



Published in final edited form as:

Nat Rev Microbiol. 2020 February ; 18(2): 97–111. doi:10.1038/s41579-019-0309-z.

Microorganisms in the reproductive tissues of arthropods

Jessamyn I. Perlmutter^{#,1,2}, Seth R. Bordenstein^{#,1,2,3,4}

¹Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee, 37232, USA.

²Vanderbilt Microbiome Initiative, Vanderbilt University, Nashville, Tennessee, 37232, USA.

³Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, Tennessee, 37232, USA.

⁴Vanderbilt Institute for Infection, Immunology, and Inflammation, Vanderbilt University, Nashville, Tennessee, 37232, USA.

Abstract

Microorganisms that reside within or transmit through arthropod reproductive tissues have profound impacts on host reproduction, health and evolution. In this Review, we discuss select principles of the biology of microorganisms in arthropod reproductive tissues, including bacteria, viruses, protists and fungi. We review models of specific symbionts, routes of transmission, and physiological and evolutionary outcomes of both hosts and microorganisms. We also identify areas in need of continuing research to answer fundamental questions remaining in fields within and beyond arthropod-microorganism associations. New opportunities for research in this area will drive a broader understanding of major concepts, biodiversity, mechanisms, and translational applications of microorganisms that interact with host reproductive tissues.

Graphical Abstract

In this Review, Perlmutter and Bordenstein discuss our current knowledge of microbial symbionts that inhabit or transmit through the reproductive tissues of arthropods, their modes of transmission, and physiological and evolutionary outcomes of the symbiotic relationships.

Introduction

In 1879, Heinrich Anton de Bary, a German microbiologist and botanist, coined the term symbiosis to mean the living together of dissimilar organisms^{1,2}. He devised the word symbiosis for his now famous talk discussing the relationships between algae, cyanobacteria and fungi that together form lichens¹. Today, the term symbiosis generally describes any

[#]Co-corresponding authors: S.R.B.: s.bordenstein@vanderbilt.edu, J.I.P.: jperlmutter16@hotmail.com.

Author contributions:

S.R.B. and J.I.P. wrote the article, reviewed and edited the manuscript before submission. J.I.P. initially drafted the article and researched data for the article.

Reviewer information:

Nature Reviews Microbiology thanks G. Hurst, I. Newton and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Competing interests statement:

The authors declare no competing interests.

relationship type between or among different organisms, including mutualism (all parties benefit), commensalism (one party benefits while the other is unaffected) and parasitism (one party benefits while another is harmed). These relationships are often context-dependent, and additional categories or subcategories exist that are not necessarily mutually exclusive, such as endosymbiosis, whereby one organism lives inside another, and hereditary symbiosis, in which microorganisms are transmitted from parent to offspring^{3–5}. Indeed, endosymbiosis and hereditary symbioses are common symbiotic relationships now recognized in many plants and animals⁵. Microorganisms of the reproductive tissues (discussed in this Review as those that reside in or transmit through reproductive tissues), including gonads, gametes and milk organs, are acquired from many different sources, including the environment and other host organisms (horizontal transmission) or from parent to offspring (vertical transmission). These microorganisms in particular can be key determinants of host fitness and offspring health because of their location and potential to be passed vertically. As microorganisms of the reproductive system are uniquely situated to alter host germlines and reproductive ability, it is crucial to understand their modes of transmission, functional relevance in hosts and effects on host evolution. Indeed, bacterial symbionts of arthropods are known to profoundly influence host reproductive strategies and physiology in ways that are often unique in the animal kingdom.

In this Review, we synthesize current knowledge on microbial symbionts that inhabit or transmit through the reproductive tissues of arthropods. We discuss which microorganisms are most often reported in these tissues, their various modes of transmission, and the influence of these symbioses on the evolution of hosts and microorganisms. We also assess widespread and specialized biological principles across various organisms and highlight major fundamental, unanswered questions in need of continued study. We then emphasize important future directions in the field, including a call for more microbial community sequencing in reproductive tissues, an increased focus on non-bacterial members of the microbiota and greater study into microorganism-microorganism interactions in reproductive tissues. New discoveries in this arena will spur innovation and discovery in both the basic and applied sciences, including vector and pest control efforts and a greater understanding of the impact of microorganisms-associated with the reproductive tract on host evolution.

Arthropod reproductive tissue microbiota

Microorganisms that inhabit arthropod reproductive tissues represent an exceedingly broad group of organisms spanning many orders of bacteria^{6–14}, fungi^{15–18}, protists^{16,19} and viruses^{16,20–22} (Figure 1). Microorganisms are present in the reproductive tissues of all of the major orders of arthropods⁵, including various insects^{15,23}, crustaceans^{24,25} and arachnids^{26,27} around the world. Spanning the entire range of symbiotic relationships with their hosts, these microorganisms vary from transient pathogens to obligate mutualists, and they perform various functions within hosts. Among these are the well-known bacterial, viral and fungal reproductive parasites that manipulate host reproduction^{6,20,23,28–30}; bacteria, viruses, fungi and protists that cause sexually transmitted diseases in their hosts¹⁶; commensal or harmful bacteria and viruses that use arthropods as vehicles to infect plants or other animals^{22,31–33}; bacterial nutritional symbionts that provide essential vitamins and other nutrients to the host^{8,13,34}; bacteria and viruses that protect hosts from

predation^{10,35–38}, and bacteria that perform nitrogen cycling for the host^{13,39}, among many others. Due to their successful survival strategies in the most speciose groups of animals, microbial inhabitants of arthropod reproductive tissues represent some of the most widespread symbioses in nature.

Though a great number of specific microorganisms and symbiotic relationships exist across the range of arthropod hosts, little is known about the complete diversity of microorganisms or microbial community interactions within host reproductive tracts with notable exceptions^{9,40–43}. Indeed, binary microorganism-host symbioses, particularly those that are hereditary, represent the majority of published research in the field. One of the few microbial community characterizations thus far was completed in the reproductive tract of two *Anopheles* mosquito species. The study reported that these mosquitoes contain on average 500 species-level bacterial OTUs in the reproductive tissues and that there is a core microbiota spanning seven genera shared among individuals of the same sex and swarm⁹. Another study showed that bedbugs exhibit a diversity of bacteria in their reproductive organs, with 31 sequence variants found across samples, although individuals harbor an average of three sequence variants⁴⁴. In addition, differences in communities occur between males and females and mated and unmated individuals, which suggests sex-specific differences and exchanges of microorganisms during copulation⁴⁴. In both studies, sequencing was not performed on contamination controls, and staining was not performed to confirm the presence of live bacteria. However, the data suggest that there may be important factors such as sex, proximity or relatedness of individuals that correlate with community structure. It also cautiously raises the potential that reproductive tissues of some arthropods may harbor a diversity of microorganisms. In addition, there are studies that focus on the interactions of several select symbiont species or strains within one host^{11,42,45}. Many of these investigations survey infection and co-occurrence rates in a population and illustrate the potential positive or negative influences that specific bacterial reproductive parasites have on each other's transmission^{40,46,47}. However, studies simultaneously assessing multiple microorganisms within arthropod reproductive tissues remain relatively scarce, and this is especially true for whole-community analyses.

We have little knowledge of how diverse the microbial communities are within arthropod host reproductive tissues, their temporal dynamics, how much community compositions vary between individuals or species, how well they correlate with host species phylogeny, what factors or conditions shape microbial communities, what kinds of interactions exist across the microbial community, or how they are acquired. In addition, surveys of non-bacterial taxa within arthropods are underrepresented in the literature, including those of viruses, fungi, protists and archaea. In particular and to the best of our knowledge, there are no studies that have investigated archaeal symbionts of arthropod reproductive tissues. Although archaeal methanogens are found in guts of termites and other insects⁴⁸, potential archaeal roles in reproductive tissues are largely unknown. Furthermore, research is far more common in insects than arachnids, crustaceans and other arthropods, although there are a few studies describing unidentified bacteria in the reproductive tissues of animals such as shrimp⁴⁹ and crabs⁵⁰, and some that identify endosymbionts of arachnids such as spiders and mites^{20,51–54}. Given the growing recognition of the role archaea have in the health of organisms, including humans⁵⁵, and an increased understanding of the role of microbial

community dynamics in the functions of diverse hosts^{56,57}, these are important research frontiers for the field to explore. Therefore, it will be crucial to better characterize the identities and dynamics of all members of the reproductive tissue microbiota of many hosts, as well as to emphasize additional research on symbiotic interactions in the context of their community rather than only in an isolated host-microorganisms relationship.

Transmission routes

Transmission routes of microorganisms within arthropod reproductive tissues have been extensively explored^{5,58}. The various transmission routes can be compared along numerous axes, including horizontal and vertical, maternal and paternal, intracellular and extracellular, sexual and non-sexual, host-driven and microorganism-driven, or transmission driven by microorganism-microorganism interactions and microorganism-host interactions, and these categories are not mutually exclusive (Figure 2). As hereditary microorganisms are arguably the best studied within the arthropod reproductive tract, a large portion of the research in this area has focused on vertical transmission routes.

One common form of vertical inheritance is transovarial transmission (inside the egg), which is typical for hereditary endosymbionts such as *Wolbachia*⁵⁹, *Rickettsia*⁶⁰, *Spiroplasma*⁶¹, *Buchnera*⁶², and *Hamiltonella*⁶³ spp., as well as certain fungi such as yeast-like symbionts (YLS)⁶⁴ and microsporidia⁶⁵, some viruses⁶⁶ and protists¹⁹. There are various mechanisms to ensure this form of passage, which are often microorganisms-driven. For example, *Wolbachia pipientis*, hereafter referred to as *Wolbachia*, infect germline stem cells⁶⁷ to spread into oocytes using host actin during oogenesis⁵⁹. They subsequently use egg microtubules to localize towards the posterior end of the embryo where cells are fated to become germline tissue⁶⁸, and the cycle repeats in the next generation. *Spiroplasma poulsonii* instead hijack the yolk uptake machinery to be endocytosed into the oocyte⁶¹. Alternatively, *Buchnera aphidicola* cells are carried by maternal bacteriocytes [G] in the midgut and are exocytosed into the extracellular space for a short period of time before selective endocytosis into the maternal blastulae [G] at ovariole tips⁶⁹. Indeed, internally transmitted parasitic microorganisms that hijack animal reproductive processes (for example, *Wolbachia* and *Spiroplasma* species) often drive their own transmission, whereas beneficial symbionts that are vertically inherited (for example, *Buchnera aphidicola*) often rely on host-driven processes that ensure passage to the next generation. In many cases in which the microorganism is vertically transmitted, the exact molecular details are not fully understood. Therefore, it will be important to continue to interrogate the molecular, genetic and biochemical mechanisms, especially among non-bacterial symbionts that have not been studied as extensively.

External vertical transmission (outside the egg) is also a typical route of transmission for bacteria and fungi, and there are many variations on this theme in nature⁷⁰. One common mechanism is smearing the symbiont on the egg as it exits the ovipositor [G]. For example in the tortoise beetle, the obligate, beneficial *Stammera* bacterial symbiont that is essential for breaking down pectin in the host's plant-based diet is transmitted from specialized reservoirs connected to the ovipositor, so eggs are covered by the time they are laid⁷¹. This is similar to the transmission of microorganisms in vaginally delivered human babies⁷².

However, there are many other modes of external transmission of symbionts that are known, particularly for bacteria. Sometimes the mother will produce secretions with beneficial microorganisms and deposit them onto eggs, which is the route of transmission of the bacterial symbiont *Candidatus Tachikawaea gelatinosa* in urostylidid stinkbugs⁷³; or the mother will secrete the substance containing the symbiont onto the surrounding area, as has been shown for necrophagous beetles⁷⁴. In other cases, the mother might package the bacteria into capsules on egg cases that are eaten as larvae emerge⁷⁵, or secrete the obligate, beneficial bacterial endosymbionts in a milky substance for her developing offspring^{12,76}. In addition, parasitic microorganisms may drive their own external transmission in some cases. For example, the male-killing endosymbiont *Arsenophonus* of *Nasonia* wasps exhibits a temporary tropism for the developing wasp oviduct and ovipositor, which promotes the external transfer of the bacteria via a transovum route to fly hosts. Subsequently, larval wasps feeding on the fly host become infected, and the transmission cycle repeats itself generation after generation⁷⁷. Despite the ever-growing knowledge of unique mechanisms of external transmission, many questions remain. For example, what is the full diversity of external transmission modes in nature? What are the external transmission routes of understudied non-bacterial symbionts, and do they differ from bacterial symbionts?

Many studies focus on the influence of host-microorganism interactions on transmission, but there is also an emerging interest in the impact of microorganism-microorganism interactions on transmission. In particular, hereditary symbionts often have the unique position of being the first microorganisms in or on the next generation of offspring. Such founding microorganisms could have the potential to shape downstream microbial community assembly and composition via positive or negative interactions with other microorganisms, and therefore, may be important determinants of offspring health. Microorganisms present in the parental reproductive tissues can notably affect which other microorganisms are passed to arthropod offspring. For example, newly acquired *Wolbachia* in *Anopheles* mosquitoes are not transmitted to the next generation⁴³ owing to negative interactions with the native microbiota. More specifically, if antibiotics are used to perturb existing members of the microbiota, *Wolbachia* are transmitted. If the resident gut and reproductive tissue bacteria, specifically of the *Asaia* genus, are supplemented back into the mosquitoes after antibiotic treatment, *Wolbachia* are no longer transmitted, which shows that bacteria in the *Asaia* genus negatively affect *Wolbachia* transmission⁴³. Moreover, certain populations of pea aphids are infected with many different hereditary endosymbiont species⁴⁰. Monitoring co-infection frequencies over time revealed that certain combinations of endosymbionts are more common than others. This suggests that microorganism-microorganism interactions within hosts have an impact on the transmission rates of these endosymbionts. In addition, microorganism-microorganism interactions can be costly or beneficial to the interacting symbionts and thus may affect increases or decreases in the proportion of individuals containing multiple symbionts in a population over time⁴⁰. Data support the notion that interactions among hereditary microorganisms can have lasting effects on which microorganisms get passed down to the next generation, which may have important implications for the health and fitness of the offspring. However, the fitness effects of these interactions have not all been fully experimentally explored. In addition, there has typically been a focus on how a select group of bacteria positively or negatively affects each

other's transmission; however, it remains to be elucidated how a microorganism or group of microorganisms affect the larger ecosystem of the symbiont and to what extent in the host and offspring.

