Eurasia Specialized Veterinary Publication

Entomological Research Letters

ISSN: 3108-639X

2024, Volume 4, Issue 2, Page No: 162-172
Copyright CC BY-NC-SA 4.0
Available online at: www.esvpub.com/

Transmission, Functional Roles, and Context Dependence Across Insect–Microbiome Partnerships During Development

Elena Petrova[1]*, Ivan Georgiev[1], Nikolay Stoyanov[2], Petar Kolev[1]

[1]Department of Microbiome Transmission and Developmental Roles, Faculty of Life Sciences, Medical University of Sofia, Sofia, Bulgaria.
[2]Department of Context Dependence in Symbiosis, Faculty of Biosciences, Technical University of Sofia, Sofia, Bulgaria.

***E-mail** ✉ elena.petrova@gmail.com

ABSTRACT

Microorganisms associated with insects can be maternally transmitted, socially transferred, environmentally reacquired, transiently ingested, or developmentally maintained in specialized host tissues. These biologically different partnerships are often grouped under the term insect microbiome, obscuring differences in persistence, functional dependence, causal support, and ecological context. This evidence-mapping review organizes recent work around three linked questions: how microbial partners are transmitted or reacquired during development, which nutritional, immune, and developmental functions have direct experimental support, and when those functions change with diet, environment, host stage, or taxonomic setting. Strong microbial dependence occurs in particular systems, including specialized nutritional symbioses and experimentally tractable gnotobiotic models, but important counterexamples exist. Environmental acquisition may be behaviorally targeted or host-filtered, microbial effects may depend on nutrient limitation or strain identity, and community change does not necessarily imply altered host fitness. We therefore propose separating microbial occurrence, persistence, transmission, functional association, perturbation response, rescue or sufficiency, mechanistic resolution, and ecological consequence as distinct evidential stages. This structure identifies where evidence is comparatively strong, where causal interpretation remains unresolved, and where laboratory mechanisms require field validation.

Keywords: Insect microbiome, Symbiont transmission, Gnotobiotic insects, Nutritional symbiosis, Host–microbe immunity, Developmental microbiology

Received: 15 November 2024
Revised: 26 December 2024
Accepted: 28 December 2024

How to Cite This Article: Petrova E, Georgiev I, Stoyanov N, Kolev P. Transmission, Functional Roles, and Context Dependence Across Insect–Microbiome Partnerships During Development. Entomol Res Lett. 2024;4(2):162-72.
https://doi.org/10.51847/xSUkww9b8t

Introduction

Microbial partnerships can affect insect development through nutrition, signaling, immune interaction, tissue specialization, and modification of the physicochemical environment experienced by the host. Yet the biological meaning of an "insect microbiome" differs substantially among systems. Some insects maintain intimate microbial associations through development, whereas others repeatedly acquire organisms from food, conspecifics, or surrounding habitat. Developmental effects therefore need to be interpreted in relation to microbial identity and the life stage at which an interaction occurs [1].

The honeybee illustrates one end of this spectrum. Its characteristic gut community is experimentally tractable, permitting defined-colonization studies, metabolic analyses, and pathogen challenges, with reproducible effects on several physiological processes. Even in this unusually well-characterized system, however, field perturbations

© 2024 Entomological Research Letters

and microbial interventions are not uniformly expressed across host ages, colonies, or bacterial lineages [2]. Strong model-system evidence establishes biological possibility more securely than generality across insects.

Caterpillars provide an important counterexample. Broad comparative sampling found low bacterial abundance, high among-individual variability, and substantial similarity between gut-associated and environmentally encountered microorganisms. Antibiotic suppression in experimentally tested *Manduca sexta* larvae did not significantly alter major growth, developmental, or survival endpoints [3]. This does not show that Lepidoptera lack meaningful symbioses; it shows that dense resident gut communities and strong microbial dependence cannot be assumed from microbial detection alone.

Comparative work similarly indicates that obligate symbioses are concentrated in nutritionally imbalanced feeding niches and that their association with host diversification varies among ecological settings [4]. A vertically inherited intracellular nutritional symbiont, an environmentally acquired gut mutualist, a facultative defensive bacterium, and a transient dietary microbe should therefore not be interpreted through a single evidential model.

This review addresses an inferential problem. Studies differ in whether they demonstrate microbial occurrence, transmission, developmental persistence, phenotype association, perturbation response, experimental rescue, mechanistic mediation, or ecological consequence. These levels are frequently compressed into statements that a microbiome "affects" development. We instead map transmission, nutritional, immune, and developmental evidence while retaining taxonomic, life-stage, dietary, environmental, and methodological boundaries. The proposed synthesis separates evidence stages without assigning a universal certainty score.

Evidence-mapping approach

Evidence was organized by biological and inferential question rather than community composition alone. The principal dimensions were transmission or acquisition mode, developmental stage, persistence, host taxon, environmental setting, measured construct, and experimental design. This matters because increasingly detailed profiling can describe community differences without establishing whether the organisms persist, perform a relevant function, or cause the measured host outcome.

