Candidatus</i> Nardonella	Intracellular	Vertical	Tyrosine precursor provisioning	In vivo bioassay, genome	4, 79

(Continued)

Table 1 (Continued)

Family	Subfamily	Beetle species	Symbiont taxon	Localization	Transmission mode	Function	Evidence for symbiont function	Key reference(s)
Curculionidae	Entiminae	<i>Pachybrachys infernalis</i>	<i>Candidatus</i> Nardonella	Intracellular	Vertical	Tyrosine precursor provisioning	In vivo bioassay, genome	4
Curculionidae	Dryophthorinae	Multiple species	<i>Candidatus</i> Nardonella	Intracellular	Vertical	Tyrosine precursor provisioning	Genome	4
Curculionidae	Dryophthorinae	<i>Strophilus</i> spp.	<i>Sodalis pierantonius</i>	Intracellular	Vertical	Tyrosine precursor provisioning, supplementation of some essential amino acids and vitamins	In vivo bioassay, genome	96, 120
Curculionidae	Scolytinae	<i>Hypothenemus hampei</i>	<i>Pseudomonas fulva</i>	Extracellular	Unknown	Detoxification of plant secondary compounds	In vitro and in vivo bioassays, genome, chemical analysis	26
Curculionidae	Scolytinae	Multiple species	Gut bacteria	Extracellular	Horizontal	Detoxification of plant secondary compounds	In vitro bioassays, genome	2
Curculionidae	Molytinae	<i>Hyllobius abietis</i>	Gut bacteria	Extracellular	Horizontal	Detoxification of plant secondary compounds	In vitro and in vivo bioassays, genome, chemical analysis	14, 17
Nosodendridae	NA	Multiple species	<i>Candidatus</i> Shikimato-generans	Intracellular	Vertical	Possibly tyrosine precursor provisioning	Close phylogenetic relationship to tyrosine-provisioning <i>Shikimato-generans</i>	55
Passalidae	NA	Multiple species	Gut bacteria	Extracellular	Unknown	Plant biomass digestion, fermentation	In vitro and in vivo bioassays, genome, chemical analysis	24, 25
Scarabaeidae	Melolonthinae	<i>Melolontha biprocassanti</i>	Gut bacteria	Extracellular	Unknown	Nitrogen fixation	Isotope labeling	3
Silphidae	Nicrophorinae	<i>Nicrophorus vespilloides</i>	<i>Yarrowia</i> yeasts and gut bacteria	Extracellular	Mixed-mode	Defense against competing microbes	In vivo bioassay, transcriptome	109, 110, 121
Silvanidae	Silvaninae	Multiple species	<i>Candidatus</i> Shikimato-generans sil-vanidophilus	Intracellular	Vertical	Possibly tyrosine precursor provisioning	In vivo bioassay, genome	40, 56
Staphylinidae	Paederinae	Multiple species	<i>Pseudomonas</i> spp.	Extracellular	Mixed-mode	Defense against predators	In vivo bioassays, genome, chemical analysis	69, 97
Tenebrionidae	Lagriinae	Multiple species	<i>Burkholderia gladioli</i> (multiple strains)	Extracellular	Mixed-mode	Defense against pathogens	In vitro and in vivo bioassays, genome, chemical analysis	46, 47

Abbreviation: NA, not applicable.