It is perhaps not surprising that interactions between microorganisms of different taxa affect their transmission, although it is a comparatively rare topic in the literature. In one intriguing case, there is a unique type of vertical transmission of the rice dwarf virus (RDV) that is vectored between plants by leafhoppers¹¹. The virus is associated with an insect obligate bacterial symbiont, *Sulcia*, and hitchhikes on the envelope of the *Sulcia* symbiont via an interaction between a viral capsid protein and an outer membrane protein of *Sulcia*. After attachment, the virus uses *Sulcia* bacteria as a vehicle for transovarial transmission¹¹. This case exquisitely highlights largely underappreciated interactions between microbes of different classifications of life that influence each other's inheritance. Indeed, the RDV-*Sulcia* interaction is probably not unique as additional studies have reported data demonstrating interactions that affect inter-taxa transmission. For example, transmission of tomato yellow leaf curl virus (TYLCV) vectored by whiteflies depends on the chaperone protein GroEL from *Hamiltonella*⁷⁸, and transmission of potato leafroll virus (PLRV) requires the aphid endosymbiont-specific protein symbionin for transmission⁷⁹. Moreover, other studies reported the negative interactions of *Wolbachia* with the vertically inherited gypsy retrovirus⁸⁰ as well as Zika virus and other viruses in cases of hosts infected with non-native strains of *Wolbachia*^{81,82}. Future investigations are required to determine how common these interactions are and between which taxa. Are there interactions that occur among or between fungi, protists or any potential archaea and other microorganisms? Are there cases whereby more than two entities directly interact in transmission? Do multipartite interactions occur in well-studied binary symbioses of host and microorganism? How would a microorganism evolve transmission dependency on another microorganism rather than the host? How would this partnership in transmission affect the evolution of each member of the system over time? Do these interactions more easily develop with obligate symbionts than facultative microorganisms, or with parasites or mutualists? Multipartite transmission interactions represent an important future research area.

Maternal transmission is the most common form of transmission for bacteria rather than paternal transmission owing to the removal of cytoplasmic material during spermatogenesis; however, there are rare cases of paternal inheritance. For example, *Rickettsia* symbionts of leafhoppers are found within sperm heads and are transferred to offspring via the sperm⁸³. In addition, bacterial endosymbionts of insects, such as those of the genus *Asaia* of *Anopheles* mosquitoes or *Sodalis* of tsetse flies, are sexually transmitted from males to females and subsequently passed on vertically to offspring^{84,85}. Although rare for bacteria, many viral endosymbionts can also be both maternally and paternally transmitted, including the well-studied cases of sigma viruses in insects⁸⁶. Sigma viruses are relatively common symbionts in insects, but they are unusual as they are one of the few known insect viruses that are exclusively vertically transmitted via gonads; the transmission from females is still much more efficient than males, probably owing to the lower number of viral particles that can be packaged in sperm compared to eggs⁸⁶. Transmission by both parents enables symbionts to persist in additional contexts compared to symbionts with only one mode of transmission. Despite this benefit, there are far fewer known cases of paternal transmission of

microorganisms in nature, even across different microbial taxa. This may either reflect actual rarity in nature or that paternal transmission is understudied in some contexts⁸⁷. In addition, biparental transmission routes can result in important consequences that differ compared to organisms that are strictly or nearly always maternally transmitted. This includes whether or not male host fitness benefits the microorganism and the different infection rates and dynamics in a host population^{88,89}. Future research could help identify additional cases of paternal transmission across microbial taxa and assess common and differing biological principles that might link to different transmission routes or microbial taxa.

The subfield of microbial transmission routes in arthropod hosts is vast, and many questions remain. In particular, although there is extensive work on vertical inheritance of single bacterial symbionts in arthropods, other categories are not as well represented. Beyond the relatively understudied non-microbial taxa previously mentioned, less is generally known about more transient microorganisms associated with arthropod reproductive tracts, excluding the many known sexually transmitted infections (STIs)¹⁶ (Box 1), and there is a need for increased investigation into opportunistic microorganisms of insect genitalia⁹⁰. Moreover, how are microorganisms that are not vertically transmitted acquired, and are there any factors that select for certain microorganisms over others? Is there a 'core' or 'healthy' microbiota associated with arthropod reproductive tissues? What types of interactions exist between transient microorganisms and non-transient or inherited members of the microbiota? Those questions will need to be addressed in future research.

Evolutionary impacts

Impact on host fitness, development and ecology.

Microorganisms that inhabit or transmit through arthropod host reproductive tissues can have a fundamental impact on host fitness and physiology. For example, they can damage or destroy reproductive tissues^{16,17,91,92}; affect fecundity^{93–95}, oogenesis^{96,97} or spermatogenesis⁹⁸; have crucial roles in nutrient provisioning³⁹; influence offspring development rate^{93,99,100}; and affect predation or pathogen susceptibility^{10,35,101–103}. These effects may also extend beyond a single generation and affect long-term physiological development, survival or evolution of the host. One interesting case is that of the rove beetle and its vertically transmitted *Pseudomonas* endosymbiont that produces the polyketide pederin, which protects the host from predators³⁸. Over time hosts may even develop unique or specialized organs or proteins that function in housing symbionts (many are referred to as bacteriomes or mycetomes)⁷, controlling their transmission¹⁰⁴ or preventing pathogen transmission during mating^{105,106}. Another example is bedbugs that have a unique and costly form of copulation¹⁰⁷ whereby females are traumatically wounded during insemination. The male organ will pass through a specialized female organ, the spermalege, that has evolved at least in part to defend against pathogens that may be introduced during traumatic insemination¹⁰⁸. Bedbugs also have a mycetome attached to the gonads that allows vertical transmission of symbionts^{109,110}. As evidenced with bedbugs, specialized organs that defend against harmful symbionts and house helpful symbionts may evolve in the same host. Certain symbionts may even affect the size or shape of reproductive organs, as evidenced by *Wolbachia*-infected crickets¹¹¹. In some populations of crickets, *Wolbachia*-

infected females have different spermathecae duct [G] lengths than their uninfected counterparts, and this difference is recoverable following antibiotic treatment that removes the *Wolbachia* infection¹¹¹. Therefore, symbionts can influence not only the long-term evolutionary development of arthropod organs but also individual reproductive organ physiology as well.

In addition to physiological and fitness effects on arthropods, microorganisms of reproductive tissues may directly affect host reproduction. In particular, reproductive parasites span diverse bacterial¹¹², fungal¹¹³ and viral^{114–116} lineages and selfishly manipulate host reproduction to facilitate their own spread at the expense of the host. Resulting phenotypes in the host can include cytoplasmic incompatibility (death of offspring from crosses between infected males and uninfected females), male killing (specific death of male offspring), feminization (genetic males physically develop and reproduce as females), masculinization (genetic females physically develop as males) and parthenogenesis (female reproduce asexually)^{23,115}. Indeed, in long-term symbioses of this nature or in cases of horizontal gene transfer from microorganism to host, the evolution of host reproduction, sex determination or sex development may be altered^{117,118}. For example, long-term parthenogenesis in wasps can lead to an obligate dependency on the reproductive parasite due to the accumulation of mutations in male-specific genes and phenotypic erosion of the ability to sexually reproduce^{117,119}. In another case, feminizing *Wolbachia* of an isopod host are not transmitted via females with YY chromosomes, which resultantly produce all-male offspring in an intriguing case whereby a host allele may have evolved on sex chromosomes to avoid population-level extinction¹²⁰. Microorganisms of reproductive tissues therefore specifically benefit from influencing reproductive features and behaviors of the host that would not necessarily be advantageous to microorganisms of other sites, and they do so in different ways. For example, some bacteria may benefit by manipulating reproduction to increase the fecundity of their transmitting hosts. However, fungi may benefit by slowly killing their transmitting host to enable many spores to develop and spread to new hosts¹²¹. Viruses may potentially do either, as they function as reproductive parasites that kill during either early or late developmental stages^{28,122}. Although there are some extraordinary cases where evolution of arthropod sex development and determination are shaped by symbiosis, it is not known how common this phenomenon is nor are all of the mechanisms fully understood.

Beyond reproductive characters, microorganisms in the reproductive tracts can substantially affect the ability of their host to occupy a particular ecological niche. In some cases, the symbiosis affects the animals or plants with which the host interacts^{123–125}, and in others, endosymbionts confer differential temperature tolerance to their hosts that may narrow the range of environments suitable for the host¹²⁶ either by increasing¹²⁷ or decreasing¹²⁸ the range of tolerable temperatures to the host. In addition, environmental temperature can determine symbiont phenotype due to cold or heat sensitivity, as well as transmission. This has consequences for the spatial distribution of the host and symbiont and their ability to spread into new populations or survive in new environments¹²⁹. However, what are the molecular mechanisms of these changes (known in some cases to be due to the induction of heat shock proteins by the symbiont¹²⁷)? How does niche specialization begin and develop

over the course of a symbiotic relationship? Are the changes generally host- or microorganism-driven, and how do the changes differentially affect the fitness of the host and microorganism?

Gene expression, gene flow and genome evolution.

Host-microorganism interactions in the reproductive tissues not only affect fecundity and sexual selection, but they also have a substantial impact on the genome and transcriptome through modulation of gene expression; interdomain transfer of genes between the interacting partners; and evolutionary pressures acting on different genomes that have intertwined fates (Figure 3).

Several studies demonstrate the effect of a microbial symbiont on host reproductive tissue gene expression and imprinting^{106,130–133}, with the noteworthy caveat that some amount of somatic tissue was sometimes pooled with reproductive tissues¹³⁰. Potentially hundreds of genes are differentially regulated in reproductive tissues in the presence of a symbiont^{130,131}, gene expression profiles are different among infected soma and germline tissues²⁴, host genes may be differentially expressed in response to an endosymbiont in male and female tissues¹³⁰, and many of the genes that are differentially expressed have a role in metabolism, immunity and sex-specific developmental processes such as spermatogenesis^{24,106,130–132,134}. The reciprocal analysis of symbiont gene expression changes in soma and germline tissues is less common and thus results cannot yet be generalized. However, it is possible for symbiont genes to be expressed differentially in male and female reproductive tissues of a host¹³⁵. Although the mechanisms underlying the transcriptional changes are not fully resolved, symbiont-mediated epigenetic changes in the host are common, particularly in parasitic relationships^{133,136,137}. The role of microbially mediated epigenetic changes in host gene expression has mainly been studied in *Wolbachia*^{136,137}. Other endosymbionts such as *Buchnera* lack genes for DNA methylation¹³⁸, so there may be differences in any putative regulatory mechanisms across organisms. This body of work has generated many questions to be more broadly explored in the future. How are all of these complex transcriptional responses and relationships regulated? In what circumstances are epigenetic changes in the host modulated, and are they changed directly or indirectly by symbionts? How do expression patterns differ across pathogens and mutualisms or dependencies? What are all of the transcriptional trends across more recently evolved symbioses compared to ancient relationships? Are the general principles different across microbial taxa (non-bacterial symbionts are currently underrepresented)? Although these questions have been answered in some cases, there is a further need to assess them in additional contexts and organisms.

Interactions between microorganisms of reproductive tissues and hosts also shape their genome content via horizontal gene transfer. Instances of gene exchange between hosts and microorganisms have attracted considerable attention because such exchanges can potentially bestow novel genes or larger sequences of DNA that are functional and/or heritable in the recipient genomes. Many such transfers have been reported for diverse microbial taxa¹³⁹. Indeed, microorganism-to-host transfer events can include single genes^{140–142}, larger genomic regions¹⁴³ and nearly entire genomes^{118,144}, with some hosts

containing genes from multiple symbionts¹⁴³. For example, a single bacterial gene encoding cytolethal distending toxin was transferred to fly and aphid genomes and is likely to function in host defense¹⁴¹, and insertion and duplication led to the presence of several megabases of *Wolbachia* DNA in the genome of the *Armadillidium pillbug* and may underpin the development of a new sex chromosome¹¹⁸. In addition, many of transferred genes confer functions such as detoxification¹⁴⁵ and thus host protection. An intriguing case of gene transfer is that of the *oskar* gene, which is present in many insects and is crucial for host germline development¹⁴⁶. Part of the gene may have bacterial origins, thus making it a rare, putative case of bacterial gene transfer that functions in host reproduction¹⁴⁶. Transfers may also occur from host to microorganisms of various taxa^{20,147,148}, although fewer cases of this phenomenon have been discovered thus far. For example, prophage WO of *Wolbachia* harbors a eukaryotic association module that is composed of genes with regions of arthropod-like DNA²⁰. This phage module is expressed within arthropod gonads and some genes of this module manipulate arthropod reproduction^{149–152}. In light of the rarity of reported host-to-microorganism transfers, it is difficult to determine whether a particular function of transferred genes is enriched. It is likely that fewer eukaryotic genes are transferred to and maintained in bacteria due to inclusion of introns, exons and other elements that do not translate well in a bacterial genome, or it is possible that eukaryotic genes are retained less often due to their generally larger size. In addition to the above trends, it is apparent that most known transfer events occurred between the host and either bacteria or fungi, and there are relatively fewer examples known among viruses or other microorganisms¹³⁹. With great progress in this area in recent years, new research questions are now at the forefront of the field. For example, is the rate of gene transfer and maintenance different between microorganisms that primarily or exclusively occupy reproductive tissues compared with microorganisms that primarily occupy soma? What are the relative rates of transfer in each direction between host and microorganism, and do these rates differ among microbial or host taxa? What underlies any putative differences among taxa? Among all DNA transfer events in either direction, how many are retained and functional?