Axenic and gnotobiotic technologies provide strong leverage because they permit microbial depletion, defined recolonization, community reconstruction, and rescue. Nevertheless, sterilization procedures, artificial diets, altered host condition, and simplified communities can generate phenotypes that would not occur under natural conditions [5]. A difference between axenic and conventional insects is therefore not automatically equivalent to a microbiome-specific mechanism.

Variation within host species is another interpretive problem. Microbiomes may differ among populations, colonies, genotypes, developmental stages, and local environments, yet such heterogeneity can be hidden by a single "core" community description [6]. Conversely, compositional variation should not automatically be treated as functional variation.

The review therefore treats causal resolution and ecological transferability as separate dimensions. Laboratory manipulation can show that a microbial partner is necessary or sufficient for a defined endpoint, while field evidence asks whether the relationship survives realistic diet, temperature, exposure, and host-state variation. We propose a descriptive evidence-stage architecture spanning occurrence, acquisition or transmission, persistence, association, perturbation, rescue or sufficiency, mechanism, and ecological consequence. It is an interpretive structure, not a validated numerical grading system.

The searches identified 1244 records across the selected databases and supplementary citation sources. After removal of duplicates, 321 unique records underwent title and abstract screening. Following eligibility assessment of full texts, 32 studies met the inclusion criteria and were incorporated into the final evidence map.

Vertical and horizontal transmission

Transmission determines whether a microbial function can persist through developmental transitions, but "vertical" and "horizontal" are often too coarse to describe the routes involved. Insects with intracellular symbionts may integrate microbial maintenance into bacteriocytes or related tissues, linking host development to symbiont housing, proliferation, and inheritance [7]. This architecture differs qualitatively from gut communities that are lost or reacquired after hatching or metamorphosis.

Symbiodolus illustrates why vertical transmission should not be equated with strict evolutionary codivergence. Detection in reproductive tissues and across life stages supports predominantly vertical maintenance, whereas

host–symbiont phylogenetic discordance is consistent with occasional horizontal transfers among lineages [8]. Developmental continuity and long-term evolutionary transmission therefore answer different questions.

Experimental reinfection provides stronger present-generation evidence than distribution alone. In pea aphids, cultured *Candidatus Fukatsuia* could be reintroduced and then maintained through transovarial transmission across generations [9]. Such experiments connect microbial identity with demonstrable inheritance.

Transmission may become still more anatomically specialized. In planthoppers, an Acetobacteraceae-associated symbiont occurs in a nested cellular arrangement during passage through the female reproductive system [10]. The localization establishes a transmission route, but complexity itself does not prove adaptive advantage or host benefit.

Environmental acquisition can be equally structured. Nymphs of the squash bug *Anasa tristis* use specialized feeding behavior to locate and ingest symbiont-rich conspecific excreta, increasing acquisition reliability [11]. Horizontal transmission is therefore not necessarily stochastic.

Sociality further blurs the vertical–horizontal dichotomy. Trophallaxis, coprophagy, direct contact, and nest material can transfer microbial partners repeatedly among individuals and generations without transovarial inheritance [12]. It is therefore useful to distinguish microbial source, acquisition route, and reliability.

Field evidence also supports host filtering of environmental sources. In *Riptortus pedestris*, gut-community assembly across sites was largely environmentally sourced, while particular *Burkholderia*-associated patterns were linked with host reproductive performance [13]. This does not imply that every environmentally encountered bacterium is beneficial; rather, repeated acquisition and enrichment can generate functional partnerships without maternal inheritance.

Source attribution must also be separated from residency. In controlled plant–soil systems, foliar-feeding insects acquired communities resembling soil more strongly than host leaves [14]. Such similarity identifies an environmental pathway but does not establish durable colonization or host benefit. The resulting transmission map therefore includes developmentally integrated maternal inheritance, predominantly vertical persistence with occasional evolutionary transfer, experimentally demonstrated transovarial maintenance, social transmission, behaviorally targeted acquisition, and broader habitat-derived assembly.

Nutritional functions

Nutrition is among the best-supported functional domains in insect–microbe research, but even here the evidence does not justify a generic claim that microbiomes provision nutrients. B vitamins are illustrative because microbial partners can synthesize or modify vitamin availability, while dependence varies among host taxa, vitamins, and diets [15].

Dietary supply and host genomic capacity provide alternatives that must be considered before microbial dependence is inferred. Some insects obtain sufficient vitamins directly from food, while others possess host-encoded pathways, including functions historically derived from microbial genes [16]. Nutrient limitation establishes a metabolic problem, not the identity of the organism solving it.

Specialized endosymbioses provide stronger evidence when genome reduction retains complementary biosynthetic capacities. Genome-eroded *Burkholderia* symbionts of scale insects preserve pathways consistent with supplementation of nutrients limited in host diets [17]. Yet biosynthetic capacity remains evidence of potential rather than direct measurement of transfer.

