Symbiont interactions bias measures of arthropod biodiversity

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Abstract

Although arthropods comprise the majority of all named species, their biodiversity is relatively understudied. Here, we highlight how interactions between arthropods and their bacterial symbionts can pose challenges for biodiversity research, especially for estimating taxonomic, functional, and genetic diversity. We also argue that recent technological developments and biomonitoring schemes provide excellent opportunities to learn more about arthropod-symbiont interactions, consequently leading to a better understanding of arthropod biodiversity.

Introduction

With one million described species[1] and about 5-10 million species in total[2,3], arthropods account for the majority of animal species. It is estimated that more than half of all arthropod species carry specialised inherited bacterial symbionts[4]. Such symbionts play a key role in shaping their diversity[5]. For example, many insects feed on nutritionally unbalanced food sources (e.g. xylem, phloem, blood) that lack essential nutrients. By supplying amino acids or vitamins, symbionts bolster their hosts' diet and shape their feeding niches[6]. Symbionts can also manipulate host reproduction[7,8], confer adaptations to abiotic stressors[9], enhance detoxification[9], and provide defense against natural enemies[10,11]. Symbionts not only impact many aspects of host biology, but also population and community dynamics[12], influencing arthropod ecology and evolution. Despite increasing efforts to catalogue host-symbiont interactions[13], we know very little about the functional role of most arthropod associates beyond model systems.

Arthropod communities are crucial for the functioning of most ecosystems[14,15], but many species are declining[16,17]. Despite their importance, arthropods are severely under-represented in biodiversity research and conservation monitoring[18]. Many taxa have yet to be described by science and countless described species are poorly understood[19]. Limited data on arthropods makes it difficult to establish historical baselines, understand phenological shifts, or estimate temporal shifts in abundance[20]. However, recent technological developments may revert this trend[18]. Molecular methods can inform the monitoring and conservation of community composition, occupancy, species richness, and species interactions[18]. Innovations such as megabarcoding of bulk samples[21] or incorporating environmental DNA alongside remote sensing[22] can make large scale biodiversity studies more accessible and standardised long-term monitoring of arthropods more feasible. These technological advances, alongside novel network analysis approaches[23], also offer exciting new opportunities to detect, monitor, and study arthropod symbionts, contributing to a better understanding of arthropod biodiversity, and how host-symbiont interactions contribute to ecosystem functioning and responses to global change.

Although symbiosis is pervasive in arthropods, its significance has so far received little attention in biodiversity research. However, with the potential for wider implementation of molecular methods for monitoring arthropod biodiversity, it is increasingly important to consider symbionts and how they may interfere with biodiversity measurements. Symbionts have the potential to affect biodiversity metrics in ways that obscure patterns of conservation interest. We argue that considering the effects of microbial symbionts is crucial for interpreting arthropod biodiversity data. First, we illustrate how symbionts influence estimates of *taxonomic diversity* by affecting the amplification and inheritance patterns of molecular markers. Next, we show that many traits used to estimate *functional diversity* in arthropod communities are directly and dynamically altered by symbionts. We argue that symbionts can bias estimates of *genetic diversity* in arthropod populations, while simultaneously providing insight into their host's functional genetic diversity. Finally, we highlight how studying symbiotic relationships can improve predictions of biodiversity change in response to global environmental change.

Taxonomic diversity

Taxonomic diversity is used to inventory the species in an area, underpinning conservation lists[24], the basis of international agreements, and nature legislation[25]. The first step of estimating taxonomic diversity is species identification--a non-trivial task for arthropods. Although arthropod species are best delimited through integrative taxonomy methods[26], DNA barcoding is increasingly replacing other approaches[27]. In principle, DNA barcoding is possible with many loci; however, in practice, a partial sequence of the mitochondrial locus *cytochrome c oxidase subunit 1* (COI) is usually the only marker employed for barcoding arthropods[28]. In metabarcoding approaches, bulk samples of arthropods (e.g., from a malaise trap) are homogenized, DNA is extracted from the homogenate, and COI is amplified via the polymerase chain reaction (PCR). High-throughput sequencing allows cost-effective recovery of COI haplotypes which can then be assigned to a species by comparing it to a reference database (e.g., BOLD[29]). Alternatively, when references are lacking or incomplete, haplotypes can be clustered into operational taxonomic units (OTUs) based on sequence identity thresholds (often 97% similarity[30]).