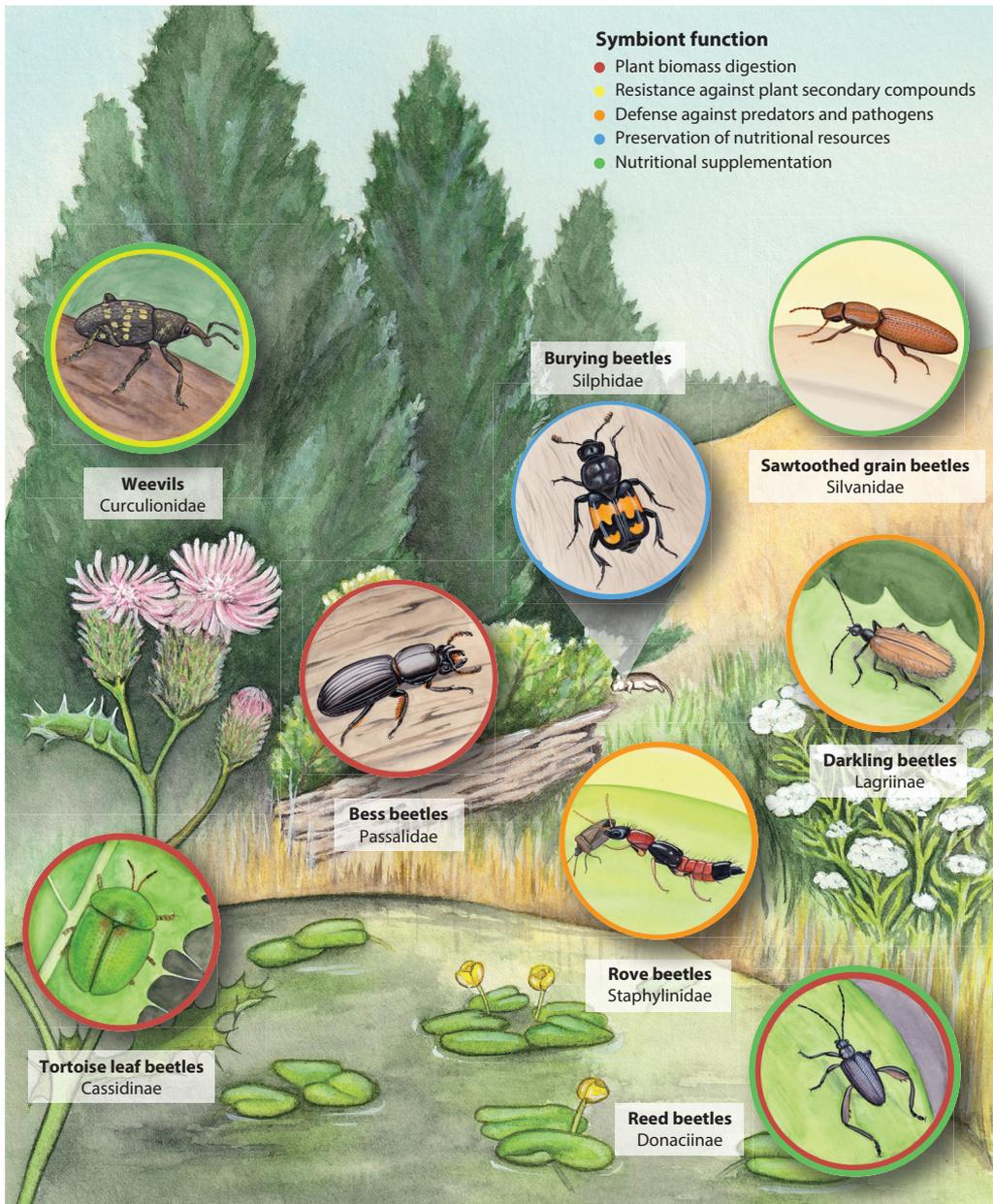


Figure 1

Specialized symbioses with bacterial symbionts promote beetle fitness and adaptation by contributing to plant biomass digestion, facilitating resistance against plant secondary compounds, conferring defense against predators and pathogens, ensuring niche preservation, and/or supplementing nutrition. Representative beetle clades are illustrated, with ring colorations corresponding to symbiont function. Illustration by Julie Johnson.

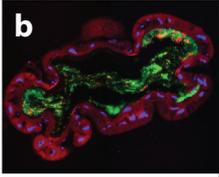
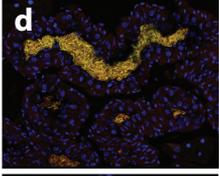
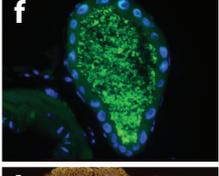
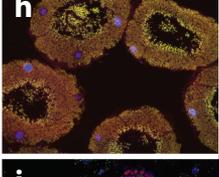
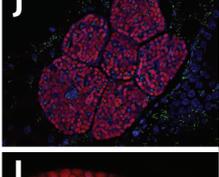
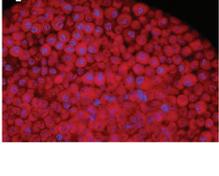
		Symbiont localization	Symbiont role	Symbiont genome size (Mb)
		Extracellular	Preservation of nutritional resources	Unknown
		Extracellular	Defense against pathogens	2.08
		Extracellular	Plant biomass digestion	0.21
		Extracellular/ intracellular	Plant biomass digestion/ nutritional supplementation	0.46
		Intracellular	Nutritional supplementation	0.31
		Intracellular	Nutritional supplementation	0.20

