Microorganisms in the reproductive tissues of arthropods

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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 the physiological and evolutionary outcomes for both hosts and microorganisms. We also identify areas in need of continuing research, to answer the fundamental questions that remain in fields within and beyond arthropod–microorganism associations. New opportunities for research in this area will drive a broader understanding of major concepts as well as the biodiversity, mechanisms and translational applications of microorganisms that interact with host reproductive tissues.

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 for his now famous talk discussing the relationships between the algae, cyanobacteria and fungi that together form lichens[1]. Today, the term generally describes any 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 symbiosis are common symbiotic relationships now recognized in many plants and animals[5]. 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 the 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 the host's reproductive strategies and physiology in ways that are often unique in the animal kingdom.

In this Review, we synthesize current knowledge on the 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 both hosts and microorganisms. We also assess both widespread and specialized biological principles across various organisms and highlight the fundamental unanswered questions in need of continued study. We then emphasize important future directions for 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 of 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, as well as a greater understanding of the impact of the microorganisms associated with the reproductive tract on host evolution.

Arthropod reproductive tissue microbiota

Microorganisms that inhabit arthropod reproductive tissues represent an exceedingly broad group, spanning many orders of bacteria[6–14], fungi[15–18], protists[16,19] and viruses[16,20–22] (FIG. 1). Microorganisms are present in the reproductive tissues of all of the major orders of arthropods[5], 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

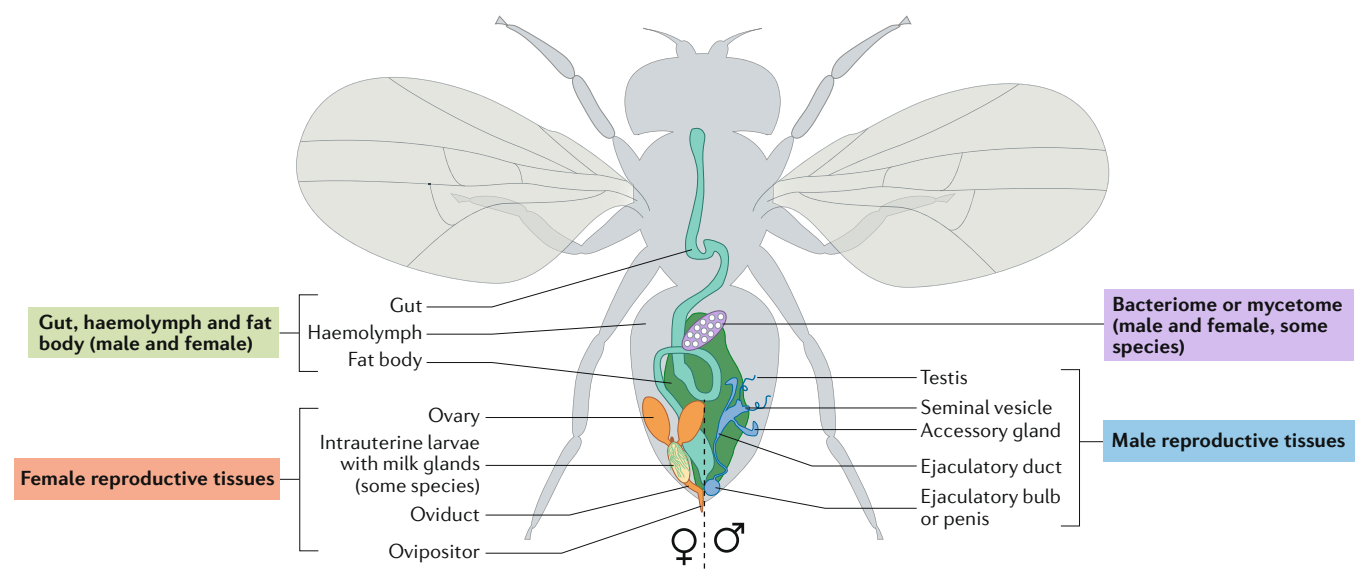[1]Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee, USA.

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Gut, haemolymph and fat body (male and female)

Gut
Haemolymph
Fat body

Bacteriome or mycetome (male and female, some species)

Testis
Seminal vesicle
Accessory gland
Ejaculatory duct
Ejaculatory bulb or penis