Inherited symbionts can interfere with DNA barcoding protocols, directly impacting taxonomic diversity estimates. This happens for two main reasons. First, most bacterial symbionts carry a homologue of the mitochondrial COI locus which can be targeted by universal COI barcoding primers, meaning that symbiont DNA competes with host DNA for barcoding primers[31]. Fortunately, symbiont sequences can be detected and removed from the data *in silico*[32,33]. Given that PCR biases are more pronounced for some taxa than for others[34], they may systematically change the relative abundance of reads in the sample, with higher potential for rare species to go undetected. Such effects could be difficult to detect and avoid in large-scale arthropod metabarcoding studies, particularly those involving dark taxa[35] where the existence or prevalence of symbionts is unknown.

Second, like mitochondria, most common bacterial symbionts of arthropods are strictly maternally inherited. This introduces linkage disequilibrium between symbionts and mitochondrial haplotypes (i.e., symbionts and specific mitochondrial variants are inherited together), and can cause indirect selection on mitochondrial DNA (mtDNA)[36]. Because of this, symbionts can artificially inflate or reduce species diversity estimates that are based exclusively on mtDNA[37]. The spread of symbionts in arthropod populations, for example through reproductive manipulation[38], therefore leads to the spread of specific mitochondrial haplotypes[39–41]. This phenomenon is very widespread in arthropods and generally leads to reduced mitochondrial diversity across individuals carrying a symbiont[36]. A uniform mitochondrial haplotype associated with symbiont presence can be mistaken for a taxonomic lineage distinct from individuals without the symbiont, and inflate species richness estimates. Similarly, multiple symbiont strains or species in a single host species can lead to multiple distinct mitochondrial haplotypes, again biasing species richness estimates upwards. Symbionts can also lead to decreased species richness estimates based on mtDNA. When symbionts spread into novel species through hybrid introgression, the associated mitochondria may also spread, resulting in multiple species sharing the same symbiont and mtDNA haplotypes[42,43].

These effects of linkage disequilibrium between symbionts and mitochondria have been discussed since the advent of DNA barcoding for species identification > 20 years ago[36]. Examples have been reported from many arthropod lineages, including flies (Diptera)[44,45], butterflies and moths (Lepidoptera)[40,46–48] , bees and wasps (Hymenoptera)[49], and beetles (Coleoptera)[50], which together comprise the bulk of arthropod species. It is therefore reasonable to expect taxonomic diversity estimates based on mtDNA to be widely affected by symbionts. Notably, symbionts may be gained, lost, or replaced throughout arthropod evolutionary history[51,52]. Thus it is impossible to conclusively assess if, and to what extent, symbionts have had an effect on mitochondrial inheritance patterns in their hosts. The inclusion of complementary nuclear markers for taxonomic identification would mitigate these issues. Protocols for amplifying nuclear markers across most arthropod taxa are available[53], and adding such markers to existing barcoding pipelines requires little additional effort. Using multiple loci for molecular species identification is already practiced in other taxa. For example, the internal transcribed spacer (ITS) is used in combination with secondary markers in fungal metabarcoding[54]. For metabarcoding arthropod communities, using additional nuclear markers would allow independent verification of diversity measurements obtained from COI barcodes, and thus alleviate biases that stem from interactions with symbionts or other sources.

Functional diversity

Functional diversity focuses on the diversity of organismal traits in a community that shapes ecosystem processes[55]. Symbionts directly influence many traits related to life history, physiology, and behavior[56], including those commonly recorded as functional traits in arthropods[57] (Supplemental Material Table S1). For many functional traits, without knowledge of symbiont presence, the presence or absence of an arthropod species supplies only part of the information. For example, symbionts heavily influence arthropod feeding niches, corresponding to the feeding guild functional trait (Table S1). Symbionts can enable hosts to exploit otherwise unavailable food sources[58,59] by aiding digestion of plant biomass[60,61], supplementing nutrients[51,62,63], and preserving nutritional resources[64], contributing productivity and nutrient cycling at the ecosystem level.