Figure 2

Diversity of symbiont localization, function, and genome evolution in beetles. (a) The burying beetle *Nicrophorus vespilloides* (Silphidae) relies on (b) gut-associated bacterial symbionts for preservation of its nutritional resources. (c) Darkling beetles (Lagriinae: *Lagriia villosa*) harbor (d) *Burkholderia* in specialized cuticle invaginations to fend off pathogenic fungi. (e) The tortoise leaf beetle, *Cassida rubiginosa* (Cassidinae), derives essential digestive enzymes facilitating folivory from (f) *Stammera*, which inhabits gut-associated symbiotic organs. (g) Reed beetles (Donaciinae; shown is *Plateumaris sericea*) harbor (h) *Macroleicola* extracellularly (shown is the symbiont of *Donacia vulgaris*) to support adult folivory, and intracellularly to supplement sap-feeding in larvae. (i) Sawtoothed grain beetles (Silvanidae: *Oryzaephilus surinamensis*) derive tyrosine precursors from (j) *Shikimatogenerans* to support cuticle biosynthesis, similar to the symbiosis between (k) weevils (Curculionidae; shown is *Pachyrhynchus infernalis*) and (l) *Nardonella*.

functions (24, 25). This is facilitated by morphologically differentiated gut compartments varying in cuticle thickness and physiochemical conditions (25). Symbiont depolymerization of lignocellulose is achieved in aerobic gut regions (midgut, posterior hindgut), while fermentation, acetogenesis, and methanogenesis are carried out by bacteria in the anaerobic hindgut (24).

The diversity of symbiont-encoded digestive functions in passalids contrasts with how members of the speciose Phytophaga clade deconstruct plant cell wall polymers (23, 72, 87). Within this group, a conserved set of cellulases, xylanases, and pectinases are horizontally acquired and encoded by most subfamilies spanning the Chrysomelidae (leaf beetles), Curculionioidea (weevils), and Cerambycidae (longhorn beetles) (87). The integration of these genes into beetle genomes followed a series of horizontal gene transfer events from bacterial and fungal donors (87). However, despite the adaptive importance of these enzymes, secondary gene losses took place in some lineages, which were offset through independent acquisitions of heritable, obligate symbionts (15, 16, 99, 101, 103).

In tortoise leaf beetles (Chrysomelidae: Cassidinae), the insect's pectinolytic metabolism is outsourced to its γ -proteobacterial symbiont *Candidatus Stammera capleta* (*Stammera* below) (101, 103) (**Figure 2e,f**). *Stammera* possesses a drastically reduced genome (0.21 Mb) that is streamlined to produce and export pectin-degrading enzymes (11, 101, 103). Symbiont loss compromises the beetle's ability to digest pectin, resulting in low larval survivorship relative to symbiont-bearing insects (101). Despite a high degree of genomic conservation, the symbionts vary across host species in the pectinases that they encode and supplement (103). While all symbiont strains encode polygalacturonase, only a subset of species additionally supplement rhamnogalacturonan lyase to their hosts. Beetles symbiotically endowed with both enzymes exhibit a broader digestive range and exploit a wider variety of host plants (103) relative to cassidines solely supplemented with polygalacturonase. Akin to the horizontal acquisition of genes encoding cellulases, xylanases, and pectinases that catalyzed the evolution of increasingly varied plant-feeding habits in beetles (87), symbiosis can have similar consequences by upgrading the digestive physiology of the insect host.

In reed beetles (Chrysomelidae: Donaciinae), variation in symbiont localization and metabolic potential reflects differences in the beneficial role of *Candidatus Macropleicola muticae* (*Macropleicola* below) (**Figure 2g,b**) relative to *Stammera* (74, 76–78, 99). While reed beetles are specialized folivores of aquatic plants during adulthood, larvae presumably subsist on sap from the host plant's submerged roots (77). *Macropleicola* is essential for donaciine survivorship (74) and encodes host-beneficial factors that appear to be specific to the beetle's developmental stages (99). Corresponding to differences in host nutritional ecology throughout development, *Macropleicola* encodes polygalacturonases to support folivory in adults feeding on pectin-rich plants while additionally supplementing essential amino acids and the B vitamin riboflavin to complement an imbalanced sap-based diet for larvae (99). This dual symbiotic role is also reflected in differences in symbiont localization throughout the host's life cycle (99). The intracellular localization of *Macropleicola* within larval midgut caeca may facilitate the efficient supplementation and exchange of micronutrients to the host, similar to obligate endosymbionts in tsetse flies, aphids, and carpenter ants (35, 36). In contrast, *Macropleicola* subsists both intra- and extracellularly within enlarged Malpighian tubules in adults, with the extracellular localization presumably facilitating the transport of polygalacturonases into the gut to aid in the digestion of foliage.

2.2. Degradation of Plant Secondary Metabolites

Plant allelochemicals, and the responses that they elicit, can drive plant–herbivore coevolution (12, 38, 39). These structurally diverse metabolites include cyanogenic glycosides, glucosinolates, alkaloids, flavonoids, and terpenoids (89). Against phytophagous beetles and other insects, they function either through direct activity or by recruiting predators or parasitoids in multitrophic alliances (42). Through the ability to detoxify, sequester, excrete, or selectively bind plant secondary metabolites, beetles can overcome the plants' defenses, exemplifying the arms race that can

govern insect–plant interactions (33). In addition to the beetles’ own detoxifying enzymes, bacterial communities are increasingly recognized as key players conferring toxin resistance to their hosts (2, 8, 14, 17, 27–29, 51, 108, 127) (**Table 1**).