Interestingly, the effects of symbionts on the host genome extend beyond nuclear genes when the microorganism is co-inherited with non-nuclear DNA (Figure 3). In particular, mitochondrial DNA (mtDNA) is co-inherited with some symbionts via the cytoplasm, and thus sometimes associates with specific bacterial or microsporidian endosymbiont infections that can lead to co-associations between symbionts and mtDNA haplotypes^{153–155}. In these cases, certain mtDNA haplotypes become overrepresented in a population¹⁵⁶, mtDNA nonsynonymous mutations increase possibly in an arms race with the symbiont,¹⁵⁷ and mtDNA diversity is reduced compared to uninfected counterparts at either a population level¹⁵⁸ or globally¹⁵⁹. Moreover, *Candidatus* Midichloria mitochondrii bacteria that infect ticks exhibit the unique ability to invade mitochondria of ovarian cells¹⁶⁰, although the exact purpose and effects are not known. Overall, the intertwined evolution of mtDNA and symbiont occur specifically when the symbiont is intracellular, and thus this association is common for the many different endosymbiotic bacteria. The association is much rarer for fungi that are less often intracellular¹⁵, and it is unknown for viruses and protists, which may or may not be a reflection of their biology and remains to be further explored.

The impact of host-microorganism symbiosis on microbial genome evolution can be substantial¹⁴ (Figure 3). Indeed, vertically transmitted and obligate intracellular bacteria frequently experience reductive genome evolution owing to the confined lifestyle¹², relaxed selection due to functional redundancy with hosts¹⁶¹, genetic drift that occurs through the bottlenecks of vertical transmission through the matriline [G]^{162,163} and accelerated sequence evolution together with altered base compositions¹⁶⁴. In general, in the cases of evolving mutualisms, genes may be lost in either the host or microorganism to avoid redundancy in the hologenome [G]. The result is genome erosion until host and symbiont genomes complement each other¹⁴, which can mean that one or more partners may be left without an essential gene and the relationship becomes obligate. However, one standing question is how non-bacterial genomes change. For example, do intracellular, vertically inherited fungi or protists exhibit the same rates of gene loss and sequence evolution as bacterial symbionts? Are they more or less likely to experience genetic changes similar to bacteria, and why?

Host behavior, sexual selection and speciation.

Given the dependency of many microorganisms on host reproduction to spread through a population, they can influence host mating behavior and mate choice to facilitate their transmission. For example, *Wolbachia* infection can lead to increased female promiscuity and male fatigue in sex-biased, male-killer populations¹⁶⁵, discrimination between infected and uninfected sister species to avoid cytoplasmic incompatibility lethality¹⁶⁶ as well as preference for mating between uninfected individuals⁵². One particularly striking case is the effect of male-killing *Wolbachia* in *Acraea encedon* butterfly hosts¹⁶⁷. Populations can become extremely female biased because of high infection rates with the male killer. With fewer opportunities to mate, females begin to form lekking swarms and exhibit mate-attracting behaviors, a departure from the canonical formation of male lekking swarms to attract females. This inverted form of sexual selection ultimately enables males to be selective about preferable female characteristics, whereas normally the opposite is true¹⁶⁷.

Importantly, manipulation of host reproductive behavior to facilitate microbial spread goes beyond mate discrimination and mating frequencies. Indeed, infection of cicadas with the fungus *Massospora cicadina* correlates with altered male wing-flick patterns that mimic females so other males are attracted and infected¹⁷, and infection of *Helicoverpa zea* moths with the gonad-specific Hz-2V virus correlates with a 5-fold to 7-fold increase in female sex pheromone production and increased mating calls to attract and infect males¹⁶⁸. Particularly for microorganisms that depend on host reproductive tissues to transmit, altering mating behavior through direct manipulation or indirect mechanisms may be a successful survival strategy. However, many questions remain. Are microorganisms of reproductive tissues more likely to influence host mating behavior than symbionts of other body sites? Do they resultantly have a unique influence on host sexual selection that other symbionts less often have? Is the impact the same among parasitic and beneficial, exclusively and non-exclusively vertically inherited, or gonad-specific and multi-tissue symbionts? What are the mechanisms that drive behavioral changes; are they direct or indirect? Are there differences among microorganisms of different classifications? What effects do these behavioral changes have on the rest of the microbial community over many host generations?

With the profound influence that symbionts may have on arthropod mating behaviors and reproduction, it follows that in some cases, they can contribute to host reproductive isolation and thus speciation¹⁶⁹. Mechanisms of symbiont-induced isolation or speciation may include mate discrimination based on infection status¹⁶⁶, hybrid sterility from microbial over-proliferation^{170,171}, hybrid lethality^{172,173} or reproductive isolation due to microorganism-mediated specialization in distinct niches¹²⁵. For example, closely related species of the parasitoid wasp genus *Nasonia* that diverged several hundred thousand to one million years ago are strongly reproductively isolated by cytoplasmic incompatibility-inducing *Wolbachia* that cause severe lethality of F1 hybrid offspring in interspecific crosses¹⁷². However, this general phenomenon is most-often demonstrated in the cases of bacterial symbionts, and it is less understood to what extent other microorganisms may play a part. In at least the cases involving *Wolbachia*-induced cytoplasmic incompatibility, a role of the phage WO genes has been established¹⁴⁹. Many additional questions still need to be addressed: what is the frequency of microbial involvement in speciation events, do the mechanisms differ for different microorganisms, and is the contribution to host speciation enriched among parasitic or gonad-specific symbionts?

Conclusions and outlook

Microorganisms of host reproductive tissues have unique relationships with their hosts. Their proximity to germline tissues enables greater probability of interaction with hereditary DNA and vertical transmission that generates a dependency on host fitness. Moreover, heritability of microorganisms in these tissues raises the potential to have multi-generational impacts that span from individual physiological effects to speciation. These characteristics have led to interactions with the host that are unique or may occur with a different frequency compared to microorganisms of other tissues. Notably, the principles from arthropod-microorganism interactions in host reproductive tissues can extend to humans (Box 2) and plants (Box 3). Building on substantial work within the field, there are many important areas for future research since a wide context is still missing. We understand much regarding bipartite interactions among certain hereditary bacteria-host pairs, but far less is known about microorganisms that fall under different criteria. To better understand the fundamental biology of microorganisms in the unique context of the reproductive tissues, the field will benefit from a vigorous attention to greater diversity of microorganism and hosts in their full ecological contexts.

Future research should emphasize investigation into the identities of non-bacterial microorganisms in the reproductive tissues of diverse host taxa, entire microbial communities in the reproductive tissues, and the transient microorganisms of the reproductive tissues. Research interest has largely focused on microorganisms such as *Wolbachia* that have importance in vector control¹⁷⁴, but research into other diverse organisms remains scarce. Non-bacterial microorganisms interact with hosts in ways that are both similar (such as reproductive parasitism⁶) and different (such as phage manipulating both bacterial and arthropod hosts¹⁴⁹, or more frequent paternal transmission among certain taxa⁸⁶). Therefore, investigation into the identity of all microorganisms, their population dynamics, mechanisms of interactions with host and other microorganisms, and their functional evolutionary consequences will be crucial in the future. This is especially

important to do across diverse host taxa to provide a more comprehensive perspective and framework to identify major biological themes across nature, since model organisms or those of importance to agriculture and health have thus far received proportionally greater attention.

Regarding communities in reproductive tissues, it will be important to not only characterize their identities, but also any putative “core” microbiota that could be important for studies of evolution, vector control initiatives, or conservation efforts. Many microbiota subfields have flourished in recent years^{56,175–177}; however, the arthropod reproductive tissue context is currently underexplored. Although whole body microbiota analyses on arthropods are not lacking in general^{43,178,179}, few studies have specifically characterized the microbiotas of reproductive tissues^{9,44}. In addition, many studies lack proper, sequenced contamination controls or assays to assess if DNA is from living microorganism, so current findings must be taken with caution, and future studies should include such controls. Building on the research foundation of bipartite symbioses, it will be important for the field to interrogate complexity in microbial networks to gain a more holistic understanding of the microorganisms in reproductive tissues.

Finally, of the microorganisms that have been most fully explored in the literature, most are hereditary or pathogens such as endosymbionts¹⁸⁰ or the infectious agents of sexually transmitted infections^{16,181}, respectively. However, very little is known about other members of the arthropod reproductive tissue microbiota (including opportunistic microorganisms, reviewed elsewhere⁹⁰), particularly those that are horizontally acquired or whose host phenotypes caused by infection are less pronounced. For example, it is difficult to answer how much of the microbiota is horizontally or vertically acquired, if these frequencies differ among different microbial taxa or hosts, what factors determine their establishment or loss in the host, how or if they interact with hereditary microorganisms, or what level of selection there is for these transient organisms. It will also be important to continue research on microorganisms that transiently colonize the reproductive tissues versus those that exclusively colonize them. How are their rates of genetic exchange with the host different? Are there biases in which genes are exchanged? Do they have broadly similar impacts on host fitness and evolution? How are these symbioses different, in terms of both the relationship with the host and other microorganisms in the reproductive tissues and throughout the body? Are they more or less likely to exhibit parallel phylogenies with the host?

Continued research to answer these questions will enable advancements not only in our understanding of fundamental biological principles, but also potentiate new applied research in areas of vector biology, agriculture and conservation.

Acknowledgements:

The authors would like to thank M. I. Hood-Pishchany, K. Ngo, B. Leigh, and the journal editorial team for constructive comments. Work in the author’s laboratory was supported by National Institutes of Health award R21 AI133522 and the Vanderbilt Microbiome Initiative to S.R.B, as well as NIH F31 AI143152 to J.I.P.

Glossary Terms:

Bacteriocyte (or mycetocyte)

Specialized fat cell found in some insects that contain endosymbiotic organisms, especially bacteria and fungi that provide essential nutrients or functions for their hosts. Bacteriocytes or mycetocytes together form bacteriomes or mycetomes, which are specialized organs in some insects that house the symbionts.

Blastulae

Hollow spheres of cells surrounding a cavity of fluid, comprising the early stages in the development of embryos.

Ovipositor

The tube-like organ at the bottom of the abdomen that females use to lay eggs.

Spermathecae

An organ in the female reproductive tract in insects that is used to store sperm post mating.

Matriline

The exclusively female line of descent from a female ancestor to a female descendant.

Hologenome

The genome of a holobiont, which is the host and all its microbial symbionts. The hologenome includes the genomes of the host and microbes.

Vas deferens

A muscular tube in the human male reproductive tract that carries sperm to the ejaculatory duct.

Coronal sulcus

The indented groove at the base of the penis head.

References

1. Oulhen N, Schulz BJ & Carrier TJ English translation of Heinrich Anton de Bary's 1878 speech, 'Die Erscheinung der Symbiose' ('De la symbiose'). *Symbiosis* 69, 131–139, (2016).
2. De Bary A Die Erscheinung der Symbiose. Vortrag auf der Versammlung der Naturforscher und Aertze zu Cassel. Trubner, Strasbourg, 21–22 (1879).
3. Pierantoni U L'origine di alcuni organi d'Icerya purchasi e la simbiosi ereditaria. *Boll. Soc. Nat. Napoli* 23, 147–150 (1909).
4. Sapp J Paul Buchner (1886–1978) and hereditary symbiosis in insects. *Int. Microbiol* 5, 145–150 (2002). [PubMed: 12362890]
5. Funkhouser LJ & Bordenstein SR Mom knows best: the universality of maternal microbial transmission. *PLoS Biol.* 11, e1001631 (2013). [PubMed: 23976878]
6. Taylor MJ, Bordenstein SR, & Slatko B Microbe profile: *Wolbachia*: a sex selector, a viral protector and a target to treat filarial nematodes. *Microbiology* 164, 1345–1347 (2018). [PubMed: 30311871]
7. Chen X, Li S & Aksoy S Concordant evolution of a symbiont with its host insect species: molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinidia*. *J. Mol. Evol* 48, 49–58 (1999). [PubMed: 9873076]

