

# Phylosymbiotic Relationship between Insects and Symbionts

Subjects: Entomology

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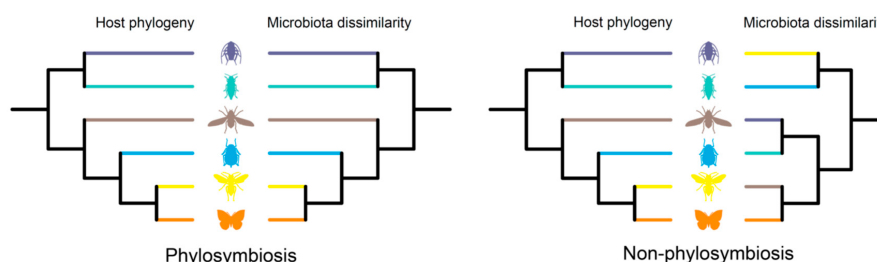
Insects harbor diverse assemblages of bacterial and fungal symbionts, which play crucial roles in host life history. Insects and their various symbionts represent a good model for studying host–microbe interactions. Phylosymbiosis is used to describe an eco-evolutionary pattern, providing a new cross-system trend in the research of host-associated microbiota. The phylosymbiosis pattern is characterized by a significant positive correlation between the host phylogeny and microbial community dissimilarities.

Keywords: microbial community structure ; phylosymbiosis pattern ; stochastic effect ; codiversification ; ecological filtering

## 1. Introduction of Phylosymbiosis

Host–microbe symbioses play a crucial role in the ecological and evolutionary history of animals <sup>[1][2]</sup>. Recent advances in the field of host–microbe interactions have demonstrated the influence of host phylogeny and ecological factors on microbial community assembly <sup>[3][4][5]</sup>. Phylosymbiosis occurs when host-associated microbiota relationships are positively associated with host phylogenetic relatedness.

Phylosymbiosis is defined as “microbial community relationship parallels the host phylogeny”, in which “phylo” refers to host lineage and “symbiosis” refers to the coexistence of hosts and microbes (**Figure 1**) <sup>[6][7]</sup>. In other words, microbial community composition dissimilarities are positively associated with the accumulation of host genetic variation. Phylosymbiosis studies focus on the entire microbiota rather than individuals within the microbiota. The persistent and intimate association between microbes and their host is not the necessary assumption of this eco-evolutionary pattern <sup>[8]</sup>.



**Figure 1.** Phylosymbiotic versus stochastic microbial community assemblages. Branches in the same color indicate the host and associated microbial community.

Pioneering studies on phylosymbiosis were performed on the parasitoid wasp genus *Nasonia* under rearing conditions <sup>[9]</sup>, in which species-specific phylosymbiotic gut bacterial communities caused lethality in interspecific hybrids <sup>[7]</sup>. Afterward, Brooks et al. <sup>[10]</sup> revealed phylosymbiosis in other animals, including deer mice (*Peromyscus*), fruit flies (*Drosophila*), and mosquitoes (i.e., *Anopheles*, *Aedes*, and *Culex*). To date, interspecific phylosymbiotic structures of microbiota have been widely reported in insects, birds, fishes, and mammals <sup>[5][10][11][12][13][14][15][16][17][18]</sup>. However, phylosymbiosis remains poorly understood at the intraspecific level. Intraspecific phylosymbiosis has only been substantiated in the microbial communities from the American pika *Ochotona princeps* <sup>[19]</sup> and the aphid *Mollitrichosiphum tenuicorpus* <sup>[20]</sup>. The host taxa in insect phylosymbiosis studies to date cover orders, families, genera, and species, and the evolutionary history of hosts spans approximately 0.3–300 million years <sup>[6][21]</sup>. The strength of the phylosymbiotic signals between the host and microbiota varies across host taxa <sup>[8]</sup>, and the phylosymbiotic relationships can be weakened with an increasing host evolutionary history <sup>[5][21]</sup>.

Phylosymbiosis analyses typically employ 16S rRNA gene amplicon sequencing data as the input data of the microbial community. Multiple beta diversity distance metrics are usually required for the robustness of the results <sup>[8]</sup>. Furthermore,

a reliable host phylogenetic tree is essential for the determination of phylosymbiosis patterns. The key to measuring phylosymbiosis is to assess the significant correlation between host phylogeny and microbiota beta diversity. Principal methods for quantifying phylosymbiosis are as follows: (1) topological congruency tests <sup>[10]</sup> utilizing the Robinson–Foulds metric <sup>[22]</sup> or matching cluster metric <sup>[23]</sup>, or (2) a matrix correlation-based approach, e.g., the Mantel test <sup>[24]</sup> and Procrustean superimposition <sup>[25]</sup>.