Caffeine, a purine alkaloid, induces paralysis and intoxication in insects by inhibiting phosphodiesterase activity (93, 118). Despite the enrichment of caffeine in beans of *Coffea* plants (93), the coffee berry borer, *Hypothenemus hampei* (Curculionidae: Scolytinae), completes its entire life cycle within the seed (49). However, the insect’s ability to detoxify caffeine is compromised after perturbation of the resident bacterial community using antibiotics (26). This corresponds to an arrested developmental profile with larvae being unable to complete pupation (26). The detoxifying function is attributed to *Pseudomonas fulva*, a caffeine demethylase–encoding bacterial species consistently isolated from the gut of *H. hampei* (26). While the transmission route of *P. fulva* is unknown, the absence of the symbiont from other bark beetles, including the close relatives *Hypothenemus crudiae* and *Hypothenemus eruditus*, reflects the specialized nutritional ecology of the coffee berry borer (26).

Conifer-feeding beetles contend with similar challenges given the enrichment of terpenoids and flavonoids in bark and cambium (67). In vitro cultivation of terpene-degrading bacterial symbionts from bark beetles highlighted the possible role of the gut microbiome in mitigating the adverse effects of terpene consumption (2). Metagenomic sequencing revealed the genetic basis of terpene degradation, uncovering a *dit* gene cluster putatively involved in this process across conifer-feeding beetles, including the bark beetle *Dendroctonus ponderosae* (Curculionidae: Scolytinae) (2) and the pine weevil *Hylobius abietis* (Curculionidae: Molytinae) (17). While *H. abietis* can degrade several diterpenes, this ability is compromised following antibiotic treatment, consistent with the loss of symbiont-encoded *dit* genes (17). The detoxifying role of the gut microbiome is further demonstrated by the way in which bark beetles contend with the induced enrichment of naringenin, a defensive flavonoid, in their diet (27). Beetles are able to detoxify naringenin by relying on the degradative capacity of the gut symbiont *Novosphingobium* (27). Given that the bacterial associates of conifer-feeding beetles display a high degree of conservation relative to other members of the Curculionidae, this overlap may reflect the adaptive importance of the gut microbiome in upgrading the nutritional quality of coniferous bark (14).

Finally, beetles can also release symbionts to suppress the plant’s antiherbivore defense. The Colorado potato beetle, *Leptinotarsa decemlineata* (Chrysomelidae: Chrysomelinae), induces the upregulation of antimicrobial plant effectors at the expense of signaling pathways dedicated to herbivore deterrence by exuding a microbial consortium consisting of *Stenotrophomonas*, *Pseudomonas*, and *Enterobacter* (28, 29). Thus, *L. decemlineata* appears to capitalize on the crosstalk and tradeoffs governing plant defensive pathways to evade the plant defenses through the deployment of its oral microbiome.

2.3. Defense Against Natural Enemies

Natural enemies exert strong selective pressures on insects to evolve effective behavioral, structural, physiological, immunological, and/or chemical defenses. In beetles, microbial symbionts can contribute to defense against predators and pathogens by enhancing cuticle formation and sclerotization, competitive exclusion of pathogens, or stimulation of the host’s immune system or through the production of bioactive secondary metabolites (46, 91, 97, 123).

Staphylinid beetles of the genus *Paederus* are chemically defended by the toxin pederin (69). While this compound has no impact on most insect predators, pederin-containing beetle larvae effectively deter wolf spiders and thereby commonly survive their attacks (69). Pederin is not produced by the beetle itself but derived from bacterial symbionts of the genus *Pseudomonas* via a

trans-AT polyketide synthase that was likely acquired through horizontal gene transfer (68, 97, 98). The highest concentrations of pederin are observed in eggs and first-instar larvae (69), possibly reflecting the vulnerability of these life stages to predation.

In addition to predators, pathogenic fungi pose an existential threat to many beetles, particularly for the immature life stages of species developing in the soil. To protect their eggs from fungal pathogens, some tenebrionid beetles of the Lagriinae engage in a defensive partnership with bacteria (45–47) (**Figure 2c,d**). Recent molecular work identified the symbionts as *Burkholderia gladioli*, with multiple strains colonizing individual beetles (45–47). Bioassays in *Lagria villosa* revealed the protective activity of the bacteria against fungal infection of the eggs, and multiple bioactive secondary metabolites were identified from different symbiont strains that could contribute to antimicrobial defense (34, 45, 47, 94). Symbiotic *Burkholderia* strains differ substantially in their genome sizes, with the most abundant strain in natural *L. villosa* populations possessing a strongly reduced genome size (2.3 Mb) (47, 124). This strain encodes a large polyketide/non-ribosomal peptide synthetase gene cluster that is responsible for the production of the bioactive compound lagriamide (47) and was likely acquired horizontally (124), akin to the secondary metabolite gene cluster in *Paederus* beetles (98).