8. Douglas AE Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol* 43, 17–37 (1998). [PubMed: 15012383]
9. Segata N et al. The reproductive tracts of two malaria vectors are populated by a core microbiome and by gender- and swarm-enriched microbial biomarkers. *Sci. Rep* 6, 24207 (2016). [PubMed: 27086581]
10. Oliver KM, Campos J, Moran NA & Hunter MS Population dynamics of defensive symbionts in aphids. *Proc. R. Soc. B* 275, 293–299 (2008).
11. Jia D et al. Insect symbiotic bacteria harbour viral pathogens for transovarial transmission. *Nat. Microbiol* 2, 17025 (2017). [PubMed: 28263320] This paper demonstrates that binding between a viral plant pathogen and an insect bacterial symbiont assists the vertical transmission of the virus through the insect.
12. Toh H et al. Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. *Genome Res.* 16, 149–156 (2006). [PubMed: 16365377]
13. Sabree ZL, Kambhampati S & Moran NA Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. *PNAS* 106, 19521–19526 (2009). [PubMed: 19880743]
14. Moran NA, McCutcheon JP & Nakabachi A Genomics and evolution of heritable bacterial symbionts. *Annu. Rev. Genet* 42, 165–190 (2008). [PubMed: 18983256] This review describes fundamental principles of symbiont transmission, genome evolution, and functions of symbiont-host interactions.
15. Gibson CM & Hunter MS Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. *Ecol. Lett* 13, 223–234 (2010). [PubMed: 20015249] This review covers the biological principles of fungal symbionts of insects.
16. Knell RJ & Webberley KM Sexually transmitted diseases of insects: distribution, evolution, ecology and host behaviour. *Biol. Rev. Camb. Philos. Soc* 79, 557–581 (2004). [PubMed: 15366763]
17. Cooley JR, Marshall DC & Hill KB A specialized fungal parasite (*Massospora cicadina*) hijacks the sexual signals of periodical cicadas (Hemiptera: Cicadidae: Magicicada). *Sci. Rep* 8, 1432 (2018). [PubMed: 29362478] This report demonstrates that *Massospora* fungi manipulate infected cicada males to exhibit female signals that in turn attract uninfected males, resulting in transmission between males during copulation attempts.
18. Gibson CM & Hunter MS Inherited fungal and bacterial endosymbionts of a parasitic wasp and its cockroach host. *Microb. Ecol* 57, 542 (2009). [PubMed: 18758845]
19. Boldbaatar D et al. Tick vitellogenin receptor reveals critical role in oocyte development and transovarial transmission of *Babesia* parasite. *Biochem. Cell Biol* 86, 331–344 (2008). [PubMed: 18756328]
20. Bordenstein SR & Bordenstein SR Eukaryotic association module in phage WO genomes from *Wolbachia*. *Nat. Commun* 7, 13155 (2016). [PubMed: 27727237] This paper reveals that large genomic modules in endosymbiont prophage WO are composed of genes derived from animal-phage gene transfers or that interact with animal biology.
21. van der Wilk F, Dullemans AM, Verbeek M & van den Heuvel JF Isolation and characterization of APSE-1, a bacteriophage infecting the secondary endosymbiont of *Acyrtosiphon pisum*. *Virology* 262, 104–113 (1999). [PubMed: 10489345]
22. Wei J et al. Vector development and vitellogenin determine the transovarial transmission of begomoviruses. *PNAS* 114, 6746–6751 (2017). [PubMed: 28607073]
23. Hurst GD & Frost CL Reproductive parasitism: maternally inherited symbionts in a biparental world. *CSH Perspect. Biol* 7, a017699 (2015).
24. Chevalier F et al. Feminizing *Wolbachia*: a transcriptomics approach with insights on the immune response genes in *Armadillidium vulgare*. *BMC Microbiol.* 12, S1 (2012). [PubMed: 22375708]
25. Haine ER, Motreuil S & Rigaud T Infection by a vertically-transmitted microsporidian parasite is associated with a female-biased sex ratio and survival advantage in the amphipod *Gammarus roeseli*. *Parasitology* 134, 1363–1367 (2007). [PubMed: 17445328]

26. Zeh DW, Zeh JA & Bonilla MM *Wolbachia*, sex ratio bias and apparent male killing in the harlequin beetle riding pseudoscorpion. *Heredity* 95, 41–49 (2005). [PubMed: 15931253]
27. Gotoh T, Noda H & Ito S *Cardinium* symbionts cause cytoplasmic incompatibility in spider mites. *Heredity* 98, 13–20 (2007). [PubMed: 17035954]
28. Kageyama D, Yoshimura K, Sugimoto TN, Katoh TK & Watada M Maternally transmitted non-bacterial male killer in *Drosophila biauraria*. *Biol. Lett* 13 (2017).
29. Ironside JE et al. Two species of feminizing microsporidian parasite coexist in populations of *Gammarus duebeni*. *J. Evol. Biol* 16, 467–473 (2003). [PubMed: 14635846]
30. Bandi C, Dunn AM, Hurst GD & Rigaud T Inherited microorganisms, sex-specific virulence and reproductive parasitism. *Trends Parasitol.* 17, 88–94 (2001). [PubMed: 11228015]
31. Perilla-Henao LM & Casteel CL Vector-borne bacterial plant pathogens: interactions with hemipteran insects and plants. *Front. Plant Sci* 7, 1163 (2016). [PubMed: 27555855]
32. Gray SM & Banerjee N Mechanisms of arthropod transmission of plant and animal viruses. *Microbiol. Mol. Biol. Rev* 63, 128–148 (1999). [PubMed: 10066833]
33. Hogenhout SA, Ammar E-D, Whitfield AE & Redinbaugh MG Insect vector interactions with persistently transmitted viruses. *Annu. Rev. Phytopathol* 46, 327–359 (2008). [PubMed: 18680428]
34. Baumann P Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol* 59, 155–189 (2005). [PubMed: 16153167]
35. Xie J, Butler S, Sanchez G & Mateos M Male killing *Spiroplasma* protects *Drosophila melanogaster* against two parasitoid wasps. *Heredity* 112, 399 (2014). [PubMed: 24281548]
36. Brownlie JC & Johnson KN Symbiont-mediated protection in insect hosts. *Trends Microbiol.* 17, 348–354 (2009). [PubMed: 19660955]
37. Degan PH & Moran NA Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. *Mol. Ecol* 17, 916–929 (2008). [PubMed: 18179430]
38. Kellner RL Molecular identification of an endosymbiotic bacterium associated with pederin biosynthesis in *Paederus sabaeus* (Coleoptera: Staphylinidae). *Insect Biochem. Molec. Biol* 32, 389–395 (2002). [PubMed: 11886773]
39. Feldhaar H et al. Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. *BMC Biol.* 5, 48 (2007). [PubMed: 17971224]
40. Rock DI et al. Context-dependent vertical transmission shapes strong endosymbiont community structure in the pea aphid, *Acyrtosiphon pisum*. *Mol. Ecol* 27, 2039–2056 (2018). [PubMed: 29215202]
41. Zhu L-Y et al. *Wolbachia* strengthens *Cardinium*-induced cytoplasmic incompatibility in the spider mite *Tetranychus piercei* McGregor. *Current Microbiol.* 65, 516–523 (2012). [PubMed: 22806335]
42. White JA, Kelly SE, Perlman SJ & Hunter MS Cytoplasmic incompatibility in the parasitic wasp *Encarsia inaron*: disentangling the roles of *Cardinium* and *Wolbachia* symbionts. *Heredity* 102, 483 (2009). [PubMed: 19223921]
43. Hughes GL et al. Native microbiome impedes vertical transmission of *Wolbachia* in *Anopheles* mosquitoes. *PNAS* 111, 12498–12503 (2014). [PubMed: 25114252]
44. Bellinvia S, Johnston PR, Reinhardt K & Otti O Bacterial communities of the reproductive organs of virgin and mated common bedbugs, *Cimex lectularius*. *Ecol. Entomol* (2019).
45. Kondo N, Shimada M & Fukatsu T Infection density of *Wolbachia* endosymbiont affected by co-infection and host genotype. *Biol. Lett* 1, 488–491 (2005). [PubMed: 17148240]
46. Skaljac M, Zanic K, Ban SG, Kontsedalov S & Ghanim M Co-infection and localization of secondary symbionts in two whitefly species. *BMC Microbiol.* 10, 142 (2010). [PubMed: 20462452]
47. Toju H & Fukatsu T Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: relevance of local climate and host plants. *Mol. Ecol* 20, 853–868 (2011). [PubMed: 21199036]
48. Tokura M, Ohkuma M & Kudo T Molecular phylogeny of methanogens associated with flagellated protists in the gut and with the gut epithelium of termites. *FEMS Microbiol. Ecol* 33, 233–240 (2000). [PubMed: 11098074]

49. Nimrat S, Bart AN, Keatsaksit A & Vuthiphandchai V Microbial flora of spermatophores from black tiger shrimp (*Penaeus monodon*) declines over long-term cryostorage. *Aquaculture* 274, 247–253 (2008).
50. Benhalima K & Moriyasu M Prevalence of bacteria in the spermathecae of female snow crab, *Chionoecetes opilio* (Brachyura: Majidae). *Hydrobiologia* 449, 261–266 (2001).
51. Duron O, Hurst GD, Hornett EA, Josling JA & Engelstädter J High incidence of the maternally inherited bacterium *Cardinium* in spiders. *Mol. Ecol* 17, 1427–1437 (2008). [PubMed: 18266629]
52. Vala F, Egas M, Breeuwer J & Sabelis M *Wolbachia* affects oviposition and mating behaviour of its spider mite host. *J. Evol. Biol* 17, 692–700 (2004). [PubMed: 15149411]
53. Martin OY & Goodacre SL Widespread infections by the bacterial endosymbiont *Cardinium* in arachnids. *J. Arachnol* 37, 106–109 (2009).
54. Vanthournout B, Vandomme V & Hendrickx F Sex ratio bias caused by endosymbiont infection in the dwarf spider *Oedothorax retusus*. *J. Arachnol*, 24–33 (2014).
55. Gaci N, Borrel G, Tottey W, O'Toole PW & Brugère J-F Archaea and the human gut: new beginning of an old story. *World J. Gastroenterol* 20, 16062 (2014). [PubMed: 25473158]
56. Gilbert JA et al. Current understanding of the human microbiome. *Nat. Med* 24, 392–400 (2018). [PubMed: 29634682]
57. Turner TR, James EK & Poole PS The plant microbiome. *Genome Biol.* 14, 209 (2013). [PubMed: 23805896]
58. Bright M & Bulgheresi S A complex journey: transmission of microbial symbionts. *Nat. Rev. Microbiol* 8, 218 (2010). [PubMed: 20157340]
59. Newton IL, Savytsky O & Sheehan KB *Wolbachia* utilize host actin for efficient maternal transmission in *Drosophila melanogaster*. *PLoS Pathog.* 11, e1004798 (2015). [PubMed: 25906062]
60. Brumin M, Levy M & Ghanim M Transovarial transmission of *Rickettsia* spp. and organ-specific infection of the whitefly *Bemisia tabaci*. *Appl. Environ. Microbiol* 78, 5565–5574 (2012). [PubMed: 22660706]
61. Herren JK, Paredes JC, Schüpfer F & Lemaitre B Vertical transmission of a *Drosophila* endosymbiont via cooption of the yolk transport and internalization machinery. *MBio* 4, e00532–00512 (2013). [PubMed: 23462112]
62. Wilkinson T, Fukatsu T & Ishikawa H Transmission of symbiotic bacteria *Buchnera* to parthenogenetic embryos in the aphid *Acyrtosiphon pisum* (Hemiptera: Aphidoidea). *Arthropod Struct. Dev* 32, 241–245 (2003). [PubMed: 18089009]
63. Dykstra HR et al. Factors limiting the spread of the protective symbiont *Hamiltonella defensa* in *Aphis craccivora* aphids. *Appl. Environ. Microbiol* 80, 5818–5827 (2014). [PubMed: 25015890]
64. Cheng D & Hou R Histological observations on transovarial transmission of a yeast-like symbiote in *Nilaparvata lugens* Stal (Homoptera, Delphacidae). *Tissue & Cell* 33, 273–279 (2001). [PubMed: 11469541]
65. Solter LF Transmission as a predictor of ecological host specificity with a focus on vertical transmission of microsporidia. *J. Invert. Pathol* 92, 132–140 (2006).
66. Huo Y et al. Transovarial transmission of a plant virus is mediated by vitellogenin of its insect vector. *PLoS Pathog.* 10, e1003949 (2014). [PubMed: 24603905]
67. Fast EM et al. *Wolbachia* enhance *Drosophila* stem cell proliferation and target the germline stem cell niche. *Science* 334, 990–992 (2011). [PubMed: 22021671]
68. Ferree PM et al. *Wolbachia* utilizes host microtubules and Dynein for anterior localization in the *Drosophila* oocyte. *PLoS Pathog.* 1, e14 (2005). [PubMed: 16228015]
69. Koga R, Meng X-Y, Tsuchida T & Fukatsu T Cellular mechanism for selective vertical transmission of an obligate insect symbiont at the bacteriocyte–embryo interface. *PNAS* 109, E1230–E1237 (2012). [PubMed: 22517738]
70. Salem H, Florez L, Gerardo N & Kaltenpoth M An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. *Proc. R. Soc. B* 282, 20142957 (2015).