## 2. Phylosymbiosis in Insects

Insects constitute the most diverse group of animals and play crucial roles in terrestrial ecosystems <sup>[26]</sup>. Insects harbor a great variety of symbionts, which contribute significantly to the survival, growth, and fecundity of the host <sup>[2][27][28]</sup>. Additionally, symbionts could facilitate host adaptation to new ecological niches and potentially drive speciation in insects <sup>[1][29]</sup>. Insect microbial community structures have been found to be correlated with environmental habitat, diet, sex, life stage, and host insect identity and phylogeny <sup>[30][31][32]</sup>. Some studies highlighted the strongest impact of insect species on the associated microbial communities <sup>[30][33]</sup>. Currently, phylosymbiosis research in insects remains in its infancy, and phylosymbiosis has been confirmed in the orders Blattodea, Coleoptera, Diptera, Isoptera, Hemiptera, and Hymenoptera (Table 1).

**Table 1.** Summary of phylosymbiosis patterns in insects.

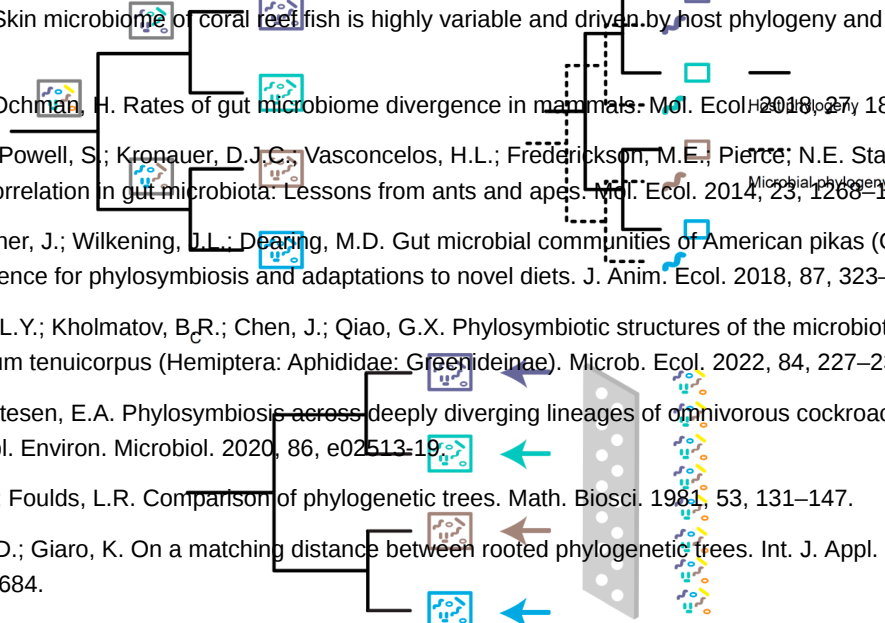
Insects Examined		No. of Species Sampled	Evolutionary Time (Mya)	Diet	Core Microbe	Obligate Symbiont	References
Blattodea		19	>300	Omnivory	Bacteroidetes, Firmicutes, and Proteobacteria	—	[21]
Coleoptera	<i>Dendroctonus frontalis</i> species complex	7	12	Phloem cell	<i>Ceratocystiopsis</i>	—	[34][35]
Diptera	<i>Anopheles</i> , <i>Aedes</i> , and <i>Culex</i>	8	100	Blood	Proteobacteria	—	[10][36]
	<i>Drosophila</i>	6	63	Decaying fruit	Proteobacteria	—	[10]
Hemiptera	Greenideinae	53	83	Phloem sap	—	<i>Buchnera aphidicola</i>	[37][38]
	<i>Mollitrichosiphum</i>	8	18–19	Phloem sap	—	<i>Buchnera aphidicola</i>	[33]
	<i>Mollitrichosiphum tenuicorpus</i>	1 (26 colonies)	11	Phloem sap	—	<i>Buchnera aphidicola</i>	[20]
	Psylloidea	102	350	Phloem sap	—	<i>Carsonella ruddii</i>	[39][40]
Hymenoptera	<i>Cephalotes</i>	13	46	Pollen and honeydew	—	<i>Cephaloticoccus</i>	[41]

Insects Examined	No. of Species Sampled	Evolutionary Time (Mya)	Diet	Core Microbe	Obligate Symbiont	References
<i>Ceratosolen</i>	6	60	Fig	<i>Wolbachia</i>	—	[42][43]
<i>Formica</i>	14	30	Honeydew and nectar	<i>Wolbachia</i> , <i>Lactobacillus</i> , <i>Liliensternia</i> , and <i>Spiroplasma</i>	—	[44][45]
<i>Nasonia</i>	4	<1	Fly puparium	Proteobacteria, Firmicutes, and Actinobacteria	—	[10]
Lepidoptera				<i>Acinetobacter</i> , <i>Apibacter</i> , <i>Asaia</i> , <i>Commensalibacter</i> , <i>Enterobacter</i> ,		
Heliconiini	23	20–30	Pollen, nectar, and fruit	<i>Enterococcus</i> , <i>Lactococcus</i> , <i>Spiroplasma</i> , and <i>Pseudomonas</i>	—	[46][47]