Intestinal symbionts can also provide colonization resistance against gut pathogens. A study in the red palm weevil, *Rhynchophorus ferrugineus* (Curculionidae: Dryophthorinae), revealed compromised immune responses and increased pathogen susceptibility in germ-free versus symbiont-bearing insects (91). Similarly, native microbial colonizers of the burying beetle gut (Silphidae, Nicrophorinae: *Nicrophorus vespilloides*) outcompete pathogenic bacteria and thereby enhance larval survival (123). Across several other beetle taxa, culture-based approaches identified microbial isolates with inhibitory activities against microbial pathogens or entomopathogenic nematodes (19, 54, 64, 112), but the chemical basis, ecological importance, and evolutionary dynamics of these associations remain to be elucidated.

2.4. Preservation of Nutritional Resources

Monopolization of rich nutritional resources represents an adaptive strategy to ensure food supply during offspring development but at the same time poses the risk of spoilage due to opportunistic or specialized pathogens (62). Burying beetles (Silphidae, Nicrophorinae) locate carcasses of small vertebrates and bury them in the soil to nourish their offspring, exhibiting variable degrees of mono- or biparental care (106). Beetle reproductive success and larval growth are higher on fresh than on decaying carcasses, indicating that microbial competitors reduce the quality of the nutritional resource for the offspring (100). Concordantly, burying beetles prefer fresh carcasses (100) and manipulate the microbial community on the carcass to prevent putrefaction (109). While this is partially due to the beetle's antimicrobial secretions that reduce the growth of antagonistic microbes (5, 32, 50), another major driver for the protection of the carcass from decay is the beetle's microbial gut community, including multiple bacterial taxa (particularly in the Enterobacteriales, Xanthomonadales, Neisseriales, Lactobacillales, and Clostridiales), as well as yeasts of the genus *Yarrowia* (66, 110, 121) (**Figure 2a,b**). These microbes are transmitted by the adult beetle onto the carcass, where they grow in a biofilm-like matrix and serve as an inoculum for the offspring (37, 109, 123). On the carcass, the microbial symbionts prevent the growth of antagonistic microbes and the concurrent build-up of toxic polyamines including putrescine and cadaverine while at the same time contributing to the production of digestive enzymes (particularly lipases) and metabolites that may aid larval digestion and nutrition (109). Removal of the microbial biofilm leads to reduced larval growth, indicating that the beetle's manipulation of the carcass microbial community ensures the nutritional quality of the offspring's food resources (109). Although most

of the experimental work on the dynamics and functional importance of microbial symbionts has been restricted to *N. vespilloides*, a comparative analysis revealed similar compositions of bacterial and fungal microbial communities across eight different Silphidae species, indicating that the microbial contribution to carcass feeding and preservation is likely widespread in carrion beetles (66).

2.5. Nutritional Supplementation

Nutritional symbioses with microbes are widespread across insects, particularly in taxa feeding on imbalanced food sources like plant sap or vertebrate blood (36). The symbionts generally provision nutrients that the host cannot obtain in sufficient quantities from the diet and lacks the metabolic capability to produce, including essential amino acids, vitamins, and—less commonly—sterols (35). In beetles, both extra- and intracellular symbionts supply their insect host with limiting nutrients. In wood-feeding longhorn beetles (Cerambycidae), bacterial gut symbionts were reported to be involved in nitrogen fixation and recycling, as well as the production of essential amino acids (6, 107). Similarly, there is evidence that gut bacteria contribute to nitrogen fixation in forest cockchafers (3) and desert-dwelling weevils (10). Nutritional associations with symbionts transitioning between intra- and extracellular localization in specialized organs occur between Anobiidae and yeasts (for a review, see 84), as well as between Donaciinae and the bacterial symbiont *Macrolepicola*, as described above (76, 99).