71. Kikuchi Y et al. Host-symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. *BMC Biol.* 7, 2 (2009). [PubMed: 19146674]
72. Dominguez-Bello MG et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *PNAS* 107, 11971–11975 (2010). [PubMed: 20566857]
73. Kaiwa N et al. Symbiont-supplemented maternal investment underpinning host's ecological adaptation. *Curr. Biol* 24, 2465–2470 (2014). [PubMed: 25264255]
74. Shukla SP, Vogel H, Heckel DG, Vilcinskas A & Kaltenpoth M Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. *Mol. Ecol* 27, 1980–1991 (2017). [PubMed: 28748615]
75. Salem H et al. Drastic genome reduction in an herbivore's pectinolytic symbiont. *Cell* 171, 1520–1531.e1513 (2017). [PubMed: 29153832] In this report, the authors describe a nutritional symbiosis between a pectin-degrading bacterial symbiont and its pectin-eating beetle host, including characteristics of the microbial genome as well as its transmission.
76. Pais R, Lohs C, Wu Y, Wang J & Aksoy S The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl. Environ. Microbiol* 74, 5965–5974 (2008). [PubMed: 18689507]
77. Nadal-Jimenez P et al. Genetic manipulation allows in vivo tracking of the life cycle of the son-killer symbiont, *Arsenophonus nasoniae*, and reveals patterns of host invasion, tropism and pathology. *Environ. Microbiol* 21, 3172–3182 (2019). [PubMed: 31237728] This study uses microscopy to in vivo track a case of symbiont-mediated vertical transmission whereby the symbiont enters offspring via larval feeding and progresses to the ovipositor in female pupae to aid transmission to the next generation.
78. Gottlieb Y et al. The transmission efficiency of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. *J. Virol* 84, 9310–9317 (2010). [PubMed: 20631135]
79. van den Heuvel JF, Verbeek M & van der Wilk F Endosymbiotic bacteria associated with circulative transmission of potato leafroll virus by *Myzus persicae*. *J. Gen. Virol* 75, 2559–2565 (1994). [PubMed: 7931143]
80. Touret F, Guiguen F & Terzian C *Wolbachia* influences the maternal transmission of the gypsy endogenous retrovirus in *Drosophila melanogaster*. *mBio* 5, e01529–01514 (2014). [PubMed: 25182324]
81. Dutra HL et al. *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. *Cell Host Microbe* 19, 771–774 (2016). [PubMed: 27156023]
82. Hegde S, Rasgon JL & Hughes GL The microbiome modulates arbovirus transmission in mosquitoes. *Curr. Opin. Virol* 15, 97–102 (2015). [PubMed: 26363996]
83. Watanabe K, Yukuhiro F, Matsuura Y, Fukatsu T & Noda H Intrasperm vertical symbiont transmission. *PNAS* 111, 7433–7437 (2014). [PubMed: 24799707] This study details the vertical transmission of a *Rickettsia* bacterial insect symbiont via inclusion within the sperm heads of its leafhopper host.
84. Damiani C et al. Paternal transmission of symbiotic bacteria in malaria vectors. *Curr. Biol* 18, R1087–R1088 (2008). [PubMed: 19081038]
85. De Vooght L, Caljon G, Van Hees J & Van Den Abbeele J Paternal transmission of a secondary symbiont during mating in the viviparous tsetse fly. *Mol. Biol. Evol* 32, 1977–1980 (2015). [PubMed: 25851957]
86. Longdon B & Jiggins FM Vertically transmitted viral endosymbionts of insects: do sigma viruses walk alone? *Proc. R. Soc. B* 279, 3889–3898 (2012).
87. Longdon B & Jiggins FM Paternally transmitted parasites. *Curr. Biol* 20, R695–R696 (2010). [PubMed: 20833306]
88. Engelstädter J & Hurst GD What use are male hosts? The dynamics of maternally inherited bacteria showing sexual transmission or male killing. *Am. Nat* 173, E159–E170 (2009). [PubMed: 19272014]
89. Ironside JE, Smith JE, Hatcher MJ & Dunn AM Should sex-ratio distorting parasites abandon horizontal transmission? *BMC Evol. Biol* 11, 370 (2011). [PubMed: 22188680]

90. Otti O Genitalia-associated microbes in insects. *Insect Sci.* 22, 325–339 (2015). [PubMed: 25388748]
91. Gauthier L et al. Viruses associated with ovarian degeneration in *Apis mellifera* L. queens. *PLoS One* 6, e16217 (2011). [PubMed: 21283547]
92. Burand JP The sexually transmitted insect virus, Hz-2V. *Virol. Sin* 24, 428 (2009).
93. Himler AG et al. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science* 332, 254–256 (2011). [PubMed: 21474763]
94. Weeks AR, Turelli M, Harcombe WR, Reynolds KT & Hoffmann AA From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS Biol.* 5, e114 (2007). [PubMed: 17439303]
95. Patot S, Lepetit D, Charif D, Varaldi J & Fleury F Molecular detection, penetrance, and transmission of an inherited virus responsible for behavioral manipulation of an insect parasitoid. *Appl. Environ. Microbiol* 75, 703–710 (2009). [PubMed: 19060167]
96. Pannebakker BA, Loppin B, Elemans CP, Humblot L & Vavre F Parasitic inhibition of cell death facilitates symbiosis. *PNAS* 104, 213–215 (2007). [PubMed: 17190825]
97. Zchori-Fein E, Borad C & Harari AR Oogenesis in the date stone beetle, *Coccotrypes dactyliperda*, depends on symbiotic bacteria. *Physiol. Entomol* 31, 164–169 (2006).
98. Snook RR, Cleland SY, Wolfner MF & Karr TL Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*: analyses of fecundity, fertility and accessory gland proteins. *Genetics* 155, 167–178 (2000). [PubMed: 10790392]
99. Ebbert MA, Marlowe JL & Burkholder JJ Protozoan and intracellular fungal gut endosymbionts in *Drosophila*: prevalence and fitness effects of single and dual infections. *J. Invert Pathol* 83, 37–45 (2003).
100. Gibson CM & Hunter MS Negative fitness consequences and transmission dynamics of a heritable fungal symbiont of a parasitic wasp. *Appl. Environ. Microbiol* 75, 3115–3119 (2009). [PubMed: 19286783]
101. Yixin HY, Woolfit M, Rancès E, O’Neill SL & McGraw EA *Wolbachia*-associated bacterial protection in the mosquito *Aedes aegypti*. *PLoS Negl. Trop. Dis* 7, e2362 (2013). [PubMed: 23951381]
102. Braquart-Varnier C et al. The mutualistic side of *Wolbachia*–isopod interactions: *Wolbachia* mediated protection against pathogenic intracellular bacteria. *Front. Microbiol* 6, 1388 (2015). [PubMed: 26733946]
103. Nikoh N et al. Genomic insight into symbiosis-induced insect color change by a facultative bacterial endosymbiont, “*Candidatus Rickettsiella viridis*”. *mBio* 9 (2018).
104. Funkhouser-Jones LJ, van Opstal EJ, Sharma A & Bordenstein SR The maternal effect gene *Wds* controls *Wolbachia* titer in *Nasonia*. *Curr. Biol* 28, 1692–1702.e1696 (2018). [PubMed: 29779872]
105. Zhong W et al. Immune anticipation of mating in *Drosophila*: Turandot M promotes immunity against sexually transmitted fungal infections. *Proc. Biol. Sci* 280, 20132018 (2013). [PubMed: 24174107]
106. Moriyama M et al. Comparative transcriptomics of the bacteriome and the spermatheca of the bedbug *Cimex lectularius* (Hemiptera: Cimicidae). *Appl. Entomol. Zool* 47, 233–243 (2012).
107. Stutt AD & Siva-Jothy MT Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. *PNAS* 98, 5683–5687 (2001). [PubMed: 11331783]
108. Reinhardt K, Naylor R & Siva-Jothy MT Reducing a cost of traumatic insemination: female bedbugs evolve a unique organ. *Proc. R. Soc. B* 270, 2371–2375 (2003).
109. BÜCHNER P Studien an intracellularen Symbionten. IV-Die Bakteriensymbiose der Bettwanze. *Arch. Protistenk* 46, 225–263 (1923).
110. Buchner P Endosymbiosis of animals with plant microorganisms. (John Wiley & Sons, Inc., 1965).
111. Marshall JL Rapid evolution of spermathecal duct length in the *Allonemobius socius* complex of crickets: species, population and *Wolbachia* effects. *PLoS One* 2, e720 (2007). [PubMed: 17684565]

112. Duron O et al. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol.* 6, 27 (2008). [PubMed: 18577218]
113. Dunn AM & Smith JE Microsporidian life cycles and diversity: the relationship between virulence and transmission. *Microbes Infect.* 3, 381–388 (2001). [PubMed: 11369275]
114. Wang F et al. A novel negative-stranded RNA virus mediates sex ratio in its parasitoid host. *PLoS Pathog.* 13, e1006201 (2017). [PubMed: 28278298]
115. Juchault P, Louis C, Martin G & Noulin G Masculinization of female isopods (Crustacea) correlated with non-Mendelian inheritance of cytoplasmic viruses. *PNAS* 88, 10460–10464 (1991). [PubMed: 11607243]
116. Nakanishi K, Hoshino M, Nakai M & Kunimi Y Novel RNA sequences associated with late male killing in *Homona magnanima*. *Proc. R. Soc. B* 275, 1249–1254 (2008).
117. Dedeine F, Bouletreau M & Vavre F *Wolbachia* requirement for oogenesis: occurrence within the genus *Asobara* (Hymenoptera, Braconidae) and evidence for intraspecific variation in *A. tabida*. *Heredity* 95, 394 (2005). [PubMed: 16118660]
118. Leclercq S et al. Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial symbiont genome. *PNAS* 113, 15036–15041 (2016). [PubMed: 27930295] This paper demonstrates that pillbugs have a 3 Mb genome insert from the endosymbiont *Wolbachia*, and the insert appears to function as a novel female-determining sex chromosomal region.
119. Stouthamer R, Russell JE, Vavre F & Nunney L Intragenomic conflict in populations infected by parthenogenesis inducing *Wolbachia* ends with irreversible loss of sexual reproduction. *BMC Evol. Biol* 10, 229 (2010). [PubMed: 20667099]
120. Becking T et al. Sex chromosomes control vertical transmission of feminizing *Wolbachia* symbionts in an isopod. *PLoS Biol.* 17, e3000438 (2019). [PubMed: 31600190]
121. Kageyama D, Narita S & Watanabe M Insect sex determination manipulated by their endosymbionts: incidences, mechanisms and implications. *Insects* 3, 161–199 (2012). [PubMed: 26467955]
122. Morimoto S, Nakai M, Ono A & Kunimi Y Late male-killing phenomenon found in a Japanese population of the oriental tea tortrix, *Homona magnanima* (Lepidoptera: Tortricidae). *Heredity* 87, 435 (2001). [PubMed: 11737291]
123. Duron O et al. Origin, acquisition and diversification of heritable bacterial endosymbionts in louse flies and bat flies. *Mol. Ecol* 23, 2105–2117 (2014). [PubMed: 24612422]
124. Hosokawa T, Kikuchi Y, Shimada M & Fukatsu T Obligate symbiont involved in pest status of host insect. *Proc. R. Soc. B* 274, 1979–1984 (2007).
125. Oliver KM, Degnan PH, Burke GR & Moran NA Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annu. Rev. Entomol* 55, 247–266 (2010). [PubMed: 19728837]
126. Morse S, Dick CW, Patterson BD & Dittmar K Some like it hot: evolution and ecology of novel endosymbionts in bat flies of cave-roosting bats (Hippoboscoidea, Nycterophiliinae). *Appl. Environ. Microbiol* 78, 02455–02412 (2012).
127. Brumin M, Kontsedalov S & Ghanim M *Rickettsia* influences thermotolerance in the whitefly *Bemisia tabaci* B biotype. *Insect Sci.* 18, 57–66 (2011).
128. Truitt AM, Kapun M, Kaur R & Miller WJ *Wolbachia* modifies thermal preference in *Drosophila melanogaster*. *Environ. Microbiol* 21, 3259–3268 (2018).
129. Corbin C, Heyworth ER, Ferrari J & Hurst GD Heritable symbionts in a world of varying temperature. *Heredity* 118, 10 (2017). [PubMed: 27703153]
130. Baião GC, Schneider DI, Miller WJ & Klasson L The effect of *Wolbachia* on gene expression in *Drosophila paulistorum* and its implications for symbiont-induced host speciation. *BMC Genomics* 20, 465 (2019). [PubMed: 31174466]
131. Zheng Y et al. Differentially expressed profiles in the larval testes of *Wolbachia* infected and uninfected *Drosophila*. *BMC Genomics* 12, 595 (2011). [PubMed: 22145623]
132. Kupper M, Stigloher C, Feldhaar H & Gross R Distribution of the obligate endosymbiont *Blochmannia floridanus* and expression analysis of putative immune genes in ovaries of the carpenter ant *Camponotus floridanus*. *Arthropod. Struct. Dev* 45, 475–487 (2016).

133. Negri I et al. Unravelling the *Wolbachia* evolutionary role: the reprogramming of the host genomic imprinting. *Proc. R. Soc. B* 276, 2485–2491 (2009).
134. Wang J & Aksoy S PGRP-LB is a maternally transmitted immune milk protein that influences symbiosis and parasitism in tsetse's offspring. *PNAS* 109, 10552–10557 (2012). [PubMed: 22689989]
135. Papafotiou G, Oehler S, Savakis C & Bourtzis K Regulation of *Wolbachia* ankyrin domain encoding genes in *Drosophila* gonads. *Res. Microbiol* 162, 764–772 (2011). [PubMed: 21726632]
136. Yixin HY et al. Infection with a virulent strain of *Wolbachia* disrupts genome wide-patterns of cytosine methylation in the mosquito *Aedes aegypti*. *PLoS One* 8, e66482 (2013). [PubMed: 23840485]
137. Bhattacharya T, Newton IL & Hardy RW *Wolbachia* elevates host methyltransferase expression to block an RNA virus early during infection. *PLoS Pathog.* 13, e1006427 (2017). [PubMed: 28617844]
138. Shigenobu S, Watanabe H, Hattori M, Sakaki Y & Ishikawa H Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. *APS. Nature* 407, 81 (2000). [PubMed: 10993077]
139. Husnik F & McCutcheon JP Functional horizontal gene transfer from bacteria to eukaryotes. *Nat. Rev. Microbiol* 16, 67 (2018). [PubMed: 29176581]
140. Moran NA & Jarvik T Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science* 328, 624–627 (2010). [PubMed: 20431015]
141. Verster KI et al. Horizontal transfer of bacterial cytolethal distending toxin B genes to insects. *Mol. Biol. Evol* (2019).
142. Metcalf JA, Funkhouser-Jones LJ, Brileya K, Reysenbach AL & Bordenstein SR Antibacterial gene transfer across the tree of life. *Elife* 3, e04266 (2014).
143. Funkhouser-Jones LJ et al. *Wolbachia* co-infection in a hybrid zone: discovery of horizontal gene transfers from two *Wolbachia* supergroups into an animal genome. *PeerJ* 3, e1479 (2015). [PubMed: 26664808]
144. Hotopp JCD et al. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* 317, 1753–1756 (2007). [PubMed: 17761848]
145. Wybouw N et al. A gene horizontally transferred from bacteria protects arthropods from host plant cyanide poisoning. *ELife* 3, e02365 (2014). [PubMed: 24843024]
146. Blondel L, Jones TE & Extavour CG Bacterial contribution to genesis of the novel germ line determinant *oskar*. *bioRxiv*, 453514 (2018).
147. Selman M et al. Acquisition of an animal gene by microsporidian intracellular parasites. *Curr. Biol* 21, R576–R577 (2011). [PubMed: 21820617]
148. Hughes AL & Friedman R Genome-wide survey for genes horizontally transferred from cellular organisms to baculoviruses. *Mol. Biol. Evol* 20, 979–987 (2003). [PubMed: 12716988]
149. LePage DP & Metcalf JA et al. Prophage WO genes recapitulate and enhance *Wolbachia*-induced cytoplasmic incompatibility. *Nature* 543, 243–247 (2017). [PubMed: 28241146]
150. Beckmann JF, Ronau JA & Hochstrasser M A *Wolbachia* deubiquitylating enzyme induces cytoplasmic incompatibility. *Nat. Microbiol* 2, 17007 (2017). [PubMed: 28248294]
151. Perlmutter JI et al. The phage gene *wmk* is a candidate for male killing by a bacterial endosymbiont. *PLOS Pathog.* 15, e1007936 (2019). [PubMed: 31504075]
152. Shropshire JD, On J, Layton EM, Zhou H & Bordenstein SR One prophage WO gene rescues cytoplasmic incompatibility in *Drosophila melanogaster*. *PNAS* 115, 4987–4991 (2018). [PubMed: 29686091]
153. Hurst GD & Jiggins FM Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proc. R. Soc. B* 272, 1525–1534 (2005).
154. Jiggins FM & Tinsley MC An ancient mitochondrial polymorphism in *Adalia bipunctata* linked to a sex-ratio-distorting bacterium. *Genetics* 171, 1115–1124 (2005). [PubMed: 16079227]