Although nutritional symbionts are often necessary for host survival (i.e., obligate symbionts), many other symbionts are facultative. Among other functions, facultative symbionts provide defense against natural enemies, manipulate host reproduction, or assist with detoxification[5,12]. The presence of facultative symbionts, their frequencies within and across populations[65], and the degree of their influence on functional traits[66] can change over time. As an example, *Bemisia tabaci,* an important agricultural pest and plant-disease vector, carries the inherited intracellular symbiont *Rickettsia.* Individuals carrying *Rickettsia* develop faster (functional trait[57]: relative growth rate) and produce more eggs (clutch size) than symbiont-free conspecifics[67].

As with other facultative endosymbionts[65], the incidence rate of *Rickettsia* has changed over time. In a North American population of *B. tabaci* changed in less than 20 years from nearly absent to near fixation, and subsequently declined to about one third[68]. At the same time, the impact of *Rickettsia* shifted from enhancing whitefly fitness to becoming costly[68]. As one of many bacterial symbiont lineages[69], *Rickettsia* is found in about one quarter of known arthropod species[4], so similar effects on functional traits are likely to be observed across host species (Supplementary Table S1). These dynamic changes pose challenges when relying on literature data to compile functional trait databases that record static species-level functional traits, as is common practice[70], because traits attributed to arthropod species may in fact be more strongly associated with symbiont presence. Without knowledge of symbiont presence and how symbionts shape arthropod phenotypes, estimates of trait diversity may not reflect functional diversity related to ecosystem processes of interest.

Genetic diversity

The potential for a large influx of arthropod genetic data available from eDNA and metabarcoding efforts has generated interest in using these data to estimate genetic diversity for research and monitoring[28,71,72]. Genetic diversity is targeted for conservation due to its importance for population persistence in the short- and long-term[73]. Although multiple types of genetic diversity can be evaluated for conservation purposes (e.g., gene-specific diversity, functional genetic diversity, or neutral genome-wide genetic diversity), neutral genome-wide diversity is one of the most widely used[74]. Neutral genome-wide diversity is useful for broad-scale, multispecies assessments because it can be linked to population fitness, capacity to respond to environmental change, and common environmental perturbations such as habitat loss and fragmentation that affect population demography and species extinction risk[75].

Despite its widespread usage, the mitochondrial DNA barcoding gene COI is not useful for measuring conservation-relevant genetic diversity outside of specific purposes[76]. Although any single gene will fail to capture genome-wide diversity or the evolutionary history of species[77] the unique biology of mitochondria (non-neutral, maternally inherited, no recombination), can decouple its diversity from that of the nuclear genome[78]. In arthropods, maternally inherited symbionts shape mitochondrial variation in ways that can further decouple it from nuclear genome-wide variation, generating mito-nuclear discordance[47,79,80]. As noted in the *Taxonomic diversity* section, many common symbionts are maternally inherited, generating strong linkage disequilibrium between symbiont and mitochondrial genomes. Inherited symbionts can cause indirect selection on the mitochondria[36] that may reduce or increase mitochondrial diversity and mitochondrial effective population sizes[81]. Selective sweeps, where symbionts (and thus linked mitochondrial haplotypes) increase their frequency or become fixed in the host population, reduce mitochondrial genetic diversity. This means that estimating genetic diversity from the COI gene or other mitochondrial barcoding loci will not reflect population demography or be associated with adaptive potential in general, and this disconnect from conservation goals is likely stronger in arthropod species with maternally inherited symbionts.

However, symbiont genetic diversity itself may be of interest[82]. Because symbionts moderate and generate arthropod phenotypes, the genetic diversity of symbiont genomes may reflect functional genetic diversity of their hosts, that is, genetic diversity with known relationships to adaptive traits. For example, in aphids defense phenotypes (e.g., production of toxins against parasitoids) vary depending on the symbiont genotype, and cannot be predicted based on the presence of the symbiont alone[83,84]. Understanding the genetic variation in symbiont genomes is therefore important for predicting host response to enemy exposure.

Predicting biodiversity change in response to global change

Identifying factors that determine the spatial distribution of species and how they may change in response to human-driven climate change is of central interest in biodiversity research. Abiotic, environmental variables such as temperature and precipitation are often used in ecological niche models to model and predict species range boundaries under future climate change scenarios. Biotic interactions, including host-symbiont interactions, can be modeled using ecological network analyses[23]. These biotic interactions shape species' ranges and range shift dynamics[85]. For example, symbionts can facilitate range expansion[5] or enhance persistence by enabling their hosts to adapt to environmental change[86,87] and natural enemies[88–90]. On the contrary, symbionts may also limit their hosts' range by constraining the host to a particular plant or temperature range[91,92].