Controlled microbiota manipulations resolve this distinction more directly. In adult *Aedes aegypti*, microbiota removal altered fecundity and metabolic physiology, while appropriate microbial or nutritional rescue restored important reproductive deficits [18]. Endpoint specificity remains important because microbial absence did not reduce every component of performance in parallel.

Defined gut colonization in honeybees altered body-weight gain, short-chain fatty-acid profiles, pollen-derived metabolites, and insulin-related signaling [19]. These findings demonstrate metabolic effects but do not make increased mass equivalent to increased colony fitness.

Diet can determine whether a microbial effect appears. Under chronic undernutrition, *Lactobacillus plantarum* promotes *Drosophila melanogaster* larval growth while the host-associated nutritional environment also supports the bacterial partner [20]. Across controlled diets, different *Drosophila*-associated bacteria compensate for different nutritional limitations [21]. Microbial partners are therefore not functionally interchangeable.

A particularly strong contrast comes from predaceous *Toxorhynchites amboinensis*. Larvae consuming axenic prey failed to develop, whereas provision of living bacteria restored gut colonization and developmental

progression [22]. This causal requirement under tested conditions contrasts with caterpillar systems in which suppression produced little measurable performance effect.

Table 1 is needed because "nutritional function" contains mechanistically different evidence states that prose can easily collapse.

Table 1. Nutritional function depends on whether evidence demonstrates metabolic capacity, conditional benefit, physiological mediation, or requirement

Nutritional state	Biological context with observed basis	Main limitation within its limit
Putative vitamin provisioning	Multiple insect taxa Biosynthetic capacity and vitamin physiology Microbial pathways can complement dietary limitation	Encoded pathways do not prove transfer Treat pathway detection as functional plausibility
Diet-or-host substitution	Insects with dietary access or host biosynthetic capacity Alternative nutrient source Requirements can be met without obligate microbes	Contributions shift among diets and stages Test microbial dependence against nonmicrobial alternatives
Genome-reduced nutritional symbiosis	Scale insects Retained symbiont biosynthetic pathways Complementary nutrient functions are preserved	Flux into the host is incompletely measured Supports targeted, not generalized, provisioning claims
Microbiota-dependent reproductive nutrition	Adult Aedes aegypti Fecundity and metabolic phenotype Depletion causes deficits that can be rescued	Endpoints respond differently Rescue strengthens causal attribution for the tested function
Diet-contingent growth promotion	Drosophila larvae Juvenile growth Benefit depends on nutrient limitation and partner identity	Defined diets simplify nature Diet is a moderator of microbial function
Living-microbe developmental requirement	Toxorhynchites larvae Developmental progression Axenic conditions prevent development; living bacteria restore it	Species- and rearing-specific Demonstrates necessity in one system, not across insects

Immune functions

Immune effects are heterogeneous because host immunity shapes microbial colonization while microorganisms also modify host immune state. In mosquitoes, midgut microorganisms interact with epithelial and systemic pathways involved in colonization, barrier function, and pathogen interactions [23]. Immune activation therefore does not automatically imply improved host survival or pathogen resistance.

Strain identity can matter. In honeybees, particular gut-associated strains differed in their ability to protect hosts challenged with *Hafnia alvei*, and stronger protection was associated with Toll-related and antimicrobial-peptide responses [24]. Protective activity should therefore not be assigned automatically to an entire bacterial genus.

Community context also matters. A defined, stably colonizing honeybee community retained inhibitory effects against *H. alvei* while modifying host immune expression [25]. This supports community-associated protection under controlled conditions but does not establish equivalent stability or protection under field diets, competing microbes, or environmental stress.

Resident communities may also inhibit pathogens through mechanisms not reducible to immune induction. An intact honeybee gut microbiota reduced *Serratia* invasion, while a candidate pathogen competition mechanism involving type-VI secretion did not fully explain the protective effect [26]. Demonstrating protection therefore does not mean the causal pathway is completely resolved.

The immune literature consequently supports at least three separable states: host immune modulation associated with colonization, strain- or community-specific pathogen protection, and community-level colonization resistance whose mechanism may remain incomplete.

Developmental functions

Development creates a moving biological target for microbiome analysis. Feeding mode, gut morphology, tissue turnover, endocrine state, immune activity, and environmental exposure can change across larval, pupal, and adult stages. Microbial succession therefore cannot automatically be interpreted as a microbial driver of development. Development itself can restructure microbial habitat, while experimental manipulation shows that microbes can alter growth, maturation, or later physiology in particular systems [27].

The causal examples differ fundamentally. Living bacteria are required for normal development in tested *Toxorhynchites* larvae, whereas *Drosophila* growth effects vary with diet and bacterial identity. In honeybees, colonization alters metabolic and endocrine physiology during early adult maturation, and in *Aedes aegypti* microbial effects extend to adult reproduction. These cases should not be collapsed into a single developmental mechanism.