## References

- Perreau, J.; Moran, N.A. Genetic innovations in animal–microbe symbioses. *Nat. Rev. Genet.* 2022, 23, 23–39.
- Brownlie, J.C.; Johnson, K.N. Symbiont-mediated protection in insect hosts. *Trends Microbiol.* 2009, 17, 348–354.
- Weinstein, S.B.; Martinez-Mota, R.; Stapleton, T.E.; Kiure, D.M.; Greenhalgh, R.; Orr, T.J.; Dale, C.; Kohl, K.D.; Dearing, M.D. Microbiome stability and structure is governed by host phylogeny over diet and geography in woodrats (*Neotoma* spp.). *Proc. Natl. Acad. Sci. USA* 2021, 118, e2108787118.
- Youngblut, N.D.; Reischer, G.H.; Walters, W.; Schuster, N.; Walzer, C.; Stalder, G.; Ley, R.E.; Farnleitner, A.H. Host diet and microbiome diversity in vertebrate species. *Nat. Commun.* 2019, 10, 2200.
- Groussin, M.; Mazel, F.; Sanders, J.G.; Smillie, C.S.; Laverone, S.; Thuiller, W.; Alm, E.J. Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nat. Commun.* 2017, 8, 14319.
- Lim, S.J.; Bordenstein, S.R. An introduction to phyllosymbiosis. *Proc. R. Soc. B Biol. Sci.* 2020, 287, 20192900.
- Brucker, R.M.; Bordenstein, S.R. The hologenome basis of speciation: Gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science* 2019, 341, 667–669.
- Brucker, R.M.; Bordenstein, S.R. The roles of host evolutionary relationships (genus: *Nasonia*) and development in structuring microbial communities. *Evolution* 2012, 66, 349–362.
- Brucker, R.M.; Bordenstein, S.R. The roles of host evolutionary relationships (genus: *Nasonia*) and development in structuring microbial communities. *Evolution* 2012, 66, 349–362.
- Brooks, A.W.; Collette, S.P.; Bruck, C.M.; Bordenstein, S.R. Phyllosymbiosis: Relationships and structured functional effects of insect-associated microbial communities across hosts of different phylogeny. *Front. Microbiol.* 2016, 7, 1420002.
- Tang, T.Y.; Ma, K.Y.; Cheung, M.K.; Yang, C.H.; Wang, Y.Q.; Hu, X.L.; Kwan, H.S.; Chu, K.H. Gut microbiota in decapod shrimp: Evidence of phyllosymbiosis. *Microb. Ecol.* 2021, 82, 994–1007.
- Ding, J.; Jiang, T.; Zhou, H.; Yang, L.; He, C.; Xu, K.; Akinyemi, F.T.; Han, C.; Luo, H.; Qin, C.; et al. The gut microbiota of pheasant lineages reflects their host genetic variation. *Front. Genet.* 2020, 11, 859.
- Dogra, M.; Morris, M.; Bordenstein, S.R. The roles of host evolutionary relationships (genus: *Nasonia*) and development in structuring microbial communities. *Evolution* 2012, 66, 349–362.
- Revelante, B.K.; Sosa, J.; Hartup, B.K.; Kohl, K.D. A bird's-eye view of phyllosymbiosis: Weak signatures of phyllosymbiosis among all 15 species of cranes. *Proc. R. Soc. B Biol. Sci.* 2020, 287, 20192988.
- Laviad-Shitrit, S.; Izhaki, I.; Lalar, M.; Halpern, M. Comparative analysis of intestine microbiota of four wild waterbird species. *Front. Microbiol.* 2019, 10, 1911.