Evidence demonstrates that environmental factors impact host-symbiont interactions[9,93–95]. Here, we focus on temperature to outline how environmental effects on symbionts may impede predictions of biodiversity change. Temperature directly affects symbiont titres[92,96], impact host fitness[93], and often alters symbiont-mediated host phenotypes such as thermal preference[97], protection against parasitoids[94,98] and reproductive manipulation[99]. Temperature can also influence the efficiency of vertical transmission[100] and the overall incidence of symbionts in host populations[101]. These effects of temperature on symbionts can add considerable uncertainty to modeling and predicting arthropod species distributions, as we suggest in the examples below.

Wolbachia, the most common heritable endosymbiont (present in 50% of terrestrial arthropod species[4]), is a common facultative associate of *Drosophila melanogaster* flies. Flies carrying *Wolbachia* prefer cooler temperatures compared to symbiont-free flies, and differences in thermal preference between flies carrying different *Wolbachia* strains can be as high as 3°C[102]. Similar trends are found across different *Wolbachia* strains and various host species[103], indicating a general pattern of *Wolbachia*-governed thermal preference in arthropods. Furthermore, in natural populations of *Drosophila melanogaster*, the predominant *Wolbachia* strain was very recently replaced, likely changing the thermal preference of infected individuals in less than 100 years[104–106]. This illustrates the inherent difficulty of estimating species distributions under climate change based on current ecological niches: upon loss or exchange of symbionts, the temperature optima for hosts may change rapidly. Although the role of symbiosis in climate change response is well documented and regularly considered in organisms such as corals[107], it is often neglected in terrestrial studies. Through host shifting, facultative symbionts may swiftly and unpredictably change the temperature preference and therefore the geographic distribution of their hosts.

Even in seemingly static obligate symbioses with long term co-evolved partners, symbiont replacements and interactions with additional symbionts may alter the thermal limits of their hosts in an unpredictable fashion. For nearly 200 million years aphids have codiversified with the nutritional symbiont *Buchnera*, relying on the bacteria to complement their plant sap diet with essential amino acids[52]. Short periods of heat strongly affect *Buchnera*, which in turn strongly reduces aphid fitness[108]. This is in part due to *Buchnera*'s reduced genome. Like many obligate endosymbionts, *Buchnera* experiences weak purifying selection, leading to an accumulation of mutations[109]. These mutations can contribute to thermal instability of proteins, triggering heat sensitivity in symbionts[110]. The heat sensitivity of *Buchnera* thus limits the thermal flexibility of their aphid hosts, which has been cited as a major reason why so few aphid species colonize the tropics[111]. In some aphid species, the acquisition of facultative symbionts may help overcome these constraints by buffering the negative effects of heat on *Buchnera*[112,113] . In other aphid species, the obligate symbiont *Buchnera* was replaced[52]. Similarly, the association of the obligate nutritional symbiont *Candidatus* Karelsulcia muelleri with auchenorrhynchan insects (cicadas, leafhoppers, planthoppers, and others) dates back more than 260 million years[114]. While still present in many of these hosts, multiple evolutionary independent replacements by other bacterial or fungal symbionts have occurred[115]. In sum, symbioses can strongly affect species distributions and have the potential to improve predictions of species responses to environmental change.

Moving forward: incorporating symbionts into biodiversity research

For many commonly applied approaches to assess arthropod biodiversity, symbionts may represent an obstacle or confounding factor not usually accounted for in biodiversity studies. At the same time, our limited knowledge on widespread arthropod-symbiont interactions means it is likely that additional symbiont-mediated effects on biodiversity metrics are yet to be discovered. Furthermore, symbionts naturally fluctuate in frequency, regularly shift host species, and impact various host traits, making their impact on biodiversity estimates dynamic and difficult to predict. For these reasons, in our opinion, it is necessary to better integrate symbiotic interactions into arthropod biodiversity research. This may be achieved without major changes to study design. For example, the taxonomy of arthropods in biodiversity studies is often ascertained through DNA barcoding. The importance of molecular identification tools will likely increase with the advent of large-scale arthropod biomonitoring schemes[116–119]. Such projects present huge opportunities to collect barcoding data not only on arthropods, but also their symbionts. Technically, this would involve just one additional PCR per sample (targeting a bacterial barcoding locus), which may be multiplexed for convenience[120].