Male reproductive tissues

Ovary
Intrauterine larvae with milk glands (some species)
Oviduct
Ovipositor

Female reproductive tissues

♀ ♂

	Gut, haemolymph and fat body (male and female)	Female reproductive tissue	Male reproductive tissue	Bacteriome or mycetome (male and female, some species)
Bacteria	● *Borrelia* (ticks, lice) ● *Francisella* (arthropods) ■ *Burkholderia* (bean and stink bugs) ■ *Sodalis* (tsetse flies) ■ *Stammera* (tortoise leaf beetles) ▶ *Pseudomonas* (beetles) ▷ *Asaia* (mosquitoes) ▷ *Candidatus* Ishikawaella (kudzu bugs) ■ ▷ *Blattabacterium* (cockroaches) ■ ▷ *Blochmannia* (ants) ■ ▷ *Rhodococcus* (reduviid bugs) ● ● ▶ *Spiroplasma* (flies)	● *Ehrlichia* (ticks) ● *Midichloria* (ticks) ● *Orientia* (ticks) ■ *Coxiella* (ticks) ■ *Nasuia* (leafhoppers) ■ *Sodalis* (tsetse flies) ■ *Stammera* (tortoise leaf beetles) ▶ *Pseudomonas* (beetles) ▷ *Asaia* (mosquitoes) ▷ *Coxiella*-like symbionts ▷ *Ehrlichia*-like symbionts ▷ *Francisella*-like symbionts ▷ *Rickettsia*-like symbionts ○ *Morganella* (grass grubs) ■ ▷ *Blochmannia* (ants) ● ▷ *Cardinium* (arthropods) ● ● ▶ *Arsenophonus* (arthropods) ● ● ▷ *Rickettsia* (arthropods) ● ● ▶ *Spiroplasma* (arthropods) ■ ▶ ▷ *Wigglesworthia* (tsetse flies) ● ● ■ *Wolbachia* (arthropods) ○ ▷	■ *Sodalis* (tsetse flies) ▷ *Asaia* (mosquitoes) ● ▷ *Cardinium* (arthropods) ■ ▷ *Wigglesworthia* (tsetse flies) ● ● ▶ *Arsenophonus* (arthropods) ● ● ▷ *Rickettsia* (arthropods) ● ● ▶ *Spiroplasma* (arthropods) ● ■ *Wolbachia* (arthropods) ○ ▷	■ *Baumannia* (sharpshooters) ■ *Buchnera* (aphids) ■ *Carsonella* (psyllids) ■ *Moranella* (mealybugs) ■ *Nardonella* (weevils) ■ *Nasuia* (arthropods) ■ *Portiera* (whiteflies) ■ *Riesia* (lice) ■ *Sulcia* (arthropods) ■ *Tremblaya* (mealybugs) ■ *Uzinura* (scales) ▶ *Hamiltonella* (aphids and other sap feeders) ▶ *Regiella* (aphids) ▶ *Serratia* (aphids) ■ ▷ *Wigglesworthia* (tsetse flies)
Fungi	● *Candida* (arthropods) ■ Yeast-like symbionts (arthropods) ● ● Microsporidia (arthropods) ● ○ *Massospora* (cicadas)	● *Candida* (arthropods) ■ Yeast-like symbionts (arthropods) ● ● Microsporidia (arthropods)	■ Yeast-like symbionts (arthropods) ● ● Microsporidia (arthropods)	■ Yeast-like symbionts (arthropods)
Viruses	● Arboviruses (mosquitoes, ticks, other vectors) ● Phage (bacteria of arthropods)	● *gypsy* retrovirus (flies) ● Iflaviruses (arthropods) ● Nyamiviruses (ticks) ● Rice dwarf virus (leafhoppers) ● Sigma viruses (dipterans) ▶ Phage APSE (*Arsenophonus* of arthropods) ★ Begomoviruses (whiteflies) ★ Polydnaviruses (wasps) ● ● Phage WO (*Wolbachia* of arthropods) ● ○ Gonad-specific or Hz-2V virus (moths)	● *gypsy* retrovirus (flies) ● Iflaviruses (arthropods) ● Nyamiviruses (ticks) ● Sigma viruses (dipterans) ▶ Phage APSE (*Arsenophonus* of arthropods) ● ● Phage WO (*Wolbachia* of arthropods) ● ○ Gonad-specific or Hz-2V virus (moth)	▶ Phage APSE (*Hamiltonella* of aphids and other sap feeders)
Protists	● *Babesia* (ticks)	● *Babesia* (ticks)		

Legend

● Parasite or pathogen
● Reproductive parasite
■ Nutritional and/or nitrogen-recycling symbiont
▶ Defence against parasitoids or predators
○ Mating behaviour
★ Domesticated for parasitization of secondary host
▷ Contributes to host development or reproduction

◄ Fig. 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 (the general category or the genus level is listed, if known) 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 it represents many known symbioses. In addition, symbionts may not be present in the same tissues or exhibit the same phenotypes with every microorganism strain or host. The microorganisms listed under 'Gut, haemolymph and fat body' or 'Bacteriome or mycetome' are present in both sexes of some species and are included because all these microorganisms contact reproductive tissues at some point (typically during transmission), even though they primarily or often reside in somatic tissues.