Applied insect-rearing research broadens the endpoints further. Microbial manipulation can affect larval growth, substrate conversion, survival, and reproduction in mass-produced insects, but responses differ among hosts, substrates, microbial treatments, and husbandry systems [28]. Production performance is therefore relevant but is not automatically equivalent to fitness in natural populations.

We propose four developmental distinctions: host-driven microbial restructuring, microbially supported development, stage-restricted functional dependence, and cross-stage carry-over. They are interpretive categories, not mutually exclusive mechanisms. Development time, body mass, pupation, adult emergence, reproduction, and survival should also remain separate endpoints unless later-life consequences are directly measured.

Table 2 reconciles developmental findings that appear contradictory when life stage, endpoint, and causal design are ignored.

Table 2. Developmental findings diverge when ontogenetic restructuring, stage-restricted dependence, conditional benefit, and carry-over are separated

Developmental distinctio with evidential standing	Evidential basis and warranted reading	Boundary condition and decisive check
Host-driven microbial restructuring **Evidence-supported distinction**	Community change across ontogeny Development may alter microbial habitat	Association does not establish direction Manipulate microbiota independently of stage
Developmentally integrated persistence **Evidence-supported distinction**	Symbionts maintained in specialized tissues Microbial continuity is embedded in host anatomy	Persistence does not show equal function at every stage Couple localization with stage-specific function
Stage-restricted requirement **Evidence-supported**	Axenic developmental arrest with microbial rescue Microbes are necessary under tested conditions	Host-, stage-, diet-, and rearing-specific Identify the required microbial process
Conditional growth promotion **Evidence-supported**	Growth benefit under nutrient constraint Developmental effect depends on diet and partner	Benefit may vanish under other diets Use diet × microbe factorial designs
Adult physiological maturation **Evidence-supported**	Colonization changes adult metabolism or reproduction Microbial effects can occur after metamorphosis	Adult endpoints are not equivalent Separate mechanisms for each endpoint
Development-to-later-life carry-over **Proposed synthesis category**	Earlier microbial state linked with later phenotype Earlier conditions may influence later performance	Direct mediation is often unresolved Use stage-specific recolonization and longitudinal tracking

Context dependence across diet and environment

Microbial function is not an intrinsic property of a taxon isolated from its environment. Host diet, microbial provenance, temperature, chemical exposure, developmental state, and surrounding microbial pools can change both community assembly and the phenotype associated with colonization.

Microbiome provenance provides one example. Adult *Aedes aegypti* receiving microbiota derived from field-caught versus laboratory-reared mosquitoes showed different gut transcriptional responses [29]. The experiment

demonstrates that source can modify host molecular response, but transcriptomic divergence alone cannot identify the community that improves survival or reproduction.

Temperature adds another layer because it acts simultaneously on insects, microorganisms, immune processes, and transmission. Changing temperature can restructure gut communities and modify host–microbe interactions, with responses dependent on host, microbe, exposure regime, and endpoint [30]. Defensive symbioses show the problem directly: temperature can change protection, symbiont cost, and transmission reliability [31].

Symbionts can therefore expand or constrain abiotic tolerance rather than simply strengthening a fixed function [32]. Altered stress tolerance should not automatically be interpreted as adaptation because laboratory phenotypes do not establish long-term fitness advantage.

Chemical perturbation similarly separates microbiome change from host consequence. In honeybees, insecticide–fungicide treatments altered gut communities and increased some opportunistic bacteria in treatment-specific ways [33]. Bumblebees exposed to glyphosate or a glyphosate-based formulation also showed microbiome changes that differed by chemical context [34]. Community disturbance does not by itself demonstrate microbiome-mediated disease or population decline.

Table 3 distinguishes context variables according to what they modify: microbial source, realized function, host response, or ecological transfer.

Table 3. Diet and environment alter whether a microbiome effect is expressed, detectable, or ecologically transferable

Biological setting with observed basis	Context distinction	Interpretation, boundary condition, and test implication
Adult Aedes aegypti **Different transcriptomic responses after field- versus lab-derived transplants**	Microbial provenance	Source modifies host molecular response Transcript change is not fitness Reciprocal transplant with standardized recipients
Developing insects **Microbial effect varies with available nutrients**	Nutritional context	Function can emerge only under constraint Defined diets simplify natural heterogeneity Factorial diet × partner experiments
Multiple insect systems **Temperature-dependent community or physiological response**	Thermal modulation	Temperature affects both partners and interaction Exposure history matters Separate direct host and microbiome-mediated effects
Symbiont-bearing insects **Protection, cost, or transmission changes with temperature**	Defensive-symbiosis temperature dependence	Defensive benefit is conditional Constant laboratory temperatures may mislead Measure protection and transmission across realistic regimes
Bees **Community restructuring after chemical exposure**	Chemical perturbation	Chemicals can alter gut ecology Direct toxicity and microbial mediation can coexist Add causal mediation or recolonization tests
Cross-system synthesis **Laboratory mechanism may weaken or change in natural settings**	Field-transfer boundary	Mechanistic validity and ecological generality are distinct Diet, climate, microbial pools, and host heterogeneity differ Validate laboratory mechanisms independently in realistic contexts

Taxonomic concentration of evidence

Mechanistically detailed evidence is concentrated in honeybees, mosquitoes, *Drosophila*, aphids, and other hemipterans with specialized symbioses. These systems are experimentally advantageous because communities or symbionts can be cultured, removed, reintroduced, localized, or genomically characterized. Their tractability can nevertheless cause a few systems to become implicit models for Insecta.