Given symbionts' broad importance for arthropod phenotypic diversity, capacity to adapt to environmental change, distributions, and ecologically relevant traits, symbiont communities can and should be direct targets of conservation and large-scale biodiversity monitoring efforts[121]. Incorporating symbionts into biodiversity monitoring is in line with the increased recognition of the importance of microbial conservation, as evidenced by the newly established Microbial Conservation Specialist Group (MCSG) in the International Union for Conservation of Nature (IUCN)[122]. Complementing arthropod with symbiont metabarcoding would give unprecedented insight into the diversity of arthropod-symbiont interactions, as well as temporal, spatial, and environmental variation in these interactions, providing a better, more integrative understanding of arthropod biodiversity and community functioning[23].

Author Contributions

All authors conceptualized the project. ED wrote the first draft of the manuscript, and all authors contributed to reviewing and editing subsequent manuscript drafts. ED created the visual.

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Competing Interests

All authors declare no financial or non-financial competing interests.

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Code Availability

Code availability is not applicable to this article as no code was generated for the current study.

Supplementary Information

Table S1: Examples of how symbionts shape arthropod functional traits.

The functional traits are grouped by category (morphology, feeding, life history, physiology, behaviour). Each trait includes an example, along with the relevant host and symbiont species. The categories and traits are based on a handbook of invertebrate functional traits[57]. Functional traits not included in the handbook are indicated with an asterisk (*). The table is comprehensive, but not exhaustive or systematic (i.e., it covers a broad range of examples, but does not include all relevant studies.)

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Supplementary information: Symbiont interactions bias measures of arthropod biodiversity

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Table S1: Examples of how symbionts shape arthropod functional traits.

The functional traits are grouped by category (morphology, feeding, life history, physiology, behaviour). Each trait includes an example, along with the relevant host and symbiont species. The categories and traits are based on a handbook of invertebrate functional traits[1]. Functional traits not included in the handbook are indicated with an asterisk (*). The table is comprehensive, but not exhaustive or systematic (i.e., it covers a broad range of examples, but does not include all relevant studies.)

Category	Functional Trait	Example	Host	Symbiont	Ref.
Morphology	Body size	Symbiont presence associated with larger progeny.	Red spider mite *Tetranychus urticae*	*Wolbachia*	2
		Higher host body size and water content compared to aposymbiotic conspecifics.	Rice weevil *Sitophilus oryzae*	*Sodalis pierantonius*	3
		Loss of symbiont results in stunted growth and reduced body size.	Southern green stink bug *Nezara viridula*	Gut bacterial symbiont (unnamed)	4
	Respiration system	Presence of symbiont triggers development of an extensive tracheal network that envelops the host's gut.	Bean bug *Riptortus pedestris*	*Burkholderia insecticola*	5
	Colour	Aposymbiotic offspring are paler compared to those with symbiont.	West Indian sweet potato weevil *Euscepes postfasciatus*	*Nardonella*	6
		After losing its symbiont, the host turns a yellowish colour.	Southern green stink bug *Nezara viridula*	Gut bacterial symbiont (unnamed)	4
	Cuticle hardness*	Symbiont provisions tyrosine, which is used for host cuticle synthesis.	Superfamily Curculionoidea	*Nardonella*	7
	Sex traits*	Presence of symbiont induces feminization in its host, including appearance of female genital apertures.	Various isopod species (e.g., *Armadillidium vulgare, A. nasatum*)	*Wolbachia*	8,9
Feeding	Feeding guild	Symbiont provisions amino acids and vitamins for sap-feeding hosts, whose diet is high in sugar but low in other nutrients.	Various Auchenorrhyncha and Sternorrhyncha species	Various bacterial symbionts *(Sulcia muelleri, Sodalis glossinidius, Buchnera aphidicola* etc.)	10–14
		Symbiont helps its host procure B vitamins, which are lacking from the host's blood-based diet.	Bed bug *Cimex lectularius*	*Wolbachia*	15
		The host's ability to digest and live on a diet of lignocellulose depends on its symbionts.	Eastern subterranean termite *Reticulitermes flavipes*	Various protist and bacterial gut microbes	16
	Ingestion rate	Symbiont infection increases feeding rate and weight gain in the host.	Silkworm *Bombyx mori*	*Cordyceps militari*	17
		After mating with symbiont infected males, host females have a lower feeding frequency.	Fruit fly *Drosophila melanogaster*	*Wolbachia*	18