16. Chiarello, M.; Auguet, J.C.; Bettarel, Y.; Bouvier, C.; Claverie, T.; Graham, N.A.J.; Rieuvilleneuve, F.; Sucre, E.; Bouvier, T.; Villegier, S. Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. *Microbiome* 2018, 6, 147.
17. Nishida, A.H.; Ochman, H. Rates of gut microbiome divergence in mammals. *Mol. Ecol.* 2018, 27, 1884–1897.
18. Sanders, J.G.; Powell, S.; Kronauer, D.J.C.; Vasconcelos, H.L.; Frederickson, M.E.; Pierce, N.E. Stability and phylogenetic correlation in gut microbiota: Lessons from ants and apes. *Mol. Ecol.* 2014, 23, 1268–1283.
19. Kohl, K.D.; Varner, J.; Wilkening, J.L.; Dearing, M.D. Gut microbial communities of American pikas (*Ochotona princeps*): Evidence for phyllosymbiosis and adaptations to novel diets. *J. Anim. Ecol.* 2018, 87, 323–330.
20. Qin, M.; Jiang, L.Y.; Kholmatov, B.R.; Chen, J.; Qiao, G.X. Phyllosymbiotic structures of the microbiota in *Mollitrichosiphum tenuicorpus* (Hemiptera: Aphididae: Greenideinae). *Microb. Ecol.* 2022, 84, 227–239.
21. Tinker, K.A.; Ottesen, E.A. Phyllosymbiosis across deeply diverging lineages of omnivorous cockroaches (order Blattodea). *Appl. Environ. Microbiol.* 2020, 86, e02513–19.
22. Robinson, D.F.; Foulds, L.R. Comparison of phylogenetic trees. *Math. Biosci.* 1981, 53, 131–147.
23. Bogdanowicz, D.; Giaro, K. On a matching distance between rooted phylogenetic trees. *Int. J. Appl. Math. Comput. Sci.* 2013, 23, 669–684.
24. Mantel, N. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 1967, 27, 209–220.



**Figure 2.** Mechanisms underlying phyllosymbiosis. (A) Gain or loss of microbes arises from stochastic processes. (B) Evolutionary processes such as host diversification, vertical data transmission, and the advantages of broad and narrow ecological niches shape the microbial community. (C) Stochastic effects such as spatial limitations, dispersal, and ecological drift.

25. Basset, Y.; Cizek, L.; Cuenoud, P.; Didham, R.K.; Guilhaumon, F.; Missa, O.; Novotny, V.; Ødegaard, F.; Roslin, T.; Schmid, J.; et al. Arthropod diversity in a tropical forest. *Science* 2012, 338, 1481–1484.

### 3.1 Stochastic Effects

26. Phyllosymbiotic microbiota can be a consequence of stochastic effects such as spatial limitations, dispersal, and ecological drift. Fluctuations in the abundance of microbes (Figure 2A) [56]. Dispersal is referred to as the movement and successful colonization of microbes across space [57]. Moeller et al. [58] revealed that the dispersal limitations of bacteria could promote the compositional divergence of gut microbial communities among mammalian species. In addition to spatial limitations, the composition of the microbial community can be disturbed by the rate and order of microbes that are added to the microbiota during dispersal processes [59]. The microbial dispersal associated with insects generally occurs through the egg surface, and capsule or jelly-like secretion transmission [60].
27. Huang, K.G.; Wang, J.; Huang, J.H.; Zhang, S.K.; Vogler, A.P.; Liu, Q.Q.; Li, Y.C.; Yang, M.W.; Li, Y.; Zhou, X.G. Host ecological drift leads to random variation in the relative abundance of species within the microbial community over time. *Front. Microbiol.* 2021, 12, 633079.
28. [61] Microbes in low abundances are more susceptible to drift with subsequent extinction. Ecological drift can generate differences in microbial community composition when deterministic processes are weak [59]. In insects, microbiota profiling varies greatly across different groups, with extremes represented by some sap-feeding insects having few gut microbes [62]. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host, but abundant intracellular symbionts and by detritivores and wood feeders harboring large and complex gut microbiota [62]. Currently, the effect of ecological drift as the sole factor structuring the microbiota has not been confirmed in any animal system. The phyllosymbiotic microbial communities of insects are typically composed of diverse microbes, some of which are abundant and resident. Therefore, the phyllosymbiosis pattern within insects is unlikely to be merely drift-driven. Ecological drift may play a part in the interactions with other community assembly processes in structuring insect microbiota.
29. Vázquez-Ortiz, K.; Pineda-Mendoza, R.M.; González-Escobedo, R.; Davis, T.S.; Salazar, K.F.; Rivera-Orduña, F.N.; Zúñiga, G. Metabarcoding of mycetangia from the *Dendroctonus frontalis* species complex (Curculionidae: Scolytinae) reveals diverse and functionally redundant fungal assemblages. *Front. Microbiol.* 2022, 13, 969230.