Exclusively intracellular symbionts harbored in bacteriomes have been found in Curculionidae, Brentidae, Silvanidae, Bostrichidae, Nosodendridae, Throscidae, and Dasytidae (4, 21, 40, 53, 55, 56, 79, 117, 125). However, functional studies and genomic analyses have so far been restricted to some species of weevils (Curculionidae) and the grain pest beetle *Oryzaephilus surinamensis* (Silvanidae) (**Figure 2i,j**). The highly eroded genomes of *O. surinamensis*'s Bacteroidetes symbiont *Candidatus* Shikimatogenerans silvanidophilus and the γ -proteobacterial symbiont *Nardonella* that occurs in two weevil families (Curculionidae and Brentidae) (**Figure 2k,l**) encode a functionally complete shikimate pathway for the biosynthesis of tyrosine and phenylalanine precursors (chorismate or prephenate), while lacking pathways for all other essential and non-essential amino acids and vitamins (4, 70). Tyrosine constitutes the precursor for the biosynthesis of the catecholamines and melanin, which are in high demand for the tanning and sclerotization of the cuticle, respectively, conferring on the cuticle its coloration and rigidity (95). As a shikimate pathway for the production of aromatic compounds is generally lacking across insects, beetles feeding on limiting diets benefit from symbiotic bacteria providing precursors for tyrosine biosynthesis, particularly given the high cuticular investment in the formation of the strongly sclerotized front wings, the elytra (80). Experimental suppression of the symbionts in *O. surinamensis*, *Euscapes postfasciatus*, and *Pachyrhynchus infernalis* (Curculionidae) resulted in beetles with thinner and less melanized cuticles, supporting the symbionts' role in cuticle sclerotization and melanization (4, 40, 56, 79). Similar effects were observed when eliminating the γ -proteobacterial symbiont *Sodalis* in *Sitophilus* species (Curculionidae) (120). However, the genome of this symbiont encodes additional amino acid and vitamin biosynthesis pathways, as the symbiosis is considerably younger than that of *Nardonella* and *Shikimatogenerans*, explaining the nascent stage of genome erosion (96). While functional studies are still lacking for the symbioses of Brentidae, Bostrichidae, Nosodendridae, Throscidae, and Dasytidae, the close phylogenetic relationships of some Brentidae symbionts with *Nardonella* (126) and of the Bostrichidae and Nosodendridae symbionts with *Shikimatogenerans* (40, 56) suggest similar functional roles and indicate that symbiont-mediated tyrosine provisioning may represent a widespread phenomenon in beetles. This is noteworthy because specialized tyrosine-supplementing symbioses appear to be rare outside of the Coleoptera, with the ant *Cardiocondyla*

obscurior and its intracellular bacteriome-localized symbiont *Candidatus* Westeberhardia cardiocondylae being, to our knowledge, the only reported case of an exclusively tyrosine-supplementing symbiosis beyond beetles (73). However, further functional analyses across beetles are necessary to understand the full extent and the evolutionary importance of tyrosine-supplementing symbioses in the most diverse insect order, as well as the implications of symbiont replacements, notably in the largest beetle family, the weevils (117).

Given the importance of the insect cuticle as a protective barrier against water loss and natural enemies, tyrosine-supplementing symbioses impact multiple ecologically relevant host traits. The deficiencies in cuticle thickness, sclerotization, and melanization in experimentally symbiont-deprived *O. surinamensis* result in impaired desiccation resistance and reduced population growth, especially under conditions of low ambient humidity (40). However, symbiont-mediated tyrosine supplementation can also impose metabolic costs on the host (41). Experimental addition of tyrosine to the diet results in reduced symbiont titers, indicating that the host mitigates the metabolic burden imposed by the symbionts' nutritional demands when they are no longer needed (70). Similarly, studies on cereal weevils (*Sitophilus oryzae*, *Sitophilus zeamais*, and *Sitophilus granarius*) revealed the host-controlled elimination of tyrosine-supplementing symbionts in adult beetles' bacteriomes following the completion of the cuticle (120). Thus, studying nutritional symbioses in beetles not only yields insights into the ecological and evolutionary importance of symbiont-mediated nutrient supplementation, but also provides opportunities to understand the regulatory mechanisms controlling mutualistic associations.

3. SYMBIONT LOCALIZATION, TRANSLOCATION, AND TRANSMISSION

The range of functions conferred by symbionts to their beetle hosts is reflected in their localizations and transmission routes (Table 1, Figure 2). Symbionts providing digestive or detoxifying enzymes are consistently localized extracellularly in the gut or in gut-associated organs (17, 26, 99, 101, 103). This facilitates the transport of enzymes into the gut lumen without the need for traversing host membranes. Symbionts that defend their host or its nutritional resource from pathogens through competitive exclusion or the production of bioactive secondary metabolites are also localized extracellularly, either in the gut (110, 123) or in specialized cuticle-lined organs connected to the outer surface (46, 105). The localization on external or internal surfaces of the host may be particularly advantageous by providing defense against pathogens at an early stage of the infection process (44). While intracellular defensive symbionts are known from other insects (92), as well as from marine invertebrates (116), most of these appear to defend their hosts against predators or parasitoids, rather than pathogens. Variation in antagonist pressures may select for different symbiont localizations in the host (44). Finally, nutrient-supplementing symbionts can be part of the extracellular gut community (6, 7), transition between intra- and extracellular localization in specialized organs (99), or be located intracellularly in bacteriomes (4, 56, 82, 125). In the last case, the provisioning of tyrosine precursors appears to be a particularly important and widespread function in beetles (4, 40, 56). In summary, while multiple localizations for symbionts of any particularly functional category occur in beetles, localization in specialized symbiont-bearing organs allows us to make predictions regarding the potential function of the symbiont (Table 1, Figure 2).