Honeybees provide deep evidence connecting community composition with metabolism, immunity, and pathogen exclusion; mosquitoes contribute axenic and gnotobiotic developmental experiments; *Drosophila* provides controlled diet–microbe manipulation; and aphids and related hemipterans reveal intracellular transmission and nutritional specialization. Caterpillars provide an important counterweight because tested larval systems show low microbial abundance and weak antibiotic-performance effects.

Evidence density is not biological prevalence. Poorly studied taxa may contain important partnerships that remain undetected, while heavily studied taxa may make their mechanisms appear disproportionately common. We therefore propose three nested questions: where microbes are detected, where transmission or function has been experimentally demonstrated, and where those functions have been challenged across realistic ecological contexts. This asymmetry has two consequences for evidence mapping. First, absence of mechanistic evidence in a poorly sampled order cannot be treated as evidence that microbial functions are absent. Second, repeated demonstration of a mechanism in a tractable model should not be converted into a presumed ancestral or universal insect condition. Within-species microbiome variation further complicates this comparison because evidence density can be high even when the functional distribution of strains or communities remains incompletely characterized [6]. The mapping problem is therefore simultaneously taxonomic and methodological: taxa differ in biology, but they also differ in how easily their microbes can be cultured, manipulated, localized, or experimentally reconstructed.

The distinction is compressed in **Figure 1** so that model-system concentration, counterexamples, uncertainty, and ecological-transfer boundaries remain visually separable.

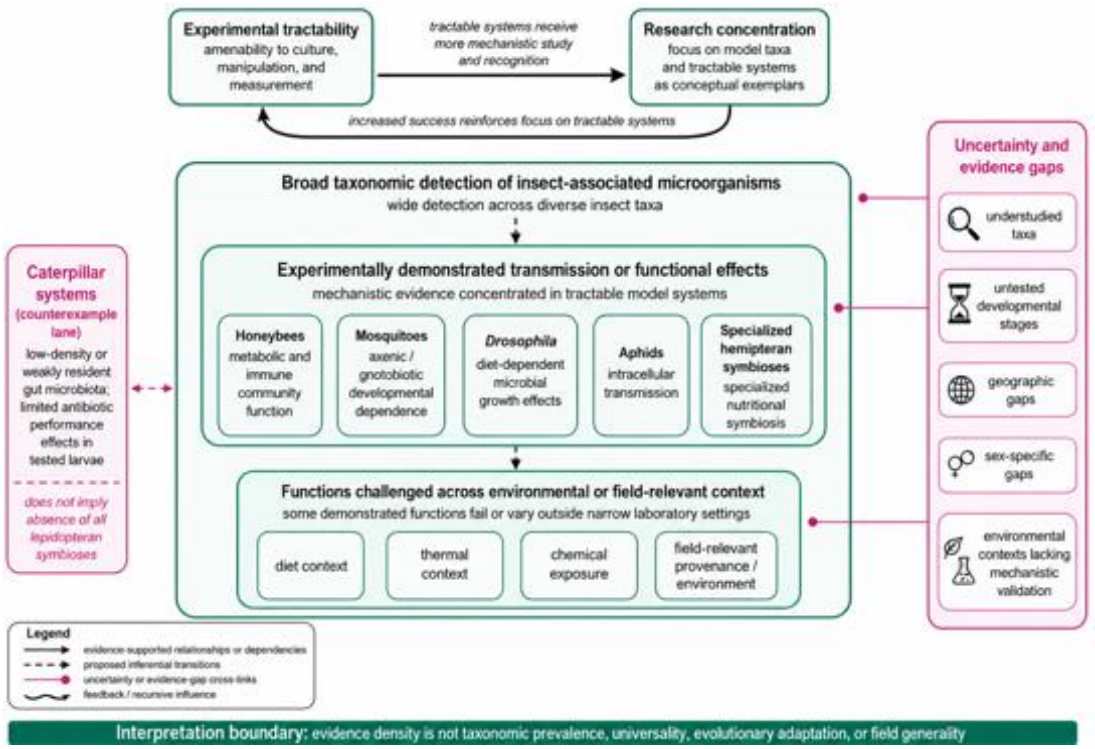


Figure 1. Taxonomic evidence concentration creates unequal confidence across transmission, function, and ecological transfer

Unresolved causality

The unresolved question is not whether microbes can affect insects; controlled experiments show that they can. The problem is determining which causal statement is justified in each system. Detection establishes association or exposure. Persistence establishes residency. Perturbation establishes sensitivity to manipulation. Rescue or defined recolonization strengthens causal attribution. Mechanistic assays identify processes. None of these alone establishes ecological or population consequence.