host reproduction[6,20,23,28–30]; bacteria, viruses, fungi and protists that cause sexually transmitted diseases in their hosts[16]; 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 other functions. Owing to their successful survival strategies in the most speciose groups of animals, the 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 operational taxonomic units in the reproductive tissues, and that a core microbiota spanning seven genera is shared among individuals of the same sex and swarm[9]. Another study showed that bedbugs exhibit a diversity of bacteria in their reproductive organs, with 31 sequence variants found across samples, although individuals harbour an average of just three sequence variants[44]. 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 copulation[44]. 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 the sex, proximity or relatedness of individuals, that correlate with community structure. The data

also raise the potential that the reproductive tissues of some arthropods could harbour a diversity of microorganisms. In addition, some studies have focused 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 that have simultaneously assessed 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 such communities correlate with host species phylogeny, what factors or conditions shape microbial communities, what kinds of interactions exist across the microbial community or how individual microorganisms are acquired. In addition, surveys of non-bacterial taxa within arthropods are under-represented in the literature, including those of viruses, fungi, protists and archaea. In particular, and to the best of our knowledge, no studies have investigated archaeal symbionts of arthropod reproductive tissues. Although archaeal methanogens are found in the guts of termites and other insects[48], potential archaeal roles in reproductive tissues are largely unknown. Furthermore, research is far more common in insects than in arachnids, crustaceans and other arthropods, although a few studies have described unidentified bacteria in the reproductive tissues of animals such as shrimp[49] and crabs[50], and some have identified endosymbionts of arachnids such as spiders and mites[20,51–54]. Given the growing recognition of the role that archaea play in the health of organisms, including humans[55], 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 of isolated host–microorganism relationships.

Transmission routes. The 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 routes driven by microorganism–microorganism interactions and microorganism–host interactions, and these categories are not mutually exclusive (FIG. 2). As hereditary microorganisms are arguably the ones that have been 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 for certain fungi, such as yeast-like symbionts (YLS)[64] and microsporidia[65], and for some viruses[66] and protists[19]. There are various mechanisms to ensure this form of passage, which are often microorganism-driven. For example, *Wolbachia pipientis*, hereafter referred to simply as *Wolbachia*, infects germline stem cells[67]
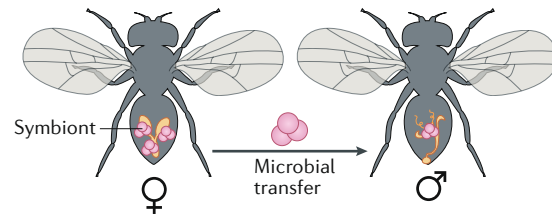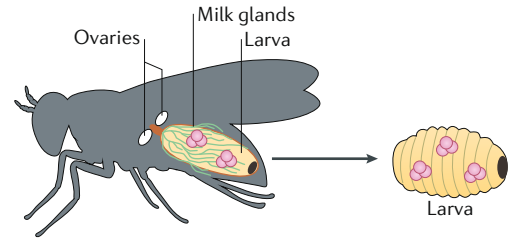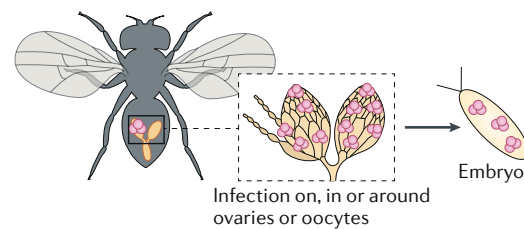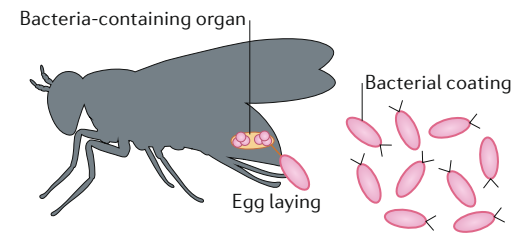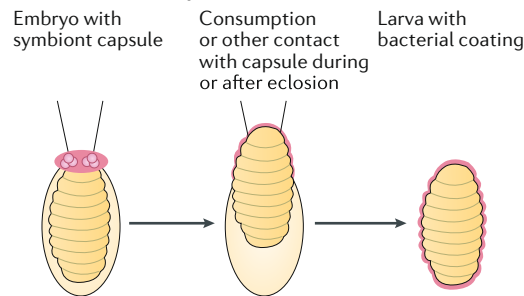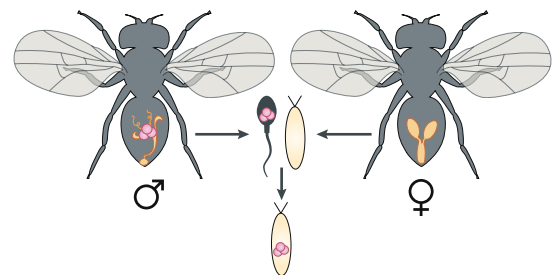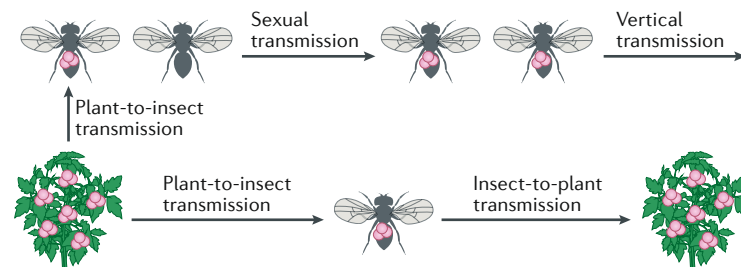