### 3.2 Evolutionary Processes

30. Havill, N.P.; Cognato, A.I.; Del Val, E.K.; Rabaglia, R.J.; Garrick, R.C. New molecular tools for *Dendroctonus frontalis* (Coleoptera: Curculionidae: Scolytinae) reveal an east-west genetic subdivision of early Pleistocene origin. *Insect Syst. Divers.* 2019, 3, 817–830.
31. [63] Cospeciation can result from coevolution and occurs when simultaneously of evolutionary changes in interacting species [63]. Cospeciation can result from coevolution and occurs when host and parasite diverge. Simón-Rodríguez, R.; De Smet, B.; Bracker, R.; McLeish, M.; Maharaj, A.; And, S.; Knights, R.; Scott, J. Microbiome dynamics: background, prevalence and rarefaction of West Nile virus. *Front. Microbiol.* 2017, 8, 526.
32. [64] [65] [66] Insects feeding on plant sap, such as species of Hemiptera, possess symbionts that can provide nutrients to compensate for deficiencies in their food source. Many phyllosymbiotic and their bacteria [67] [68] [69] [70] [71] rely on the biosynthetic and metabolic complementarity of essential nutrition to maintain intimate associations [29] [69] [70] [71]. For instance, the primary endosymbiont *Buchnera aphidicola* has highly coadapted to and evolved with aphids for millions of years [72] [73] [74]. Likewise, such coevolutionary examples have been identified from extracellular gut symbionts that enable nutrient

39. Martonfi F., Bulthuis K. Paeppe A. Mate P. Imola A. Playkin B. Gs. A. H. Standg. R. K. F. K. h. e. z. i. p. h. y. l. a. g. e. s. y. c. i. s. t. u. r. a. s. t. h. e. h. a. s. t. e. r. i. a. l. c. o. m. m. u. n. i. t. i. e. s. i. n. t. h. e. m. i. c. r. o. b. i. o. m. e. o. f. p. s. y. l. l. i. d. s. (Hemiptera: Psylloidea) in Aotearoa New Zealand. PLoS ONE 2023, 18, e0285587.

Codiversification represents another evolutionary process that underlies phyllosymbiosis (**Figure 2B**). It occurs when hosts and microbes exhibit congruent phylogenetic trees but does not necessarily imply an occurrence of coevolution [72]. Codiversification can be a consequence of unidirectional selection; that is, microbes adapt to the evolutionary changes

imposed by their hosts but not vice versa. In the social corbiculate bees, a strain-level phylogenetic association between the core gut bacteria *Lactobacillus* Firm-5 and the host bees was observed, which suggested host-microbe codiversification [76]. Other adaptation processes, such as host-shift speciation [79] and shared geographic isolation [80], can also contribute to matching phylogenies of microbes and host lineages.

42. Li, J.; Wei, X.; Huang, D.W.; Xiao, J. The phyllosymbiosis pattern between the fig wasps of the same genus and their associated microbial flora. *Microbiol.* **2022**, *12*, 8001992. [CrossRef](https://doi.org/10.1093/mic/miab292) [PubMed]

high fidelity, it seems unlikely that all microbiota members are involved in the aforementioned evolutionary processes driving phyllosymbiosis. Early arriving species can affect the ability of late arriving species to establish themselves during the fig-wasp symbiosis. *Proc. R. Soc. B Biol. Sci.* 2009, **272**, 2593–2599.

community assembly, which is referred to as priority effects [81]. The importance of priority effects in shaping microbial community composition has been reviewed [82]. Moreover, multiple studies have revealed that highly connected keystone phyllosymbionts in Formica ants. Front. Microbiol. 2023. 14. 1044286.

or hub microbes can determine the overall community structure via interspecific interactions [83][84][85]. The evolutionary processes underlying phyllosymbiosis represented by the evolution of social parasitism in Formica ants revealed by a global phylogeny. Proc. Natl. Acad. Sci. USA 2021, 118, e2026029118.

46 example, *B. t. chondri* is located on specialized VZC-type erythrocytes and maintained within a specialized generation and a phage-mediated  
transmitted at a late stage of infection (see Appendix 1, Table 1, 2020-03, 2020-03-20). The symbiont *Rickettsia* is vertically

transmitted to offspring paternally via an intrasperm passage as well as maternally via an ovarial passage [80]. Additionally, 47. Cicconardi, F.; Milanetti, E.; Pinheiro de Castro, E.C.; Mazo-Vargas, A.; Van Belleghem, S.M.; Ruggieri, A.A.; Rastas, P.; Hanly, J.; Evans, E.; Jiggins, C.D.; et al. Evolutionary dynamics of genome size and content during the adaptive radiation of Helicoverpa from acanthosomatid stinkbugs, which is maternally transmitted via egg smearing [89]. For social insects,

e.g., *Acromyrmex* leaf-cutting ants [90] and the honey bee *Apis mellifera* [91], social acquisition of beneficial microbes is

critical for specificity and partner fidelity in host–bacterial associations. These initial colonizing symbionts with vertical transmission may have served as keystone for humans and are responsible for the host species-specific microbial Archia. E. A. Social networks predict gut microbiome composition in wild baboons. *eLife* 2015, 4, e05224.

50. Xu, T.T.; Jiang, L.Y.; Chen, J.; Qiao, G.X. Host plants influence the symbiont diversity of Eriosomatinae (Hemiptera: Aphididae). *Insects* **2020**, *11*, 217.