Genomic capacity illustrates the distinction. Biosynthetic genes can make provisioning plausible, yet direct flux, metabolite transfer, rescue, or loss-of-function evidence is needed to demonstrate realized contribution. Immune-gene induction after colonization establishes host response, not necessarily pathogen resistance. Transcriptomic differences after microbiome transplantation establish molecular sensitivity without identifying net fitness effects. The article therefore proposes an evidence-stage architecture rather than a certainty score. Evidence may progress from occurrence to persistence, association, perturbation, rescue or sufficiency, mechanism, and ecological consequence, but stages need not always occur in this order and no single method is mandatory. Obligately

intracellular symbionts may resist culture or removal, requiring triangulation among genomics, microscopy, physiology, and natural variation.

Causal inference also depends on the counterfactual being tested. Removing an entire community asks a different question from deleting one strain, disrupting one microbial pathway, supplementing one metabolite, or changing an environmental source. Concordant results across these perturbations can strengthen a mechanistic interpretation, whereas disagreement can reveal redundancy, host compensation, or indirect treatment effects. The proposed architecture therefore treats triangulation as especially valuable when no single manipulation can isolate the biologically relevant process. It also preserves a separate validation boundary between a demonstrated organismal phenotype and claims about lifetime fitness, transmission success, or population persistence.

Table 4 converts these causal gaps into discriminating tests rather than numerical grades.

Table 4. Causal transitions in insect–microbiome research and the experiments required to distinguish them

Discriminating test	Observable evidence	Interpretation permitted within its limit
	Detection → residency	
	Proposed inference boundary	
Track persistence after source removal	Repeated detection in host samples	Microbe is associated with host Repeated exposure may mimic colonization
	Residency → function	
	Proposed inference boundary	
Selective depletion or defined recolonization	Stable colonization plus phenotype association	Resident community correlates with host state Host state may structure microbiota independently
	Perturbation → microbial causation	
	Evidence-supported causal test principle	
Rescue with defined organisms or product	Phenotype changes after antibiotic or axenic treatment	Phenotype is sensitive to microbial manipulation Treatment may alter host nutrition or physiology
	Rescue → specific function	
	Evidence-supported causal test principle	
Pathway-specific disruption	Microbe or metabolite restores phenotype	Tested factor is sufficient for a defined endpoint Parallel microbial functions may remain
	Genomic capacity → realized provisioning	
	Proposed inference boundary	
Metabolomics, isotope tracing, pathway disruption	Symbiont retains biosynthetic pathway	Nutrient contribution is plausible Expression, flux, and uptake remain uncertain
	Immune response → protection	
	Proposed inference boundary	
Pathway perturbation plus pathogen challenge	Colonization changes immune transcription	Microbes alter host immune state Immune change may not cause suppression
	Trait change → fitness consequence	
	Proposed inference boundary	
Multi-stage lifetime or demographic measurement	Growth, fecundity, survival, or molecular phenotype changes	Defined trait is microbiome-sensitive Trade-offs can reverse net lifetime effect
	Laboratory mechanism → field generality	
	Proposed inference boundary	
Field-realistic factorial validation	Mechanism replicates under controlled conditions	Causal relation is valid in that context Diet, temperature, provenance, and community complexity may modify it

Future experimental priorities

Future work should combine causal resolution with ecological realism rather than treating them as competing objectives. Axenic and gnotobiotic systems remain indispensable where feasible because they permit depletion, recolonization, rescue, and defined-community reconstruction. Their strongest use is to identify what a microbial partner contributes, followed by validation under realistic diets, developmental states, and environmental regimes. Nutritional studies should move beyond pathway annotation toward metabolite exchange, flux, rescue, and pathway-specific disruption. Immune studies should distinguish microbial induction of host signaling from direct microbial competition and then determine whether either mechanism changes infection, survival, or reproduction. Developmental studies should use longitudinal designs that separate host-driven microbial succession from microbe-driven developmental effects.

Field transfer deserves particular attention because microbiota from laboratory and field hosts can generate different responses, temperature can modify community and defensive functions, and abiotic stressors can act on host and microbe simultaneously. Chemical-exposure studies likewise need to separate compositional perturbation from demonstrated physiological mediation.

Applied microbiome manipulation should be evaluated for reproducibility, transmission stability, ecological compatibility, and measurable host outcomes. Cross-system synthesis identifies substantial potential for microbiome-informed insect production or management while emphasizing that application potential is not equivalent to validated intervention efficacy [35]. The central priority is therefore not simply to manipulate microbiomes, but to determine when a defined microbial effect survives developmental and environmental variation.