Life history	Ontogeny	Symbiont proliferates dramatically after metamorphosis and synthesizes amino acids needed for building a protective exoskeleton.	*Cereal weevil* *Sitophilus spp.*	*Sodalis pierantonius*	19
		Individuals with symbionts developed faster than those without.	Silverleaf whitefly *Bemisia tabaci*	*Rickettsia*	20
	Clutch size	Individuals with symbionts produce more offspring compared to uninfected individuals.	Silverleaf whitefly *Bemisia tabaci*	*Rickettsia*	20
		Individuals with symbionts have more eggs in their ovaries compared to aposymbiotic individuals.	Fruit fly *Drosophila neotestacea*	*Spiroplasma*	21
	Egg size	Maternal fitness and egg size increase when infected with one symbiont, but decrease when infected by a different symbiont.	*Kelly's citrus thrips* *Pezothrips kellyanus*	*Cardinium* (increase), *Wolbachia* (decrease)	22
		Egg size increases with symbiont infection, which in turn promotes fertilization.	Red spider mite *Tetranychus urticae*	*Wolbachia*	2
	Egg production*	Symbiont plays a role in egg production. Following antibiotics treatment, the host has lower fecundity and eggs are no longer viable.	*Rice water weevil* *Lissorhoptrus oryzophilus*	*Wolbachia*	23
	Life span	Individuals with the symbiont infection were more likely to survive into adulthood.	Silverleaf whitefly *Bemisia tabaci*	*Rickettsia*	20
		Symbiont infection decreases larval survival and adult longevity.	Mill moth *Ephestia kuehniella*	*Wolbachia*	24
		Symbiont infection decreases longevity.	Fruit fly *Drosophila melanogaster*	*Spiroplasma*	25,26
	Age at maturity	Individuals with symbiont reach adulthood faster than those without.	Silverleaf whitefly *Bemisia tabaci*	*Rickettsia*	27
	Reproductive mode	Parthenogenesis: Symbiont infection is linked to parthenogenesis (asexual reproduction, where embryos develop from unfertilized eggs).	Broad-nosed weevils Tribe *Naupactini*	*Wolbachia*	28,29
			Parasitoid wasp *Pnigalio soemius*	*Rickettsia*	30
		Cytoplasmic incompatibility (CI): Symbiont infection triggers CI (mating between infected males and uninfected females results in embryo death.)	Spider mite *Eotetranychus suginamensis*	*Cardinium*	31
			Parasitoid wasp *Encarsia inaron*	*Cardinium, Wolbachia*	32

		Male-killing (MK): Symbiont infection results in MK (males die during embryogenesis).	Ladybird beetle *Adalia bipunctata*	*Rickettsia*	33,34
			Pierre's acraea butterfly *Acraea encedana*	*Wolbachia*	35
			Fruit fly *Drosophila melanogaster*	*Spiroplasma*	36
	Voltinism	Univoltine and multivoltine populations are associated with different symbiont strains.	Plum curculios *Conotrachelus nenuphar*	*Wolbachia*	37
Physiology	Resting metabolic rate	Facultative symbionts are energetically costly and increase the standard metabolic rate of the host.	Pea aphid *Acyrthosiphon pisum*	*Hamiltonella defensa,* *Regiella insecticola*	38
	Relative growth rate	Killing the symbiont reduces the host’s growth rate.	Cotton stainers *Dysdercus fasciatus* Red firebugs *Pyrrhocoris apterus*	*Actinobacteria*	39
		Hosts without symbionts have lower growth rates.	Saw-toothed grain beetle *Oryzaephilus surinamensis*	*Sulcia*-like bacteria	40
	Desiccation resistance	Symbiont contributes to host’s cuticle production and increases cuticle thickness, thus preventing transpiration and enhancing desiccation resistance.	Saw-toothed grain beetle *Oryzaephilus surinamensis* Rice weevil *Sitophilus oryzae*	*Sulcia*-like bacteria; *Sodalis pierantonius*	3,40
		Artificially introduced symbiont confers desiccation resistance to the host.	Yellow fever mosquito *Aedes aegypti*	*Wolbachia*	41
	Salinity resistance	Symbionts of hosts living in arid, saline-alkali areas are more resistant to saline-alkali stress.	Beetles Assorted Coleoptera	Various bacterial species	42
	Temperature tolerance	Shifts in symbiont heat sensitivity contribute to variation in heat tolerance of the host.	Aphids *Aphis gossypii, Ap. fabae, Acyrthosiphon kondoi, Ac. pisum*	*Buchnera*	43
		Hosts infected with different symbiont strains have different temperature preferences. Individuals infected with symbionts prefer lower temperatures compared to aposymbiotic individuals.	Fruit fly *Drosophila melanogaster*	*Wolbachia*	44