155. Ironside JE, Dunn A, Rollinson D & Smith J Association with host mitochondrial haplotypes suggests that feminizing microsporidia lack horizontal transmission. *J. Evol. Biol* 16, 1077–1083 (2003). [PubMed: 14640399]
156. Charlat S et al. The joint evolutionary histories of *Wolbachia* and mitochondria in *Hypolimnas bolina*. *BMC Evol. Biol* 9, 64 (2009). [PubMed: 19317891]
157. Cariou M, Duret L & Charlat S The global impact of *Wolbachia* on mitochondrial diversity and evolution. *J. Evol. Biol* 30, 2204–2210 (2017). [PubMed: 28977708]
158. Jiggins FM Male-killing *Wolbachia* and mitochondrial DNA: selective sweeps, hybrid introgression and parasite population dynamics. *Genetics* 164, 5–12 (2003). [PubMed: 12750316]
159. Kapantaidaki DE et al. Low levels of mitochondrial DNA and symbiont diversity in the worldwide agricultural pest, the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *J. Heredity* 106, 80–92 (2014).
160. Beninati T et al. A novel alpha-Proteobacterium resides in the mitochondria of ovarian cells of the tick *Ixodes ricinus*. *Appl. Environ. Microbiol* 70, 2596–2602 (2004). [PubMed: 15128508]
161. Van Ham RC et al. Reductive genome evolution in *Buchnera aphidicola*. *PNAS* 100, 581–586 (2003). [PubMed: 12522265]
162. Funk DJ, Wernegreen JJ & Moran NA Intraspecific variation in symbiont genomes: bottlenecks and the aphid-*Buchnera* association. *Genetics* 157, 477–489 (2001). [PubMed: 11156972]
163. Abbot P & Moran NA Extremely low levels of genetic polymorphism in endosymbionts (*Buchnera*) of aphids (*Pemphigus*). *Mol. Ecol* 11, 2649–2660 (2002). [PubMed: 12453247]
164. Moran NA Accelerated evolution and Muller’s ratchet in endosymbiotic bacteria. *PNAS* 93, 2873–2878 (1996). [PubMed: 8610134]
165. Charlat S et al. Male-killing bacteria trigger a cycle of increasing male fatigue and female promiscuity. *Curr. Biol* 17, 273–277 (2007). [PubMed: 17276921]
166. Jaenike J, Dyer KA, Cornish C & Minhas MS Asymmetrical reinforcement and *Wolbachia* infection in *Drosophila*. *PLoS Biol.* 4, e325 (2006). [PubMed: 17032063]
167. Jiggins FM, Hurst GD & Majerus ME Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. *Proc. R. Soc. B* 267, 69–73 (2000).
168. Burand JP, Tan W, Kim W, Nojima S & Roelofs W Infection with the insect virus Hz-2v alters mating behavior and pheromone production in female *Helicoverpa zea* moths. *J. Insect Sci* 5, 6 (2005). [PubMed: 16299596]
169. Brucker RM & Bordenstein SR Speciation by symbiosis. *Trends Ecol. Evol* 27, 443–451 (2012). [PubMed: 22541872]
170. Miller WJ, Ehrman L & Schneider D Infectious speciation revisited: impact of symbiont-depletion on female fitness and mating behavior of *Drosophila paulistorum*. *PLoS Pathog.* 6, e1001214 (2010). [PubMed: 21151959]
171. Krueger CM, Degrugillier ME & Narang SK Size difference among 16S rRNA genes from endosymbiotic bacteria found in testes of *Heliothis virescens*, *H. subflexa* (Lepidoptera: Noctuidae), and backcross sterile male moths. *Fla. Entomol.* 382–390 (1993).
172. Bordenstein SR, O’Hara FP & Werren JH *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature* 409, 707–710 (2001). [PubMed: 11217858]
173. Gebiola M, Kelly SE, Hammerstein P, Giorgini M & Hunter MS “Darwin’s corollary” and cytoplasmic incompatibility induced by *Cardinium* may contribute to speciation in *Encarsia* wasps (Hymenoptera: Aphelinidae). *Evolution* 70, 2447–2458 (2016). [PubMed: 27530694]
174. O’Neill SL et al. Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses. *Gates Open Res* 2 (2018).
175. Fierer N Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol* 15, 579 (2017). [PubMed: 28824177]
176. Byrd AL, Belkaid Y & Segre JA The human skin microbiome. *Nat. Rev. Microbiol* 16, 143 (2018). [PubMed: 29332945]
177. van Oppen MJ & Blackall LL Coral microbiome dynamics, functions and design in a changing world. *Nat. Rev. Microbiol* 17, 557–567 (2019). [PubMed: 31263246]

178. Gibson J et al. Simultaneous assessment of the macrobiome and microbiome in a bulk sample of tropical arthropods through DNA metasytematics. *PNAS* 111, 8007–8012 (2014). [PubMed: 24808136]
179. Degli Esposti M & Romero EM The functional microbiome of arthropods. *PLoS One* 12, e0176573 (2017). [PubMed: 28475624]
180. Weinert LA, Araujo-Jnr EV, Ahmed MZ & Welch JJ The incidence of bacterial endosymbionts in terrestrial arthropods. *Proc. R. Soc. B* 282, 20150249 (2015).
181. Mann RS, Pelz-Stelinski K, Hermann SL, Tiwari S & Stelinski LL Sexual transmission of a plant pathogenic bacterium, *Candidatus Liberibacter asiaticus*, between conspecific insect vectors during mating. *PLoS One* 6, e29197 (2011). [PubMed: 22216209]
182. Bolling BG, Olea-Popelka FJ, Eisen L, Moore CG & Blair CD Transmission dynamics of an insect-specific flavivirus in a naturally infected *Culex pipiens* laboratory colony and effects of co-infection on vector competence for West Nile virus. *Virology* 427, 90–97 (2012). [PubMed: 22425062]
183. Moran NA & Dunbar HE Sexual acquisition of beneficial symbionts in aphids. *PNAS* 103, 12803–12806 (2006). [PubMed: 16908834]
184. Sookar P, Bhagwant S & Allymamod M Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of mycotic infection. *J. Insect Sci* 14, 100 (2014). [PubMed: 25201230]
185. Adamo SA, Kovalko I, Easy RH & Stoltz D A viral aphrodisiac in the cricket *Gryllus texensis*. *J. Exp. Biol* 217, 1970–1976 (2014). [PubMed: 24625650]
186. García-Munguía AM, Garza-Hernández JA, Rebollar-Tellez EA, Rodríguez-Pérez MA & Reyes-Villanueva F Transmission of *Beauveria bassiana* from male to female *Aedes aegypti* mosquitoes. *Parasites & Vectors* 4, 24 (2011). [PubMed: 21352560]
187. Lung O, Kuo L & Wolfner MF *Drosophila* males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. *J. Insect Physiol* 47, 617–622 (2001). [PubMed: 11249950]
188. Esteves E et al. Antimicrobial activity in the tick *Rhipicephalus (Boophilus) microplus* eggs: cellular localization and temporal expression of microplusin during oogenesis and embryogenesis. *Dev. Comp. Immunol* 33, 913–919 (2009). [PubMed: 19454333]
189. Kaya M et al. New chitin, chitosan, and O-carboxymethyl chitosan sources from resting eggs of *Daphnia longispina* (Crustacea); with physicochemical characterization, and antimicrobial and antioxidant activities. *Biotechnol. Bioproc. E* 19, 58–69 (2014).
190. Peng Y, Grassl J, Millar AH & Baer B Seminal fluid of honeybees contains multiple mechanisms to combat infections of the sexually transmitted pathogen *Nosema apis*. *Proc. R. Soc. B* 283, 20151785 (2016).
191. Bradford LL & Ravel J The vaginal mycobiome: a contemporary perspective on fungi in women's health and diseases. *Virulence* 8, 342–351 (2017). [PubMed: 27657355]
192. Wylie KM et al. The vaginal eukaryotic DNA virome and preterm birth. *Am. J. Obstet. Gynecol* 219, 189. e181–189. e112 (2018). [PubMed: 29738749]
193. Ravel J et al. Vaginal microbiome of reproductive-age women. *PNAS* 108 Suppl 1, 4680–4687 (2011). [PubMed: 20534435]
194. Aagaard K et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One* 7, e36466 (2012). [PubMed: 22719832]
195. Zhang R et al. Qualitative and semiquantitative analysis of Lactobacillus species in the vaginas of healthy fertile and postmenopausal Chinese women. *J. Med. Microbiol* 61, 729–739 (2012). [PubMed: 22301614]
196. Shiraishi T et al. Influence of menstruation on the microbiota of healthy women's labia minora as analyzed using a 16S rRNA gene-based clone library method. *Jpn. J. Infect. Dis* 64, 76–80 (2011). [PubMed: 21266747]
197. Noyes N, Cho K-C, Ravel J, Forney LJ & Abdo Z Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. *PLoS One* 13, e0191625 (2018). [PubMed: 29364944]

198. Hunt KM et al. Characterization of the diversity and temporal stability of bacterial communities in human milk. *PLoS One* 6, e21313 (2011). [PubMed: 21695057]
199. Hochreiter WW, Duncan JL & Schaeffer AJ Evaluation of the bacterial flora of the prostate using a 16S rRNA gene based polymerase chain reaction. *J. Urol* 163, 127–130 (2000). [PubMed: 10604329]
200. Porter CM, Shrestha E, Peiffer LB & Sfanos KS The microbiome in prostate inflammation and prostate cancer. *Prostate Cancer Prostatic Dis.* 21, 345–354 (2018). [PubMed: 29795140]
201. Liu CM et al. Male circumcision significantly reduces prevalence and load of genital anaerobic bacteria. *mBio* 4, e00076–00013 (2013). [PubMed: 23592260]
202. Nelson DE et al. Bacterial communities of the coronal sulcus and distal urethra of adolescent males. *PloS One* 7, e36298 (2012). [PubMed: 22606251]
203. Mändar R et al. Seminal microbiome in men with and without prostatitis. *Int. J. Urol* 24, 211–216 (2017). [PubMed: 28147438]
204. Pellati D et al. Genital tract infections and infertility. *Eur. J. Obstet. Gynecol. Reprod. Biol* 140, 3–11 (2008). [PubMed: 18456385]
205. Payne MS & Bayatibojakhi S Exploring preterm birth as a polymicrobial disease: an overview of the uterine microbiome. *Front. Immunol* 5, 595 (2014). [PubMed: 25505898]
206. Onderdonk AB, Delaney ML & Fichorova RN The human microbiome during bacterial vaginosis. *Clin. Microbiol. Rev* 29, 223–238 (2016). [PubMed: 26864580]
207. Weng SL et al. Bacterial communities in semen from men of infertile couples: metagenomic sequencing reveals relationships of seminal microbiota to semen quality. *PLoS One* 9, e110152 (2014). [PubMed: 25340531]
208. Sastry KS Seed-borne plant virus diseases. (Springer Science & Business Media, 2013).
209. Saikkonen K, Faeth SH, Helander M & Sullivan T Fungal endophytes: a continuum of interactions with host plants. *Annu. Rev. Ecol. Evol. S* 29, 319–343 (1998).
210. Gao F.-k., Dai C.-c. & Liu X.-z. Mechanisms of fungal endophytes in plant protection against pathogens. *Afr. J. Microbiol. Res* 4, 1346–1351 (2010).
211. Truyens S, Weyens N, Cuypers A & Vangronsveld J Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environ. Microbiol* 7, 40–50 (2014).
212. Cankar K, Kraigher H, Ravnkar M & Rupnik M Bacterial endophytes from seeds of Norway spruce (*Picea abies* L. Karst). *FEMS Microbiol. Lett* 244, 341–345 (2005). [PubMed: 15766788]
213. Compant S, Mitter B, Colli-Mull JG, Gangl H & Sessitsch A Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb. Ecol* 62, 188–197 (2011). [PubMed: 21625971]
214. Kaga H et al. Rice seeds as sources of endophytic bacteria. *Microbes Environ.* 24, 154–162 (2009). [PubMed: 21566368]
215. Lopez-Lopez A, Rogel MA, Ormeno-Orrillo E, Martinez-Romero J & Martinez-Romero E *Phaseolus vulgaris* seed-borne endophytic community with novel bacterial species such as *Rhizobium endophyticum* sp. nov. *Syst. Appl. Microbiol* 33, 322–327 (2010). [PubMed: 20822874]
216. Mundt JO & Hinkle NF Bacteria within ovules and seeds. *Appl. Environ. Microbiol* 32, 694–698 (1976). [PubMed: 984839]
217. Mano H et al. Culturable surface and endophytic bacterial flora of the maturing seeds of rice plants (*Oryza sativa*) cultivated in a paddy field. *Microbes Environ.* 21, 86–100 (2006).
218. Shade A, McManus PS & Handelsman J Unexpected diversity during community succession in the apple flower microbiome. *mBio* 4, e00602–00612 (2013). [PubMed: 23443006]
219. Obersteiner A et al. Pollen-associated microbiome correlates with pollution parameters and the allergenicity of pollen. *PLoS One* 11, e0149545 (2016). [PubMed: 26910418]
220. Ambika Manirajan B et al. Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity. *Environ. Microbiol* 18, 5161–5174 (2016). [PubMed: 27612299]
221. Manirajan BA et al. Diversity, specificity, co-occurrence and hub taxa of the bacterial–fungal pollen microbiome. *FEMS Microbiol. Ecol* (2018).