Fig. 2 | **Transmission routes of microorganisms in the reproductive tract in arthropods.** Depicted are representative methods for the transmission of microorganisms in the reproductive tract between individuals. Pink circles represent symbionts, and pink outlines or coatings indicate outer coverage by symbionts. **a** | Horizontal transmission can spread microorganisms between the 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 the 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.

to spread into oocytes using host actin during oogenesis[59]. They subsequently use egg microtubules to localize towards the posterior end of the embryo, where cells are fated to become germline tissue[68], and the cycle thus repeats in the next generation. *Spiroplasma poulsonii* instead hijack the yolk uptake machinery, to be endocytosed into the oocyte[61]. Alternatively, *Buchnera aphidicola* cells are carried by maternal bacteriocytes in the midgut and are exocytosed into the extracellular space for a short period, before selective endocytosis into the maternal blastulae at ovariole tips[69]. 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, *B. 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 of vertical transmission, 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 nature[70]. One common mechanism is smearing the symbiont on the egg as it exits the ovipositor. 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 laid[71,72]. This is similar to the transmission of microorganisms in vaginally delivered human babies[73]. However, many other modes of external transmission of symbionts 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 stinkbugs[74]; or the mother will secrete the substance containing the symbiont onto the surrounding area, as has been shown for necrophagous beetles[75]. In other cases, the mother might package the bacteria into capsules on egg cases that are eaten as larvae emerge[72], 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 generation[77]. 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 those of bacterial symbionts?

Many studies have focused on the influence of host–microorganism interactions on transmission, but interest is also emerging 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. The 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 generation[43], 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* transmission[43]. Moreover, certain populations of pea aphids are infected with many different hereditary endosymbiont species[40]. Monitoring co-infection frequencies over time has 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 time[40]. 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 explored experimentally. 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 extents in the host and offspring.

It is perhaps not surprising that interactions between microorganisms of different taxa affect each other's transmission, although it is a comparatively rare topic in the literature. In one intriguing case, the rice dwarf virus (RDV) is vectored between plants by leafhoppers via a unique type of vertical transmission[11]. The virus is associated with an insect obligate bacterial symbiont, *Sulcia*, and the virus hitchhikes on the envelope of the *Sulcia* symbiont via an interaction between a viral capsid protein and an outer membrane protein

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Bacteriocytes
Or mycetocytes. Specialized fat cells found in some insects that contain endosymbiotic organisms, especially bacteria (or 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 female arthropods use to lay eggs.

of *Sulcia*. After attachment, the virus uses *Sulcia* bacteria as a vehicle for transovarial transmission[11]. This case exquisitely highlights the largely underappreciated interactions between microorganisms 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, the transmission of tomato yellow leaf curl virus, vectored by whiteflies, depends on the chaperone protein GroEL from *Hamiltonella*[78], and the transmission of potato leafroll virus requires the aphid endosymbiont-specific protein symbionin for transmission[79]. Moreover, other studies have reported negative interactions of *Wolbachia* with the vertically inherited *gypsy* retrovirus[80], as well as with Zika virus and other viruses, in cases of hosts infected with non-native strains of *Wolbachia*[81,82]. Future investigations will be required in order to determine how common these interactions are and between which taxa. Do interactions occur among or between fungi, protists or any potential archaea and other microorganisms? Are there cases in which 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 with 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 sperm[83]. 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 this is rare for bacteria, many viral endosymbionts can also be transmitted both maternally and paternally, including the well-studied cases of sigma viruses in insects[86]. Sigma viruses are relatively common symbionts in insects, but they are unusual in that they are one of the few known types of insect viruses that are transmitted exclusively vertically, via the gonads; transmission is still much more efficient from females than from males, probably owing to the lower number of viral particles that can be packaged in sperm than in eggs[86]. Transmission by both parents enables symbionts to persist in additional contexts, compared with symbionts with only one mode of transmission. Despite this benefit, far fewer cases of paternal transmission of microorganisms are known in nature, even across different microbial taxa. This may reflect either actual rarity in nature or that paternal transmission is understudied in some contexts[87]. In addition, biparental transmission routes can result in important consequences that differ from those for organisms that are strictly or nearly always transmitted maternally. These consequences include whether or not male host fitness benefits the microorganism, as well as 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 has been 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 genitalia[90]. Moreover, how are microorganisms that are not vertically transmitted acquired, and do any factors select for certain microorganisms over others? Is 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? These questions will need to be addressed in future research.