Conclusion

Insect–microbiome partnerships cannot be interpreted through microbial presence alone. Transmission route, developmental persistence, microbial identity, host life stage, nutritional environment, immune context, and abiotic exposure determine whether a detected association becomes functionally important. Strong causal evidence exists in particular nutritional symbioses, defined gut communities, and gnotobiotic developmental models, but counterexamples show that dense resident microbiota and strong microbial dependence are not general properties of insects.

The principal contribution of this evidence map is inferential separation. Detection is not residency; residency is not function; function is not necessarily mechanism; a laboratory mechanism is not automatically a field consequence; and an altered trait is not automatically a fitness effect. The proposed evidence-stage architecture preserves these boundaries while allowing different experimental routes to causal understanding.

Progress will depend on combining stage-specific manipulation, mechanistic rescue, environmental realism, and taxonomically broader sampling. This approach can determine not merely which microorganisms occur with insects, but when, how, and under what biological conditions those partnerships matter.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Chabanol E, Gendrin M. Insects and microbes: Best friends from the nursery. Curr Opin Insect Sci. 2024;66:101270. doi:10.1016/j.cois.2024.101270
2. Motta EVS, Moran NA. The honeybee microbiota and its impact on health and disease. Nat Rev Microbiol. 2024;22(3):122-37. doi:10.1038/s41579-023-00990-3
3. Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N. Caterpillars lack a resident gut microbiome. Proc Natl Acad Sci U S A. 2017;114(36):9641-6. doi:10.1073/pnas.1707186114

4. Cornwallis CK, van 't Padje A, Ellers J, Klein M, Jackson R, Kiers ET, et al. Symbioses shape feeding niches and diversification across insects. Nat Ecol Evol. 2023;7(7):1022-44. doi:10.1038/s41559-023-02058-0
5. Wu J, Wang Q, Wang D, Wong ACN, Wang GH. Axenic and gnotobiotic insect technologies in research on host-microbiota interactions. Trends Microbiol. 2023;31(8):858-71. doi:10.1016/j.tim.2023.02.007
6. Lange C, Boyer S, Bezemer TM, Lefort MC, Dhami MK, Biggs E, et al. Impact of intraspecific variation in insect microbiomes on host phenotype and evolution. ISME J. 2023;17(11):1798-807. doi:10.1038/s41396-023-01500-2
7. Luan JB. Insect bacteriocytes: Adaptation, development, and evolution. Annu Rev Entomol. 2024;69:81-98. doi:10.1146/annurev-ento-010323-124159
8. Wierz JC, Dirksen P, Kirsch R, Krüsemer R, Weiss B, Pauchet Y, et al. Intracellular symbiont Symbiodolus is vertically transmitted and widespread across insect orders. ISME J. 2024;18(1). doi:10.1093/ismejo/wrae099
9. Maeda GP, Kelly MK, Sundar A, Moran NA. Intracellular defensive symbiont is culturable and capable of transovarial, vertical transmission. mBio. 2024;15(6). doi:10.1128/mbio.03253-23
10. Michalik A, Franco DC, Szklarzewicz T, Stroiński A, Łukasik P. Facultatively intrabacterial localization of a planthopper endosymbiont as an adaptation to its vertical transmission. mSystems. 2024;9(7). doi:10.1128/msystems.00634-24
11. Villa SM, Chen JZ, Kwong Z, Acosta A, Vega NM, Gerardo NM. Specialized acquisition behaviors maintain reliable environmental transmission in an insect-microbial mutualism. Curr Biol. 2023;33(13):2830-8.e4. doi:10.1016/j.cub.2023.05.062
12. Onchuru TO, Martinez AJ, Ingham CS, Kaltenpoth M. Transmission of mutualistic bacteria in social and gregarious insects. Curr Opin Insect Sci. 2018;28:50-8. doi:10.1016/j.cois.2018.05.002
13. Shan HW, Xia XJ, Feng YL, Wu W, Li HJ, Sun ZT, et al. The plant-sucking insect selects assembly of the gut microbiota from environment to enhance host reproduction. NPJ Biofilms Microbiomes. 2024;10(1):64. doi:10.1038/s41522-024-00539-z
14. Hannula SE, Zhu F, Heinen R, Bezemer TM. Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. Nat Commun. 2019;10(1):1254. doi:10.1038/s41467-019-09284-w
15. Serrato-Salas J, Gendrin M. Involvement of microbiota in insect physiology: Focus on B vitamins. mBio. 2023;14(1). doi:10.1128/mbio.02225-22
16. Douglas AE. The B vitamin nutrition of insects: The contributions of diet, microbiome and horizontally acquired genes. Curr Opin Insect Sci. 2017;23:65-9. doi:10.1016/j.cois.2017.07.012
17. Michalik A, Bauer E, Szklarzewicz T, Kaltenpoth M. Nutrient supplementation by genome-eroded Burkholderia symbionts of scale insects. ISME J. 2023;17(12):2221-31. doi:10.1038/s41396-023-01528-4
18. Harrison RE, Yang X, Eum JH, Martinson VG, Dou X, Valzania L, et al. The mosquito Aedes aegypti requires a gut microbiota for normal fecundity, longevity and vector competence. Commun Biol. 2023;6(1):1154. doi:10.1038/s42003-023-05545-z
19. Zheng H, Powell JE, Steele MI, Dietrich C, Moran NA. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proc Natl Acad Sci U S A. 2017;114(18):4775-80. doi:10.1073/pnas.1701819114
20. Storelli G, Strigini M, Grenier T, Bozonnet L, Schwarzer M, Daniel C, et al. Drosophila perpetuates nutritional mutualism by promoting the fitness of its intestinal symbiont Lactobacillus plantarum. Cell Metab. 2018;27(2):362-77.e8. doi:10.1016/j.cmet.2017.11.011
21. Consuegra J, Grenier T, Baa-Puyoulet P, Rahioui I, Akherraz H, Gervais H, et al. Drosophila-associated bacteria differentially shape the nutritional requirements of their host during juvenile growth. PLoS Biol. 2020;18(3). doi:10.1371/journal.pbio.3000681
22. Coon KL, Valzania L, Brown MR, Strand MR. Predaceous Toxorhynchites mosquitoes require a living gut microbiota to develop. Proc Biol Sci. 2020;287(1919):20192705. doi:10.1098/rspb.2019.2705
23. Cai JA, Christophides GK. Immune interactions between mosquitoes and microbes during midgut colonization. Curr Opin Insect Sci. 2024;63:101195. doi:10.1016/j.cois.2024.101195
24. Lang H, Duan H, Wang J, Zhang W, Guo J, Zhang X, et al. Specific strains of honeybee gut Lactobacillus stimulate host immune system to protect against pathogenic Hafnia alvei. Microbiol Spectr. 2022;10(1). doi:10.1128/spectrum.01896-21