	Pesticide tolerance*	Hosts infected with symbiont have increased susceptibility to pesticides.	Silverleaf white fly *Bemisia tabaci* Brown plant hopper *Nilaparvata lugens*	*Arsenophonus, Rickettsia, Wolbachia*	45–47
	Nitrogen recycling*	Symbionts located in bacterial pouches are used for nitrogen recycling.	Slender ants *Tetraponera* spp.	Various bacterial species	48
	Pathogen resistance*	Symbiont protects the host from fungal infections.	Beewolf *Philanthus triangulum*	*Streptomyces*	49
		Symbiont protects the host from RNA viral infections.	Fruit fly *Drosophila melanogaster*	*Wolbachia*	50
	Protection from natural enemies*	Symbiont protects its host from parasitic nematodes.	Fruit fly *Drosophila neotestacea*	*Spiroplasma*	21
		Symbiont protects its host from parasitoid wasps.	Fruit fly *Drosophila melanogaster*	*Spiroplasma*	51
Behaviour	Activity time	Depending on the symbiont infection, the time the host sleeps increases or decreases.	Fruit fly *Drosophila melanogaster*	*Wolbachia* (increases), *Spiroplasma* (decreases)	52,53
		Nymphs with a higher symbiont titer spend more time resting whereas those without symbionts spend more time wandering.	Japanese common stinkbug *Megacopta punctatissima*	*Candidatus* Ishikawaella capsulata	54
	Aggregation	Symbionts produce the components of host aggregation pheromones.	Source bark beetle *Ips typographus* Desert locust *Schistocerca gregaria*	*Grosmannia spp.* *Pantoea agglomerans*	55,56
		Presence of microbes, not host pheromones, causes hosts to aggregate.	Firebrats *Thermobia domestica*	*Mycotypha microspora, Enterobacter cloacae*	57
	Dispersal mode	Flight activity of the host is contingent on the amino acids and vitamins procured by symbiont.	Grain weevil *Sitophilus oryzae*, *S. zeamais*	Enterobacteriaceae gut endosymbiont	58

		Long-distance dispersal via ballooning is influenced by symbionts. Certain symbionts decrease long-distance ballooning.	Money spider *Erigone atra*	*Wolbachia, Rickettsia* *Spiroplasma, Cardinium*	59
	Locomotion speed	Gut symbiont increases intrinsic movement, dispersal, and velocity.	Black bean aphid *A. fabae*	*Serratia symbiotica*	60
	Sociality	By enabling hosts to subsist on nutrient-poor wood, symbionts partially enabled cooperative societies in wood-dwelling insects.	Cockroaches, termites, beetles Blattodea, Isoptera, Coleoptera: *Passalidae*, Coleoptera: *Curculionidae* Coleoptera: *Tenebrionidae*	Various bacterial, fungal, protistan symbionts	61
	Defensive behavior*	Hosts infected with symbiont show less aggression and escape behavior when in the presence of parasitoid wasps.	Pea aphid *Acyrthosiphon pisum*	*Hamiltonella defensa*	62
	Kin recognition*	Conspecifics with a more similar gut bacteria avoided mating with each other.	Fruit fly *D. melanogaster*	Gut bacteria	63

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