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 STIs 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 forms of transmission, vertically and sexually transmitted organisms share a dependency on host reproductive activity, and thus they share many of the same biological principles related to host fitness and evolution. For example, STIs can affect host fitness by altering egg production rates[184] or sperm motility[185]. 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 defences that are often unique to the reproductive tissues[187] or are specifically modulated to prevent STIs during mating activity[105]. These immune responses are likely the results of an evolutionary arms race between STIs and their hosts. Indeed, antimicrobial peptides are commonly found on eggs[188,189] and seminal fluid[190], 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 behaviours in order to facilitate their spread, such as viruses that correlate with quicker mating rates in males[185]. Importantly, STIs 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 transmission[183]. When experimentally tested, the bacteria carried by male aphids could be sexually transferred to their female partners and subsequently transmitted vertically via the matriline. Furthermore, these bacteria could replace other symbionts already carried by the mothers[183]. This suggests that some current-day symbioses may have originally begun as STIs. Therefore, STIs have much the same potential to affect host evolution as the well-studied vertically inherited symbionts. However, there are some differences, including that STIs tend to more often be 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].

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 spermatogenesis[98]; have crucial roles in nutrient provisioning[39]; influence offspring development rates[93,99,100]; and affect predation or pathogen susceptibility[10,35,101–103]. These effects may also extend beyond a single generation and may affect the 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 predators[38]. Over time, hosts may even develop unique or specialized organs or proteins (many are referred to as bacteriomes or mycetomes) that function in housing symbionts[7], controlling their transmission[104] or preventing pathogen transmission during mating[105,106]. Another example is bedbugs, which have a unique and costly form of copulation[107] 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 insemination[108]. 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, such as with *Wolbachia*-infected crickets[111]. In some populations of crickets, *Wolbachia*-infected females have different spermathecae duct lengths than their uninfected counterparts, and this difference is recoverable following antibiotic treatment that removes the *Wolbachia* infection[111]. 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 bacterial[112], fungal[113] and viral[114–116] lineages and selfishly manipulate host reproduction to facilitate their own spread at the expense of the host. The resulting phenotypes in the host can include cytoplasmic incompatibility (leading to the death of offspring from crosses between infected males and uninfected females), male killing (the specific death of male offspring), feminization (genetic males physically developing and reproducing as females), masculinization (genetic females physically developing as males) and parthenogenesis (females reproducing 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, owing 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, which is an intriguing case whereby a host allele may have evolved on sex chromosomes to avoid population-level extinction[120]. Microorganisms of reproductive tissues therefore specifically benefit from influencing the reproductive features and behaviours of the host in ways 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 in order to increase the fecundity of their transmitting hosts. However, fungi may benefit by slowly killing their transmitting host in order to enable many spores to develop and spread to new hosts[121]. 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 in which the evolution of arthropod sex development and determination has been 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 influence the range of environments suitable for the host[126], either by increasing[127] or decreasing[128] the range of temperatures tolerable 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 environments[129]. However, what are the molecular mechanisms of these changes (known in some cases to be owing to the induction of heat shock proteins by the symbiont[127])? How does niche specialization begin and develop over the course of a symbiotic relationship? Are the changes generally host-driven 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 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 (FIG. 3).

Several studies have demonstrated 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 has sometimes been pooled with reproductive tissues[130]. Potentially hundreds of genes are differentially regulated in reproductive tissues in the presence of a symbiont[130,131],