54. Yan, T. and Sloan, J. Z. Long-dispersed Diapriids: Diversity patterns consistent with simple ecological filtering without any  
 55. Anthridae Insect Sci. 2021;28:165–179.  
 In principle, some host traits can function as filters that exert a selective role on

environmental microbes, and the microbes suitable according to these selective forces can coexist with the host (Figure 52). Diamond, J.M. Assembly of Species Communities. In Ecology and Evolution of Communities, Eddy, M., Diamond, J.M., Eds. It is possible that hosts maintain host-species-specific microbial communities via a strong selection of environmental

microbes and then yield phyllosymbiotic microbiota. Closely related hosts have similar physiological characteristics, immune systems, or microbial defense mechanisms <sup>87, 1465–1471</sup>, which may bring about the tendency to harbor similar microbial communities.

54. Weiher, E., Clarke, G.D.P., Keddy, P.A. Community assembly, rules, morphological dispersion, and the coexistence of plant species. *Oikos* 1998, 81, 309–322.

55. Veary, P. et al. Bionestology: A method for studying the effects of species interactions on the fitness of insects. *Proc. Natl. Acad. Sci. USA* 2013, 110, 12804–12809.

### 3.3.1. Immune System

56. Kohl, K.D. Ecological and evolutionary mechanisms underlying patterns of phyllosymbiosis in host-associated microbial communities. *Ecology Letters* 2015, 18, 1069–1080. [CrossRef]

composition [95][96][97][98]. Insects rely on physiological barriers and innate immune responses to defend themselves against pathogens [99][100]. The innate immune system of insects is composed of cellular immune responses by circulating hemocytes [101] and humoral immune responses. Although the hemocyte categories involved in cellular immune

58. Moeller, A.H.; Suzuki, T.A.; Lin, D.; Lacey, E.A.; Wasser, S.K.; Nachman, M.W. Dispersal limitation promotes the responses vary among different insect species, hemocyte functions primarily include phagocytosis, modulation, and diversification of the mammalian gut microbiota. *Proc. Natl. Acad. Sci. USA* 2017, 114, 13768–13773. [\[CrossRef\]](#)

59. Nigam, S.; Diksha, S.; Singh, S.; Sankar, A.; O'Neill, S.P.; Billinski, S.N.; Star, S.K.; SFAK, K.; and, prophylactic oxidase (PPO) pathway, Wiley, 2013; The expression and progress of these pathways subsequently result in a Microbial Microbial Reproductive (AMP) product 356 reactive oxygen species (ROS) generation, and melanization. Insects depend on two pathways to regulate

60. Salem, H.; Flores, E.; Cerardo, N.; Kattenpoth, M. An out-of-body experience: The extracellular dimension for the antimicrobial peptide generation, namely, the Toll pathway, which responds to fungi and most Gram-positive bacteria, and the MAMP pathway, which is induced by Gram-negative bacteria. *Eur. J. Biol. Sci.* 2015, 282, 20142957. [CrossRef]

61 Chase, J. M., Myers, J. A. Disentangling the importance of ecological niches from stochastic processes across scales. *The insect-immune system not only defends against pathogens but also plays an important role in maintaining host-microbe symbiosis*. *BioRxiv* 2021.11.06.235123. <https://doi.org/10.1101/235123>.  
 62 Phillips, Trans, R. S. Q. *Bo* 2001.11.06.235123. Serving as one of the model systems in Hemiptera, aphids lack several immune-related genes that are suspected to be essential in arthropod immunity <sup>[140]</sup>. Previous studies suggested that the reduced