222. Sugio A et al. Diverse targets of phytoplasma effectors: from plant development to defense against insects. *Annu. Rev. Phytopathol* 49, 175–195 (2011). [PubMed: 21838574]
223. MacLean AM et al. Phytoplasma effector SAP54 hijacks plant reproduction by degrading MADS-box proteins and promotes insect colonization in a RAD23-dependent manner. *PLoS Biol.* 12, e1001835 (2014). [PubMed: 24714165] This report presents a case where a phytoplasma bacterial pathogen of *Arabidopsis* directly manipulates plant reproduction via production of the SAP54 transcription factor that degrades host proteins critical to reproductive tissue development. This converts plant reproductive structures into leaves that better attract insect hosts for oviposition and infection, thus spreading the infection.
224. Sugio A, Kingdom HN, MacLean AM, Grieve VM & Hogenhout SA Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. *PNAS* 108, E1254–1263 (2011). [PubMed: 22065743]
225. Puente ME, Li CY & Bashan Y Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. *Environ. Exp. Bot* 66, 402–408 (2009).
226. Puente ME, Li CY & Bashan Y Rock-degrading endophytic bacteria in cacti. *Environ. Exp. Bot* 66, 389–401 (2009).
227. Sánchez-López AS et al. Community structure and diversity of endophytic bacteria in seeds of three consecutive generations of *Crotalaria pumila* growing on metal mine residues. *Plant & Soil* 422, 51–66 (2018).
228. Carlier AL & Eberl L The eroded genome of a *Psychotria* leaf symbiont: hypotheses about lifestyle and interactions with its plant host. *Environ. Microbiol* 14, 2757–2769 (2012). [PubMed: 22548823]
229. Partida-Martinez LP & Hertweck C Pathogenic fungus harbours endosymbiotic bacteria for toxin production. *Nature* 437, 884 (2005). [PubMed: 16208371]

Box 1:**Sexually transmitted infections of arthropods**

Sexually transmitted infections (STIs) of arthropods are diverse and span many different bacteria, fungi and viruses and have been extensively reviewed elsewhere^{16,90}. Some exhibit mixed modes of inheritance (that is they are both sexually and vertically transmitted^{182,183}), so the two modes are not mutually exclusive. Despite the different form of transmission, vertically and sexually transmitted organisms share dependency on host reproductive activity and thus share many of the same biological principles related to host fitness and evolution. For example, STIs can affect host fitness by altering egg production rates¹⁸⁴ or sperm motility¹⁸⁵. They may also sterilize the host^{185,186}, thus sharing the consequence of reduced offspring that is characteristic of symbionts that manipulate host reproduction. Beyond direct fitness impacts, interactions between hosts and STIs have led to reproduction-specific immune responses and defenses that are often unique to the reproductive tissues¹⁸⁷ or are specifically modulated to prevent STIs during mating activity¹⁰⁵. These immune responses are a likely to be a result of an evolutionary arms race between STIs and their hosts. Indeed, antimicrobial peptides are commonly found on eggs^{188,189} as well as seminal fluid¹⁹⁰ to help protect females and offspring from infection.

Beyond a direct fitness effect, STIs share some important host evolutionary consequences with vertically inherited symbionts. Indeed, STIs may induce altered host mating behaviors to facilitate their spread, such as viruses that correlate with quicker mating rates in males¹⁸⁵. Importantly, they may also represent an environmental reservoir for the establishment of new host-microorganism symbioses. For example, one study demonstrated that aphids may acquire new beneficial symbionts initially through sexual transmission¹⁸³. When experimentally tested, bacteria carried by male aphids could be sexually transferred to their female partners and subsequently transmitted vertically via the matriline. Further, they could replace other symbionts already carried by the mothers¹⁸³. This suggests that some current day symbioses may have originally begun as STIs. Therefore, STIs have much of the same potential to affect host evolution as the well-studied vertically inherited symbionts. However, there are some differences, including that STIs tend to be more often pathogenic, may have a wider host range, rely on host males and females for dispersal more equally and are horizontally rather than vertically transmitted^{16,90}.

Box 2:**Microorganisms of human reproductive tissues**

Unlike arthropods, characterization of the microbiota of human reproductive tissues is common, but less is understood about their function or evolutionary consequences. In humans, bacteria are the best studied and are the most abundant in reproductive tissue samples. The vaginal microbiota also contains a smaller proportion of diverse fungi¹⁹¹ and viruses¹⁹², but non-bacterial microorganisms are not fully characterized in men and women and merit further study. In contrast, bacteria are well-studied, particularly within women. Bacterial 16S rRNA gene sequencing has revealed that, within groups of reproductive-age women that have been studied thus far, the vaginal microbiota is diverse and often dominated by *Lactobacillus* species, including *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus jensenii* and *Lactobacillus gasseri*¹⁹³. In a minority of women tested in these studies, the vaginal microbiota comprises diverse anaerobic bacteria such as *Streptococcus* with no single dominant species, whereas others are dominated by anaerobic bacteria such as the *Prevotella*, *Atopobium* and *Gardnerella* genera¹⁹³. Many factors contribute to inter-individual variation in the vaginal microbiota, including ethnicity¹⁹³, pregnancy¹⁹⁴, menopause¹⁹⁵, menstruation¹⁹⁶, hygiene¹⁹⁷, use of birth control¹⁹⁷ and age¹⁹⁷. In addition, women contain microorganisms in breastmilk that are important for offspring health¹⁹⁸.

Less is known about the reproductive tract microbiota in men compared with women. However, the upper male genital tract (prostate and vas deferens [G]) is considered to be germfree except during infection^{199,200} and the lower genital tract (urethra and coronal sulcus [G]) contains dozens of bacterial families, of which Clostridiales and Prevotellaceae are most abundant²⁰¹. Common genera include *Corynebacterium*, *Anaerococcus*, *Staphylococcus* and *Prevotella*²⁰¹, which indicates some overlap between genera found in the female reproductive tracts, whereas some are more common in men. 16S rRNA gene sequencing indicates that there is likely no 'core' penis microbiota²⁰², a result that parallels the extensive inter-individual variation discussed above for the vaginal microbiota. In addition, circumcision²⁰², prostatitis²⁰³ and prostate cancer²⁰⁰ associate with changes in the reproductive tract microbiota.

Microorganisms in the reproductive tract also have an impact on human health and fitness. For example female genital tract infections (GTIs) are associated with pelvic inflammatory disease, ovarian abscesses, tissue scarring, and infertility among other conditions²⁰⁴. Other microbial infections can also cause complications in pregnancy and birth such as stillbirth, sepsis or preterm birth²⁰⁵. In addition, disruption of vaginal microbiota homeostasis is a hallmark of bacterial vaginosis, whereby the community changes from *Lactobacillus* dominance to more diverse communities of mostly obligate anaerobes²⁰⁶. Therefore, *Lactobacilli* are likely to have important beneficial roles, but it is unclear what functions other microorganisms may have. Microorganisms in the male reproductive tract also affect male fitness. Indeed, the abundances of certain microbial genera such as *Prevotella* and *Pseudomonas* are associated with poor semen quality, including reduced motility, volume and concentration, whereas the abundances of *Gardnerella* and *Lactobacillus* species are associated with normal semen quality²⁰⁷.

Additionally, GTIs in men correlate with poor sperm quality and are associated with infertility²⁰⁴. Bacterial DNA sequences are detected in semen of healthy men²⁰³, so future studies could determine if any living, resident microorganisms confer benefits to male reproduction. In addition, long-term evolutionary consequences of these symbioses on the host are poorly understood compared to arthropod symbioses and remain open questions.

Box 3:**Microorganisms of plant reproductive tissues**

Plant reproductive tissues contain a diverse range of microorganisms. The bacterial microbiota of plant reproductive tissues includes various endophytes (microbial symbionts that inhabit plants without any apparent harm) and phytopathogens (microbial symbionts that parasitize plants). In addition, the plant microbiota comprises many known viral²⁰⁸ and fungal²⁰⁹ pathogens, as well as many protective fungal endophytes (reviewed in Ref.²¹⁰). Among bacteria, the best surveyed group of plant microorganisms, a comprehensive compilation of seed endophytes from 2014 spans 131 bacterial genera and four phyla that occur in 25 different plants²¹¹. The most common phyla were Proteobacteria, followed by Actinobacteria and Firmicutes. Cultivation or sequencing methods across multiple studies demonstrated that 17 of the plant species seeds contained multiple phyla, whereas the remaining eight only contained a single bacterial phylum²¹¹. A plant seed may also contain several species within the same phylum or genus²¹². Bacteria commonly sequenced in seeds include various species of *Pantoea*, *Methylobacterium*, *Bacillus*, *Stapylococcus*, *Pseudomonas* and *Paenibacillus*^{211,213–215}. Notably cultivation methods in many plant species result in only one or a small number of bacterial species²¹⁶. This may either reflect the inability to culture most microorganisms of seeds, or the presence of few bacterial cells. Known factors affecting the sequence diversity of seed microbiota include plant species²¹¹, location outside or inside the seed²¹⁷, and the stage of seed maturation²¹⁷. Other reproductive structures of plants have site-specific microbial communities as well, such as flowers²¹⁸, fruits²¹³ and pollen²¹⁹. For example, pollen of diverse host species has many common bacterial genera, including *Rosenbergiella*, *Pseudomonas*, *Methylobacterium*, *Friedmanniella* and *Bacillus*^{219–221}, which represent some shared and distinct symbionts compared to those commonly found in seeds.

Interestingly, plant-microorganism symbioses are known to share many of the same evolutionary principles demonstrated in arthropod-microorganism symbioses. For example, symbiotic modulation of host gene expression in reproductive tissues is known in plants. One extraordinary case is that of phytoplasma, which are obligate intracellular bacteria that are transmitted from plant to plant via insect vectors, such as leafhoppers and psyllids²²². Phytoplasma cause symptoms such as yellowing of leaves, greening of flowers or even sterility²²². In *Arabidopsis thaliana*, phytoplasma produce an effector protein, SAP54, which changes the flowers into leaf-like vegetative structures²²³. Both SAP54 and phytoplasma effector protein SAP11 manipulate plant host transcription factors that regulate normal flower development²²⁴. Leafhoppers then preferentially choose infected plants for oviposition due to the physical changes in the plant²²³. This enables the bacteria to spread by attracting its insect vector. In addition, bacterial seed endophytes in plants can also determine the environmental niche of the host. For example, endophytic bacteria of the giant cardon cactus help seedlings develop on sites of barren rock²²⁵. When the endophytes are eliminated by antibiotic treatment, the seeds fail to develop, but when sterile seeds are re-inoculated with the endophytes, their growth is rescued²²⁵. The endophytes are able to accomplish this remarkable feat by performing

two tasks: fixing nitrogen for the host, and producing various organic acids that weather the rock and release the life-sustaining minerals required for growth in this harsh environment²²⁶. In addition, vertically inherited bacterial seed endophytes of the rattlebox shrub include microorganisms that contribute to growth and resilience, which probably promotes the ability of the host to survive in metal-contaminated mining sites²²⁷. Furthermore, some of these beneficial relationships may result in microbial genome erosion and development of obligate associations, as described for arthropods. One example is the case of *Burkholderia* endosymbionts of *Psychotria* plants that are vertically transmitted and are likely to have a role in protection from predation or pathogens²²⁸. Additionally, it is known that microorganisms of different taxa may cooperatively interact in host reproductive tissues. For example, members of the fungal genus *Rhizopus* that cause rice seedling blight contain their endosymbiotic bacteria that produce a phytotoxin that is critical in pathogenesis. When the toxin damages host tissues, both the fungal host as well as the bacterial symbionts of the fungi benefit from plant nutrients, in a striking case of inter-taxa dependence and function in parasitization of host reproductive tissues²²⁹. Thus, plant-microorganism symbioses in reproductive tissues of plants share many important evolutionary characteristics to that of arthropod-microorganism symbioses and merit further study.