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Spermathecae

An organ in the female reproductive tract in insects that is used to store sperm post-mating.
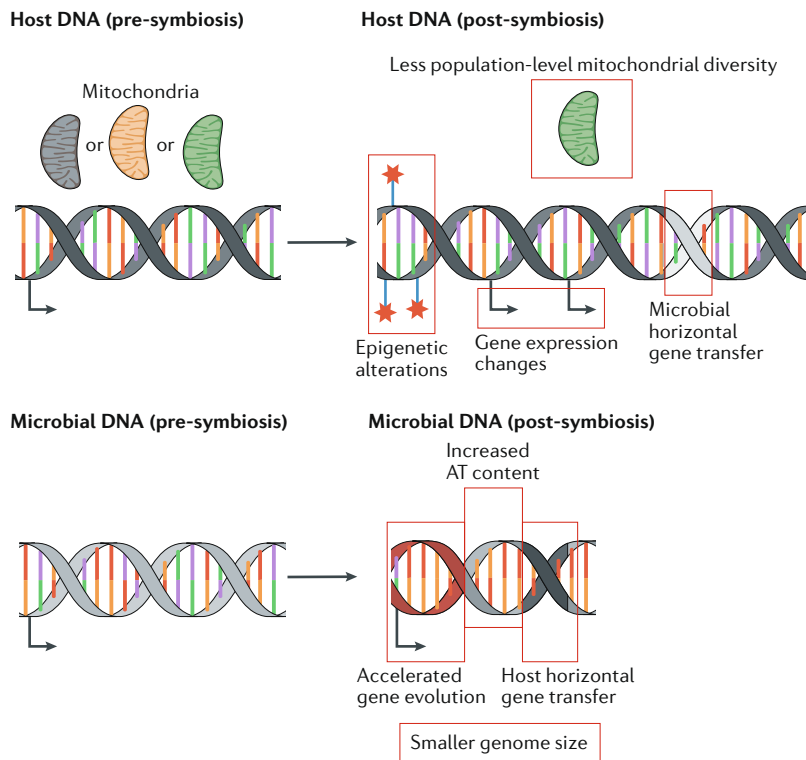
Fig. 3 | Effects on the genomes and transcriptomes of hosts and microorganisms. Each effect is not universal to all symbioses but instead represents changes known in some systems. The top panels show changes that can occur in host DNA, such as epigenetic alterations, changes in gene expression in the presence of symbionts, fewer mitochondrial DNA haplotypes in the population and horizontal gene transfer from microorganisms to host. The bottom panels show changes that can occur in microbial DNA, such as accelerated gene evolution, increased AT content, horizontal gene transfer from host to microorganism and an overall reduced genome size. The post-symbiosis panels show population-level changes many generations since pre-symbiosis.

gene expression profiles are different among infected soma and germline tissues[24], host genes may be differentially expressed in response to an endosymbiont in male and female tissues[130], and many of the genes that are differentially expressed have roles 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 these results cannot yet be generalized. However, it is possible for symbiont genes to be expressed differentially in male and female reproductive tissues of a host[135]. 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 methylation[138], so there may be differences in any putative regulatory mechanisms across organisms. This body of work has generated many questions to be explored more broadly in the future. How are all of these complex transcriptional responses and relationships regulated and mediated? 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 (since non-bacterial symbionts are currently underrepresented)? Although these questions have been answered in some cases, there is a need to assess them in additional contexts and organisms.

Interactions between microorganisms of reproductive tissues and their 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 taxa[139]. Indeed, microorganism-to-host transfer events can include single genes[140–142], larger genomic regions[143] and nearly entire genomes[118,144], with some hosts containing genes from multiple symbionts[143]. For example, a single bacterial gene encoding cytolethal distending toxin was transferred to the fly and aphid genomes and is likely to function in host defence[141], 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 chromosome[118]. In addition, many transferred genes confer functions such as detoxification[145] 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 development[146]. Part of the gene may have bacterial origins, thus making it a rare, putative case of bacterial gene transfer that functions in host reproduction[146]. 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* harbours a eukaryotic association module that is composed of genes with regions of arthropod-like DNA[20]. 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 the transferred genes is enriched. It is likely that fewer eukaryotic genes are transferred to and maintained in bacteria due to the 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 trends above, it is apparent that most known transfer events occurred between the host and either bacteria or fungi, and relatively fewer examples are known among viruses or other microorganisms[139]. With the great progress in this area in recent years, new research questions are now at the forefront of the field. For example, are the rates of gene transfer and maintenance different between microorganisms that primarily or exclusively occupy reproductive tissues and 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 (FIG. 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 over-represented in a population[156]; mtDNA non-synonymous mutations increase, possibly in an arms race with the symbiont[157]; and mtDNA diversity is reduced compared with uninfected counterparts, either at a population level[158] or globally[159]. Moreover, *Candidatus* Midichloria mitochondrii bacteria that infect ticks exhibit the unique ability to invade the mitochondria of ovarian cells[160], although the exact purpose and effects are not known. Overall, the intertwined evolution of mtDNA and symbiont occurs 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, which are less often intracellular[15], 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 substantial[14] (FIG. 3). Indeed, vertically transmitted and obligate intracellular bacteria frequently experience reductive genome evolution, owing to their confined lifestyle[12], relaxed selection due to functional redundancy with hosts[161], genetic drift that occurs through the bottleneck of vertical transmission through the matriline[162,163] and accelerated sequence evolution together with altered base compositions[164]. In general, in the cases of evolving mutualisms, genes may be lost in either the host or a microorganism so as to avoid redundancy in the hologenome. The result is genome erosion until the host and symbiont genomes complement each other[14], which can mean that one or more of the 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 those in bacteria and why?