87. Kogan R., Mengol G., Tsipouras T., Fukatsu T. Cellular mechanisms of phyletic host–symbiont coadaptation: obligate mutualisms, including symbiosis at the bacteriocyte–embryo interface. *Proc. Natl. Acad. Sci. USA* 2012, 109, E1230–E1237.
88. Watanabe, K., Yukuniri, F., Matsuura, T., Fukatsu, T.; Noda, H. IntraspERM vertical symbiont transmission. *Proc. Natl. Acad. Sci. USA* 2014, 111, 7433–7437.
89. Kikuchi, Y.; Ohbayashi, T.; Jang, S.; Mergaert, P. *Burkholderia insecticola* triggers midgut closure in the bean bug *Riptortus pedestris* to prevent secondary bacterial infections of midgut crypts. *ISME J.* 2020, 14, 1627–1638.
90. Marsh, S.E.; Poulsen, M.; Pinto-Tomas, A.; Currie, C.R. Interaction between workers during a short time window is required for bacterial symbiont transmission in *Acromyrmex* leaf-cutting ants. *PLoS ONE* 2014, 9, e103269.
91. Powell, J.E.; Martinson, V.G.; Urban-Mead, K.; Moran, N.A. Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl. Environ. Microbiol.* 2014, 80, 7378–7387.
92. Smith, W.P.J.; Wucher, B.R.; Nadell, C.D.; Foster, K.R. Bacterial defences: Mechanisms, evolution and antimicrobial resistance. *Nat. Rev. Microbiol.* 2023, 21, 519–534.
93. Mason, C.J.; Raffa, K.F. Acquisition and structuring of midgut bacterial communities in gypsy moth (Lepidoptera: Erebidae) larvae. *Environ. Entomol.* 2014, 43, 595–604.
94. Franzenburg, S.; Walter, J.; Künzel, S.; Wang, J.; Baines, J.F.; Bosch, T.C.; Fraune, S. Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc. Natl. Acad. Sci. USA* 2013, 110, E3730–E3738.
95. Kwong, W.K.; Mancenido, A.L.; Moran, N.A. Immune system stimulation by the native gut microbiota of honey bees. *R. Soc. Open Sci.* 2017, 4, 170003.
96. Guarneri, A.A.; Schaub, G.A. Interaction of triatomines, trypanosomes and microbiota. In *Triatominae—The Biology of Chagas Disease Vectors*; Guarneri, A.A., Lorenzo, M.G., Eds.; Springer Nature: New York, NY, USA, 2021; pp. 345–386.
97. Mistry, R.; Kounatidis, I.; Ligoxygakis, P. Interaction between familial transmission and a constitutively active immune system shapes gut microbiota in *Drosophila melanogaster*. *Genetics* 2017, 206, 889–904.
98. Hooper, L.V.; Littman, D.R.; Macpherson, A.J. Interactions between the microbiota and the immune system. *Science* 2012, 336, 1268–1273.
99. Chen, K.; Lu, Z. Immune responses to bacterial and fungal infections in the silkworm, *Bombyx mori*. *Dev. Comp. Immunol.* 2018, 83, 3–11.
100. Lemaitre, B.; Hoffmann, J. The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* 2007, 25, 697–743.
101. Strand, M.R. The insect cellular immune response. *Insect Sci.* 2008, 15, 1–14.
102. Arteaga, B.L.A.; Crispim, J.S.; Fernandes, K.M.; de Oliveira, L.L.; Pereira, M.F.; Bazzolli, D.M.S.; Martins, G.F. Differential cellular immune response of *Galleria mellonella* to *Actinobacillus pleuropneumoniae*. *Cell Tissue Res.* 2017, 370, 153–168.
103. Wu, G.Q.; Liu, Y.; Ding, Y.; Yi, Y.H. Ultrastructural and functional characterization of circulating hemocytes from *Galleria mellonella* larva: Cell types and their role in the innate immunity. *Tissue Cell* 2016, 48, 297–304.
104. Azambuja, P.D.; Garcia, E.S.; Ratcliffe, N.A. Aspects of classification of Hemiptera hemocytes from six triatomine species. *Mem. Inst. Oswaldo Cruz.* 1991, 86, 1–10.
105. Evans, J.D.; Aronstein, K.; Chen, Y.P.; Hetru, C.; Imler, J.L.; Jiang, H.; Kanost, M.; Thompson, G.J.; Zou, Z.; Hultmark, D. Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol. Biol.* 2006, 15, 645–656.
106. Ali Mohammadie Kojour, M.; Han, Y.S.; Jo, Y.H. An overview of insect innate immunity. *Entomol. Res.* 2020, 50, 282–291.
107. Nichols, H.L.; Goldstein, E.B.; Saleh Ziabari, O.; Parker, B.J. Intraspecific variation in immune gene expression and heritable symbiont density. *PLoS Pathog.* 2021, 17, e1009552.
108. Pan, X.; Pike, A.; Joshi, D.; Bian, G.; McFadden, M.J.; Lu, P.; Liang, X.; Zhang, F.; Raikhel, A.S.; Xi, Z. The bacterium *Wolbachia* exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito *Aedes aegypti*. *ISME J.* 2018, 12, 277–288.
109. Nyholm, S.V.; Graf, J. Knowing your friends: Invertebrate innate immunity fosters beneficial bacterial symbioses. *Nat. Rev. Microbiol.* 2012, 10, 815–827.
110. Gerardo, N.M.; Altincicek, B.; Anselme, C.; Atamian, H.; Barribeau, S.M.; de Vos, M.; Duncan, E.J.; Evans, J.D.; Gabaldon, T.; Ghanim, M.; et al. Immunity and other defenses in pea aphids, *Acyrtosiphon pisum*. *Genome Biol.* 2010, 11, R21.