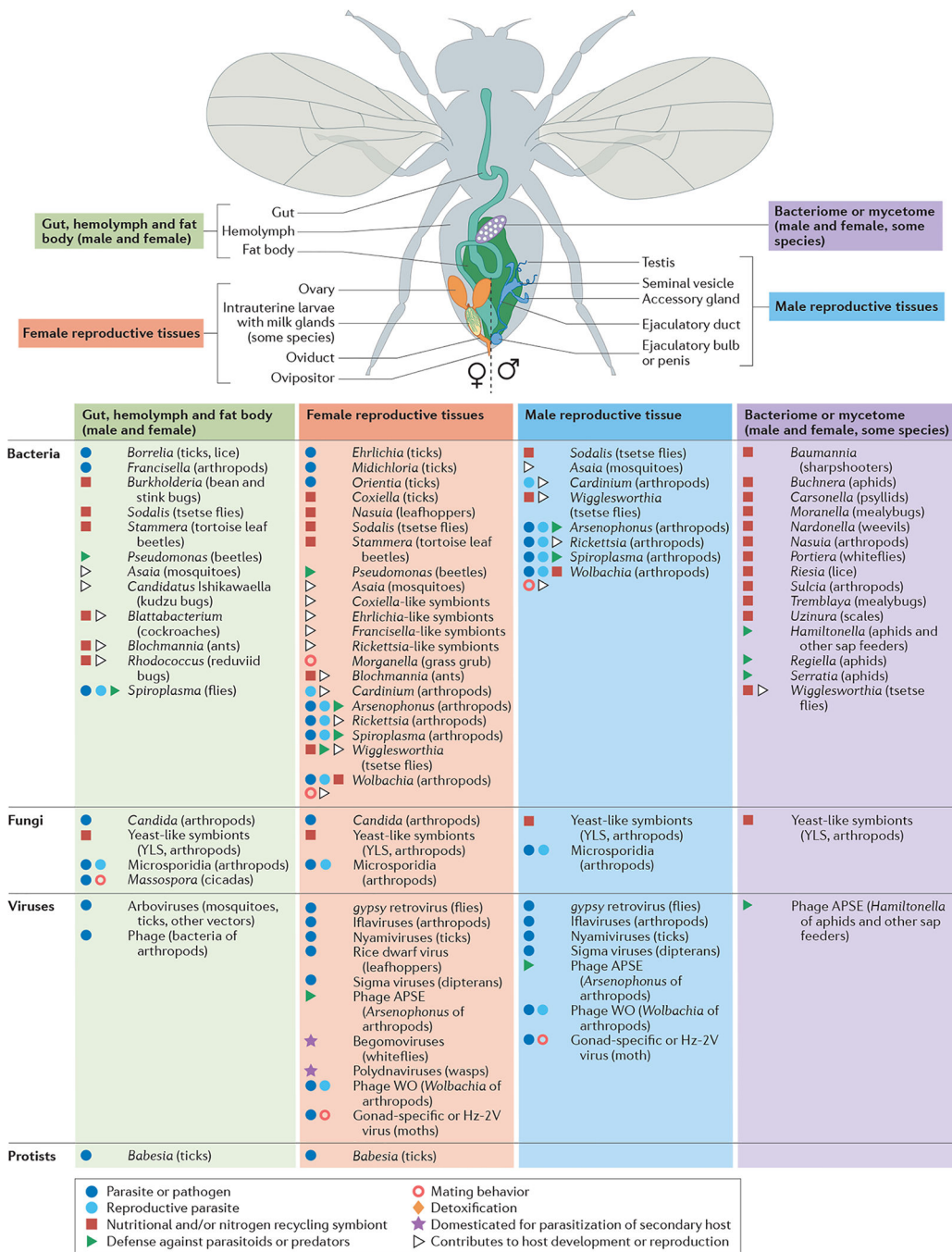


Figure 1. Example microorganisms associated with arthropod reproductive tissues.

The silhouette is a representative image that includes organs from both males and females, as well as various species of insects, and is therefore not anatomically accurate for any given arthropod species, and neither is it to scale to enable visualization of all organs. Select microorganisms and viruses are listed in their primary or additional densely populated body sites. The list is not comprehensive of all symbionts, all tissue localizations, or all functions, but represents many known symbioses. In addition, symbionts may not be present in the same tissues or exhibit the same phenotypes in every strain or host. The microorganisms that

are listed under the ‘gut, hemolymph and fat body’ as well as the ‘bacteriome or mycetome’ are present in both sexes of some species and are included because all contact reproductive tissues at some point (typically during transmission) even though they primarily or often reside in other tissues. The function is listed as “unknown phenotype or function” if the presence of the symbiont is known, but the effect on the arthropod host is not well established. In addition, a function of a symbiont may only apply in some circumstances (for example, different hosts or strains).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

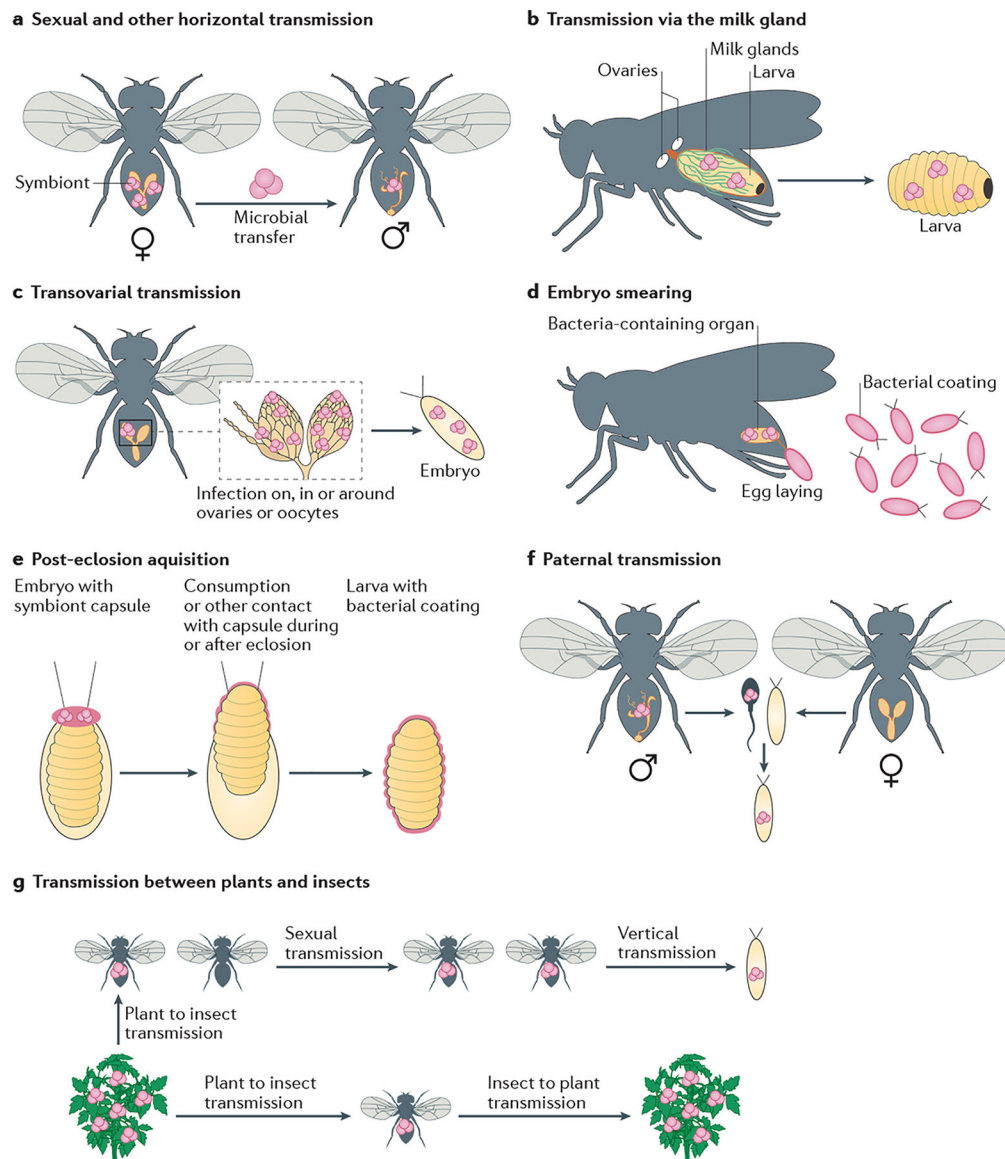


Figure 2. Transmission routes of microorganisms in the reproductive tract in arthropods. Depicted are representative methods for transmission of microorganisms in the reproductive tract between individuals. Pink circles represent symbionts, and pink outlines or coatings indicate outer coverage by symbionts. a) Horizontal transmission can spread microorganisms between reproductive tissues of different host individuals, usually through copulation. b) Certain hereditary microorganisms can be vertically transmitted from mother to offspring via the milk glands, as has been reported for the tsetse fly symbiont *Wigglesworthia glossinidia*. c) Hereditary endosymbionts, including many common reproductive parasites, can be vertically transmitted via infection in the ovarian tissues and passage internally to embryos. d) Bacteria in specialized organs can be smeared onto embryos as they are laid so that offspring are coated with the microorganisms when they eclose. e) Hereditary symbionts may be acquired post egg-laying through various mechanisms, including passage through symbiont capsules during eclosion. f) Microorganisms may be paternally transmitted via

various mechanisms, including packaging within sperm heads that enables infection of offspring. G) Certain microorganisms may also be cyclically transmitted through both insect and plant hosts. The insects often carry these microorganisms on their genitalia and can pass them sexually to other insects, horizontally to new plants or vertically to offspring.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

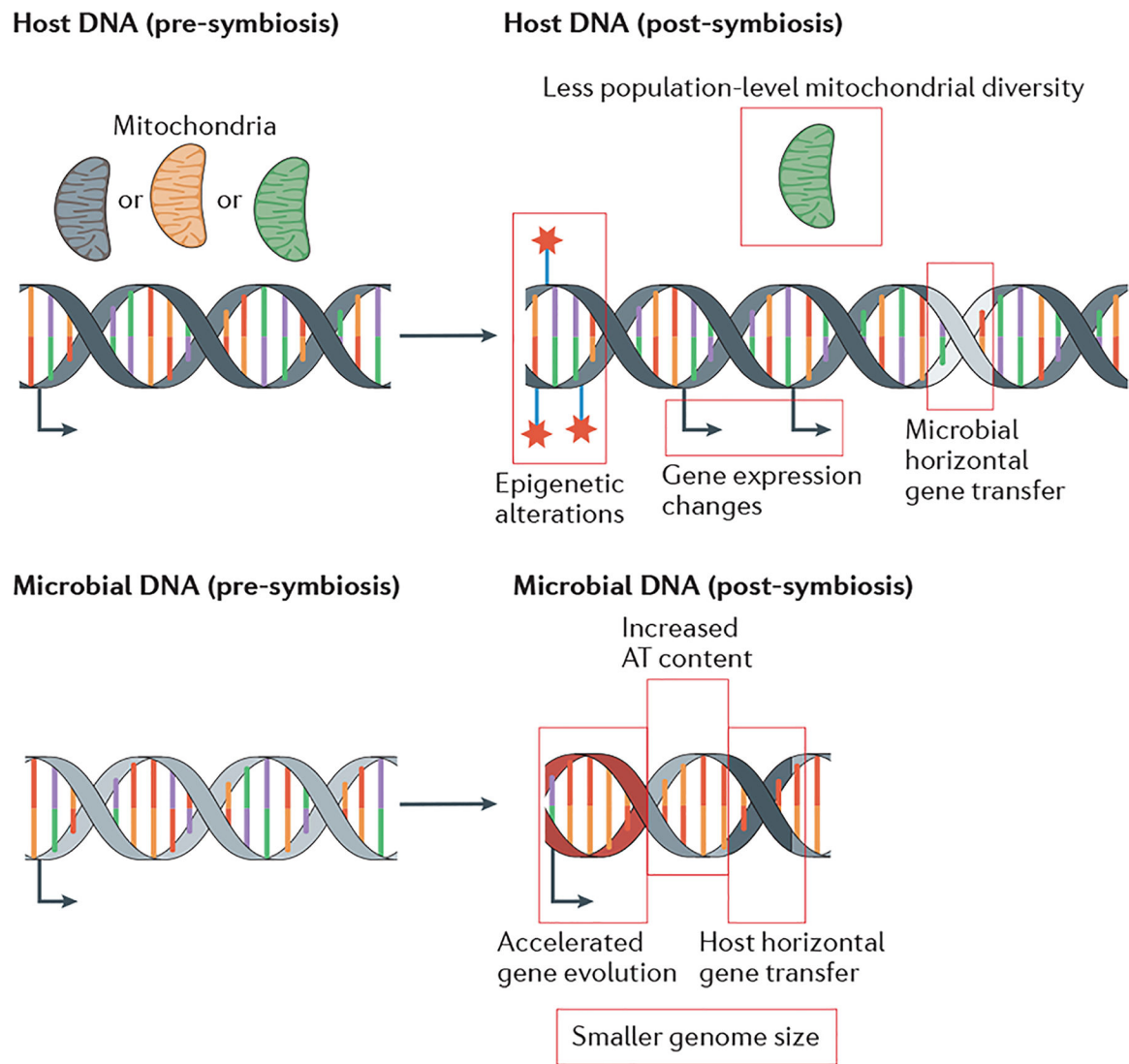


Figure 3. Effects on the genomes and transcriptomes of hosts and microorganisms.

Post-symbiosis sections demonstrate population-level changes many generations since pre-symbiosis. Each effect is not universal to all symbioses and instead represents changes known in some systems. The top panel shows changes that can occur in host DNA, where there can be epigenetic alterations, changes in gene expression in the presence of symbionts, fewer mitochondrial DNA haplotypes in the population, and horizontal gene transfer from microbe to host. The bottom panel shows changes that can occur in microbial DNA, where there can be accelerated gene evolution, increased AT content, horizontal gene transfer from host to microbe, and an overall reduced genome size.