Host behaviour, sexual selection and speciation. Given the dependency of many microorganisms on host reproduction in order to spread through a population, they can influence host mating behaviour and mate choice so as to facilitate their transmission. For example, *Wolbachia* infection can lead to increased female promiscuity and male fatigue in sex-biased, male-killer populations[165]; discrimination between infected and uninfected sister species, to avoid cytoplasmic incompatibility lethality[166]; and a preference for mating between uninfected

individuals[52]. One particularly striking case is the effect of male-killing *Wolbachia* in *Acraea encedon* butterfly hosts[167]. 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 behaviours, 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 true[167].

Importantly, manipulation of host reproductive behaviour in order 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 infected[17], and infection of *Helicoverpa zea* moths with the gonad-specific Hz-2V virus correlates with a fivefold to sevenfold increase in female sex pheromone production and increased mating calls to attract and infect males[168]. Particularly for microorganisms that depend on host reproductive tissues in order to transmit, altering mating behaviour 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 behaviour than symbionts at other body sites? Do they resultantly have a unique influence on host sexual selection that other symbionts have less often? 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 behavioural changes — are they direct or indirect interactions? Are there differences among microorganisms of different classifications? What effects do these behavioural changes have on the rest of the microbial community over many host generations?

With the profound influence that symbionts may have on arthropod mating behaviours and reproduction, it follows that in some cases they can contribute to host reproductive isolation and thus speciation[169]. Mechanisms of symbiont-induced isolation or speciation may include mate discrimination based on infection status[166], hybrid sterility from microbial over-proliferation[170,171], hybrid lethality[172,173] or reproductive isolation owing to microorganism-mediated specialization in distinct niches[125]. 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 crosses[172]. However, this general phenomenon is most-often demonstrated in the cases of bacterial symbionts, and the extent to which other microorganisms may play a part is less understood. In at least the cases involving *Wolbachia*-induced cytoplasmic incompatibility, a role of the phage WO genes has been established[149]. Many additional questions still need to be addressed: what is the frequency of microbial involvement in speciation

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 its microorganisms.

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 a greater probability of interaction with hereditary DNA and vertical transmission that generates a dependency on host fitness. Moreover, the 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 than among microorganisms of other tissues.

Notably, the principles from arthropod–microorganism interactions in host reproductive tissues can extend to both humans (BOX 2) and plants (BOX 3). Building on substantial work within the field, many areas for future research are important, since a wide context is still missing. We understand much regarding the 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 vigorous attention to the greater diversity of microorganisms 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. Much research interest has focused on microorganisms, such as *Wolbachia*, that have importance in vector control[174], but research into other diverse organisms often remains scarce. Non-bacterial microorganisms interact with hosts in ways that are both similar to (such as reproductive parasitism[6]) and different (such as a phage manipulating both bacterial and arthropod hosts[149], or more frequent paternal transmission among certain taxa[86]) from the ways that bacteria do. Therefore, investigations into the identity of all microorganisms, their population dynamics, their mechanisms of interaction with the 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, as 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 characterize not only their identities but also any putative 'core' microbiota that could be important for studies of evolution, vector control initiatives or conservation efforts. Many microbiota-related 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 microbiota of reproductive tissues[9,44]. In addition, many studies lack proper, sequenced contamination controls or assays to assess whether DNA is from living microorganisms, so current findings must be interpreted 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 in order 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 endosymbionts[180] 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

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Box 2 | Microorganisms of human reproductive tissues

Unlike with 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 most abundant taxa in reproductive tissue samples[191]. The vaginal microbiota also contains a smaller proportion of diverse fungi[192] and viruses[193], 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 the groups of reproductive-age women who have been studied thus far, the vaginal microbiota are diverse and often dominated by *Lactobacillus* species, including *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus jensenii* and *Lactobacillus gasseri*[191]. In a minority of the women tested in these studies, the vaginal microbiota comprise diverse anaerobic bacteria such as *Streptococcus*, with no single dominant species, whereas other microbiota are dominated by anaerobic bacteria such as those in the *Prevotella*, *Atopobium* and *Gardnerella* genera[191]. Many factors contribute to interindividual variation in the vaginal microbiota, including ethnicity[191], pregnancy[194], menopause[195], menstruation[196], hygiene[197], use of birth control[197] and age[197]. In addition, women contain microorganisms in breast milk that are important for offspring health[198].