During their development, beetles undergo complete metamorphosis, entailing the restructuring of most of the internal organs. This poses the problem of symbiont maintenance and translocation in the pupal stage but may also provide an opportunity for adaptive decoupling of symbiont localization and function (52). In some cases, bacteriomes harboring intracellular symbionts are

largely unchanged throughout this process (82). The symbionts of reed beetles, however, translocate from an intracellular localization in larval mid-gut organs to an intra- and extracellular occurrence in adult Malpighian tubules, coinciding with the aforementioned change in function (Section 2.1) (99). In other cases, symbiotic organs degenerate in the adult stage when symbiont contributions are no longer needed (120). The problem of symbiont maintenance and translocation during metamorphosis, as well as their relocation to specialized organs for transgenerational symbiont transmission, is solved in a multitude of ways across beetles, a thorough synthesis of which merits a separate review. We refer the interested reader to the histological examinations by Paul Buchner and his students (see 21), which provide detailed accounts of symbiont localization, translocation, and transmission for many beetle symbioses that are still being deciphered on the functional level. In this section, we briefly highlight a few key points. (a) In contrast to previous claims that the gut microbiota is completely removed during metamorphosis, recent studies in Lepidoptera demonstrate the survival of gut symbionts during the reorganization of the digestive tract, enabling the maintenance of a core gut microbiota throughout holometabolous development (63). (b) Extracellular and external symbionts can be translocated outside of the beetle's body (110), circumventing the challenges associated with tissue reorganization during metamorphosis but necessitating mechanisms for symbiont reacquisition on the host side and adaptations for survival outside of the host on the part of the symbiont. (c) Bacteriome-localized symbionts can experience translocation events through the movement of entire bacteriomes, transport of bacteriocytes, or release and translocation of symbionts to their novel destination, but the mechanisms guiding and controlling these processes remain poorly understood. Maire et al. (82) provide valuable insights into the process and molecular basis of bacteriocyte translocation along the gut during metamorphosis and the formation of adult bacteriomes in *S. oryzae* (Curculionidae). Bacteriocyte movement coincides with changes in cell morphology and the upregulation of host genes involved in cell adhesion, cell motility, cellular shape, and cytoskeletal reorganization (82). At the bacteriocytes' final destination, the symbionts express virulence genes and invade stem cells that subsequently differentiate into the adult beetle's bacteriocytes (82).

Symbiont acquisition routes in beetles are similarly varied, facilitating the horizontal, vertical, or mixed-mode transmission of beneficial microbes among host individuals (**Table 1**). Horizontal exchange is especially prevalent among herbivorous beetle clades harboring detoxifying symbionts (61). This is evident in the compositional overlap observed between the beetles' gut bacterial communities and environmental microbes subsisting on their host plants (14, 17, 108). As many detoxifying symbionts appear to be acquired from the environment every generation, the plant-associated microbiome is increasingly recognized as a source of metabolic innovation to detoxify noxious secondary metabolites in plant-based diets (61). Mixed-mode transmission is most commonly described in insect clades supplying their offspring with symbionts extracellularly during the egg stage (58, 65, 71, 104). Smearred through specialized secretions, symbiont populations are propagated vertically, but horizontal exchange among con- and heterospecifics can also take place (102). This dynamic governs the defensive partnership between darkling beetles and *Burkholderia*, where the symbiont is transmitted both vertically via maternal secretions over the egg surface and horizontally through the host plant (45, 46). As with many obligate partnerships described between hemipteran, hymenopteran, and dipteran insects and their nutritional endosymbionts (20, 75, 81), a subset of beetle clades evolved adaptations that ensure the strict vertical transmission of beneficial microbes. These beetles have elaborate transmission organs in adult females, and symbiont populations are transmitted intracellularly during oogenesis (56, 79) or encapsulated and supplied to the egg surface for later uptake by the hatching larvae (74, 101) (**Table 1**). The restricted localization within the host throughout development and vertical transmission to the offspring often entail severe population bottlenecks and offer minimal opportunities for genetic

recombination (85). Consequently, convergent patterns of reductive genome evolution commonly afflict beetle symbionts, as noted for *Nardonella* (0.2 Mb) (4), *Stammera* (0.21 Mb) (11, 101, 103), *Macropoleicola* (0.46 Mb) (99), and *Shikimatogenerans* (0.31 Mb) (70) (**Table 1**).