25. Wang J, Lang H, Zhang W, Zhai Y, Zheng L, Chen H, et al. Stably transmitted defined microbial community in honeybees preserves Hafnia alvei inhibition by regulating the immune system. Front Microbiol. 2022;13:1074153. doi:10.3389/fmicb.2022.1074153
26. Steele MI, Motta EVS, Gattu T, Martinez D, Moran NA. The gut microbiota protects bees from invasion by a bacterial pathogen. Microbiol Spectr. 2021;9(2). doi:10.1128/Spectrum.00394-21
27. Girard M, Luis P, Valiente Moro C, Minard G. Crosstalk between the microbiota and insect postembryonic development. Trends Microbiol. 2023;31(2):181-96. doi:10.1016/j.tim.2022.08.013
28. Jordan HR, Tomberlin JK. Microbial influence on reproduction, conversion, and growth of mass produced insects. Curr Opin Insect Sci. 2021;48:57-63. doi:10.1016/j.cois.2021.10.001
29. Hegde S, Brettell LE, Quek S, Etebari K, Saldaña MA, Asgari S, et al. Aedes aegypti gut transcriptomes respond differently to microbiome transplants from field-caught or laboratory-reared mosquitoes. Environ Microbiol. 2024;26(2). doi:10.1111/1462-2920.16576
30. Mason CJ, Shikano I. Hotter days, stronger immunity? Exploring the impact of rising temperatures on insect gut health and microbial relationships. Curr Opin Insect Sci. 2023;59:101096. doi:10.1016/j.cois.2023.101096
31. Hudson CM, Stalder D, Vorburger C. Clines of resistance to parasitoids: The multifarious effects of temperature on defensive symbioses in insects. Curr Opin Insect Sci. 2024;64:101208. doi:10.1016/j.cois.2024.101208
32. Lemoine MM, Engl T, Kaltenpoth M. Microbial symbionts expanding or constraining abiotic niche space in insects. Curr Opin Insect Sci. 2020;39:14-20. doi:10.1016/j.cois.2020.01.003
33. Al Naggar Y, Singavarapu B, Paxton RJ, Wubet T. Bees under interactive stressors: The novel insecticides flupyradifurone and sulfoxaflor along with the fungicide azoxystrobin disrupt the gut microbiota of honey bees and increase opportunistic bacterial pathogens. Sci Total Environ. 2022;849:157941. doi:10.1016/j.scitotenv.2022.157941
34. Helander M, Jeevannavar A, Kaakinen K, Mathew SA, Saikkonen K, Fuchs B, et al. Glyphosate and a glyphosate-based herbicide affect bumblebee gut microbiota. FEMS Microbiol Ecol. 2023;99(7). doi:10.1093/femsec/fiad065
35. Jang S, Kikuchi Y. Impact of the insect gut microbiota on ecology, evolution, and industry. Curr Opin Insect Sci. 2020;41:33-9. doi:10.1016/j.cois.2020.06.004