111. Altincicek, B.; Gross, J.; Vilcinskas, A. Wounding-mediated gene expression and accelerated viviparous reproduction of the pea aphid *Acyrtosiphon pisum*. *Insect Mol. Biol.* 2008, 17, 711–716.
112. Laughton, A.M.; Garcia, J.R.; Gerardo, N.M. Condition-dependent alteration of cellular immunity by secondary symbionts in the pea aphid, *Acyrtosiphon pisum*. *J. Insect Physiol.* 2016, 86, 17–24.
113. Guo, L.; Tang, J.; Tang, M.; Luo, S.; Zhou, X. Reactive oxygen species are regulated by immune deficiency and Toll pathways in determining the host specificity of honeybee gut bacteria. *Proc. Natl. Acad. Sci. USA* 2023, 120, e2219634120.
114. Alessandri, G.; Rizzo, S.M.; Ossiprandi, M.C.; van Sinderen, D.; Ventura, M. Creating an atlas to visualize the biodiversity of the mammalian gut microbiota. *Curr. Opin. Biotechnol.* 2022, 73, 28–33.
115. Song, S.J.; Sanders, J.G.; Delsuc, F.; Metcalf, J.; Amato, K.; Taylor, M.W.; Mazel, F.; Lutz, H.L.; Winker, K.; Graves, G.R.; et al. Comparative analyses of vertebrate gut microbiomes reveal convergence between birds and bats. *mBio* 2020, 11, e02901-19.
116. Hicks, A.L.; Lee, K.J.; Couto-Rodriguez, M.; Patel, J.; Sinha, R.; Guo, C.; Olson, S.H.; Seimon, A.; Seimon, T.A.; Ondzie, A.U.; et al. Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nat. Commun.* 2018, 9, 1786.
117. Muegge, B.D.; Kuczynski, J.; Knights, D.; Clemente, J.C.; Gonzalez, A.; Fontana, L.; Henrissat, B.; Knight, R.; Gordon, J.I. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 2011, 332, 970–974.
118. Xu, S.F.; Jiang, L.Y.; Qiao, G.X.; Chen, J. The bacterial flora associated with the polyphagous aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) is strongly affected by host plants. *Microb. Ecol.* 2020, 79, 971–984.
119. Brady, C.M.; Asplen, M.K.; Desneux, N.; Heimpel, G.E.; Hopper, K.R.; Linnen, C.R.; Oliver, K.M.; Wulff, J.A.; White, J.A. Worldwide populations of the aphid *Aphis craccivora* are infected with diverse facultative bacterial symbionts. *Microb. Ecol.* 2014, 67, 195–204.
120. Hammer, T.J.; Janzen, D.H.; Hallwachs, W.; Jaffe, S.P.; Fierer, N. Caterpillars lack a resident gut microbiome. *Proc. Natl. Acad. Sci. USA* 2017, 114, 9641–9646.
121. Amato, K.R.; Sanders, J.G.; Song, S.J.; Nute, M.; Metcalf, J.L.; Thompson, L.R.; Morton, J.T.; Amir, A.J.; McKenzie, V.; Humphrey, G. Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. *ISME J.* 2019, 13, 576–587.
122. Lanan, M.C.; Rodrigues, P.A.P.; Agellon, A.; Jansma, P.; Wheeler, D.E. A bacterial filter protects and structures the gut microbiome of an insect. *ISME J.* 2016, 10, 1866–1876.
123. McLoughlin, K.; Schluter, J.; Rakoff-Nahoum, S.; Smith, A.L.; Foster, K.R. Host selection of microbiota via differential adhesion. *Cell Host Microbe* 2016, 19, 550–559.
124. Hooper, L.V.; Gordon, J.I. Glycans as legislators of host–microbial interactions: Spanning the spectrum from symbiosis to pathogenicity. *Glycobiology* 2001, 11, 1R–10R.
125. Mikaelyan, A.; Thompson, C.L.; Hofer, M.J.; Brune, A. Deterministic assembly of complex bacterial communities in guts of germ-free cockroaches. *Appl. Environ. Microbiol.* 2016, 82, 1256–1263.
126. Xu, Y.; Jiang, J.Y.; Lin, X.J.; Shi, W.P.; Cao, C. Identification of diverse viruses associated with grasshoppers unveils the parallel relationship between host phylogeny and virome composition. *Virus Evol.* 2022, 8, veac057.
127. Leigh, B.A.; Bordenstein, S.R.; Brooks, A.W.; Mikaelyan, A.; Bordenstein, S.R. Finer-scale phyllosymbiosis: Insights from insect viromes. *mSystems* 2018, 3, e00131-18.
128. Jiang, D.; Armour, C.R.; Hu, C.; Mei, M.; Tian, C.; Sharpton, T.J.; Jiang, Y. Microbiome multi-omics network analysis: Statistical considerations, limitations, and opportunities. *Front. Genet.* 2019, 10, 995.