4. PARALLELS AND CONTRASTS TO BEETLE–FUNGAL MUTUALISMS

Obligate symbioses between beetles and fungi arose independently at least 16 times (18, 60). The stability and persistence of these partnerships—the oldest dating from more than 100 Mya (119)—reflect the intertwined evolutionary history between two diverse eukaryotic clades (60). Fungal mutualists are a key source of adaptations in the Coleoptera (48), converging functionally with many of the aforementioned bacterial partnerships (**Table 1**) by contributing digestive (31, 59) and detoxifying enzymes (30), as well as defensive compounds to fend off pathogenic infections (122). However, one notable distinction broadly separating fungiculture and bacterial–beetle symbioses centers on whether the microbial partner can also be classified as food (57). Fungiculture defines some of the best-studied beetle–fungal mutualisms and has evolved in clades specializing on poor nutritional substrates, including phloem or wood (90). In exploiting a dietary niche rich in carbon, but deficient in nitrogen, phosphorus, and other elements (35), these beetles propagate, grow, and consume their cultivars as a primary source of nutrition (90), as demonstrated in other fungus-farming insect clades, including termites (1), ants (90), and stingless bees (88). Because filamentous fungal symbionts are able to synthesize essential sterols, vitamins, and amino acids, in addition to concentrating trace elements from the environment, they can sustain their hosts as a nutritional resource throughout the insect’s life cycle (111). While bacterial gut symbionts may fulfill similar roles for their beetle hosts (57), highly streamlined mutualisms may be constrained by the limited metabolic potential of symbionts possessing tiny genomes (85). The beetle may lyse its symbiont to extract specific nutrients or recycle the microbe when it is no longer needed (120), but the beneficial factors defining these partnerships complement the host’s diet, in contrast to the sustaining and broad nutritional value of fungal cultivars (57).

5. CONCLUSIONS AND OUTLOOK

Beetles engage in diverse associations with symbiotic bacteria that can confer a range of benefits to their hosts. By providing digestive and detoxifying capacities, nutritional supplementation, or protection for the host or its nutritional resources against natural enemies, microbial symbioses can expand ecological niche space for host lineages (**Table 1, Figure 1**). This is reflected in the fact that bacterial symbionts promote host fitness across ecologically diverse beetles, spanning herbivorous (4, 17, 24, 28, 40, 56, 99, 101, 103, 120), necrophagous (109, 110), omnivorous (45, 46), and predacious taxa (97, 98) (**Figure 1**). However, drastic genome erosion experienced by symbiotic microbes may also constrain beneficial contributions to the insect host (99), limiting their adaptive potential (13). A striking example is provided by reed beetles and their nutritional symbionts: While the capacity to produce many of the essential amino acids is retained across the symbionts of all host species, both pectinase-encoding genes have been lost at least three times independently, always in beetle lineages feeding on pectin-poor Poales plants (99). However, reversals to pectin-rich diets have not been observed, indicating that the loss of pectinases constrained the potential of the beetles to expand their dietary niche (99).

While the ecological and evolutionary consequences of functionally similar symbioses have been described across several other insect orders, beetles stand out due to their particularly broad diversity of mutualistic partnerships. As such, beetle–bacteria symbioses provide excellent opportunities for elucidating the general principles underlying symbiotic associations from the

molecular level to the phenotypic consequences in the natural environment. Dissecting the gradient of host–symbiont integration is possible due to the availability of experimentally tractable associations, even including highly intimate intra- and extracellular symbioses (46, 56, 101, 103), which is a rare feature in other insect orders. Similarly, the diversity of localizations within and outside of host cells enables detailed studies connecting symbiont localization and function and provides opportunities for elucidating the molecular basis of symbiont translocation events during metamorphosis (82), as well as transitions between extra- and intracellular lifestyles (99).

Finally, the large number of independently evolved symbioses in beetles present many incidences of convergence in localization and/or function (4, 40, 56, 120) that allow for comparative analyses to derive general principles regarding the evolutionary trajectories of host–symbiont associations. Intensive research efforts over the past decades on similarly dynamic associations in Hemiptera have provided important insights into the coevolutionary process and its consequences in mutualisms (13, 115). Future research directed at gaining a comprehensive picture of symbiont functionalities within and across beetle families and expanding the molecular toolbox for experimentally tractable systems (4, 82, 120) will continue to yield insights into both the molecular basis of host–symbiont interactions and the importance of microbial symbionts for adaptation and diversification of the most speciose animal order.

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