Less is known about the reproductive tract microbiota in men than in women. However, the upper male genital tract (prostate and vas deferens) is considered to be germ free, except during infection[199,200], and the lower genital tract (urethra and coronal sulcus) contains dozens of bacterial families, of which Clostridiales and Prevotellaceae are the most abundant[201]. Common genera include *Corynebacterium*, *Anaerococcus*, *Staphylococcus* and *Prevotella*[201], which indicates some overlap with the genera found in female reproductive tracts, although some are more common in men. 16S rRNA gene sequencing indicates that there is likely no 'core' penis microbiota[202], a result that parallels the extensive interindividual variation discussed above for the vaginal microbiota. In addition, circumcision[202], prostatitis[203] and prostate cancer[200] 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 conditions[204]. Other microbial infections can also cause complications in pregnancy and birth, such as stillbirth, sepsis or preterm birth[205]. 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 anaerobes[206]. Therefore, Lactobacilli are likely to have important beneficial roles, but it is unclear what functions other microorganisms might 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 quality[207]. Additionally, GTIs in men correlate with poor sperm quality and are associated with infertility[204]. Bacterial DNA sequences are detected in the semen of healthy men[203], so future studies could determine whether any living, resident microorganisms confer benefits to male reproduction. In addition, the long-term evolutionary consequences of these symbioses on the host are poorly understood compared with 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 include various endophytes (microbial symbionts that inhabit plants without any apparent harm) and phytopathogens (microbial symbionts that parasitize plants). In addition, plant microbiota comprise many known viral[208] and fungal[209] 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 plants[211]. The most common phylum was Proteobacteria, followed by Actinobacteria and Firmicutes. Cultivation or sequencing methods across multiple studies have demonstrated that 17 of the plant species seeds contained multiple phyla, whereas the remaining eight only contained a single bacterial phylum[211]. A plant seed may also contain several species within the same phylum or genus[212]. Bacteria commonly sequenced in seeds include various species of *Pantoea*, *Methylobacterium*, *Bacillus*, *Staphylococcus*, *Pseudomonas* and *Paenibacillus*[211,213–215]. Notably, cultivation methods in many plant species result in only one or a small number of bacterial species[216]. This might reflect either the inability to culture most microorganisms of seeds or the presence of few bacterial cells. Known factors that affect the sequence diversity of seed microbiota include the plant species[211], location outside or inside the seed[217] and the stage of seed maturation[217]. Other reproductive structures of plants have site-specific microbial communities as well, such as flowers[218], fruits[213] and pollen[219]. 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 with 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 psyllids[222]. Phytoplasma cause symptoms such as yellowing of leaves,

greening of flowers or even sterility[222]. In *Arabidopsis thaliana*, phytoplasma produce an effector protein, SAP54, that changes the flowers into leaf-like vegetative structures[223]. Both SAP54 and the phytoplasma effector protein SAP11 manipulate the plant host transcription factors that regulate normal flower development[224]. Leafhoppers then preferentially choose infected plants for oviposition owing to physical changes in the plant[223]. This enables the bacteria to spread by attracting their 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 rock[225]. 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 rescued[225]. 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 environment[226]. 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 sites[227]. Furthermore, some of these beneficial relationships may result in microbial genome erosion and the development of obligate associations, as we described for arthropods. One example is the case of *Burkholderia* endosymbionts of *Psychotria* plants, which are transmitted vertically and are likely to have a role in protection from predation or pathogens[228]. 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 endosymbiotic bacteria that produce a phytotoxin that is critical in pathogenesis. When the toxin damages host tissues, both the fungal host and the bacterial symbionts of the fungi benefit from the plant nutrients, in a striking case of inter-taxa dependence and function in parasitization of host reproductive tissues[229]. Thus, plant–microorganism symbioses in the reproductive tissues of plants share many important evolutionary characteristics with arthropod–microorganism symbioses and merit further study.

(including opportunistic microorganisms, reviewed elsewhere[90]), particularly those that are acquired horizontally or whose host phenotypes caused by infection are less pronounced. For example, it is important to discover how much of the microbiota is acquired horizontally or vertically, whether these frequencies differ among different microbial taxa or hosts, what factors determine the establishment or loss of symbionts in the host, how or whether newly acquired symbionts 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 the rates of genetic exchange with the host different between these

types? Are there biases in which genes are exchanged? Do the different types have broadly similar impacts on host fitness and evolution? How are these symbioses different, in terms of the relationships with both the host and other microorganisms, in the reproductive tissues and throughout the body? Are the different types of symbionts more or less likely to exhibit parallel phylogenies with the host?

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

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