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## A complex journey: transmission of microbial symbionts

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### Abstract

The perpetuation of symbioses through host generations relies on symbiont transmission. Horizontally transmitted symbionts are taken up from the environment anew by each host generation, and vertically transmitted symbionts are most often transferred through the female germ line. Mixed modes also exist. In this Review we describe the journey of symbionts from the initial contact to their final residence. We provide an overview of the molecular mechanisms that mediate symbiont attraction and accumulation, interpartner recognition and selection, as well as symbiont confrontation with the host immune system. We also discuss how the two main transmission modes shape the evolution of the symbiotic partners.

Symbiont transmission maintains symbioses through host generations and has a pivotal role in their evolution<sup>1–5</sup>. Despite growing evidence that microbial associations are present in diverse animals and plants in virtually every environment, our knowledge of the mechanisms of symbiont transmission is restricted to a few model systems. Most of these involve bacteria and some archaea<sup>6,7</sup> (Supplementary information S1 (table)). Two fundamentally different modes of transmission can be distinguished: horizontal (that is, from an environmental, free-living symbiont source) and vertical (that is, inheritance of the symbiont from the mother or, more rarely, from both parents). However, there is great variation, and transmission can also be mixed, involving both vertical and horizontal transfers from the environment and intraspecific or interspecific host switching (FIG. 1).

This Review focuses on those associations that maintain protracted physical contact and involve most of the host population. We use de Bary's definition of symbiosis: living together of differently named organisms<sup>8</sup>, irrespective of the effects of the interaction on the fitness of the partners<sup>9</sup>. The key question is how the symbiont is transferred to the host progeny, regardless of the type of symbiosis. Reviews are already available for a range of symbiotic systems: plants<sup>10–16</sup>, sponges<sup>17, 18</sup>, chemosynthetic bacteria–marine invertebrates<sup>19, 20</sup>, entomopathogenic nematodes<sup>21, 22</sup>, annelids<sup>23, 24</sup>, insects<sup>25–30</sup>, squid<sup>31, 32</sup>

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#### Competing interests statement

The authors declare no competing financial interests.

#### DATABASES

**Entrez Genome Project:** <http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj> *Bacteroides thetaiotaomicron* | *Buchnera aphidicola* | *Candidatus Endoriftia persephone* | *Candidatus Hamiltonella defensa* | *Candidatus Regiella insecticola* | *Candidatus Riesia pediculicola* | *Candidatus Serratia symbiotica* | *Nostoc punctiforme* | *Photorhabdus luminescens* | *Prochloron didemni* | *Sinorhizobium meliloti* | *Sodalis glossinidius* | *Vibrio fischeri* | *Wigglesworthia glossinidia* | *Xenorhabdus nematophila*

#### FURTHER INFORMATION

Monika Bright's homepage: <http://www.hydrothermalvent.com>

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#### SUPPLEMENTARY INFORMATION

See online article: [S1](#) (table)

and the vertebrate gut<sup>33-36</sup>. Here, we review how the conversation between partners, which ultimately integrates the symbiont into the host's life cycle, is initiated. By identifying the similarities and differences between the two principal types of symbiont transmission, we explore their evolutionary implications.

## Horizontal transmission

The cyclic occurrence of aposymbiotic (that is, before the symbiont is acquired) and symbiotic phases in the host's life cycle is intrinsic to horizontally transmitted symbionts (Boxes 1,2; FIG. 2) (Supplementary information S1 (table)). In animal hosts, the aposymbiotic phase includes germ cells (eggs and sperm) and embryonic development. When present, larvae are also aposymbiotic during their pelagic dispersal (in tubeworms, lucinid and bathymodiolin mussels). When development is direct, symbionts can be acquired by juveniles (for example, in squid). Similarly, in plants germ cells and zygotes are aposymbiotic, as are seeds in legumes, and sporophytes and spores in hornworts.

For horizontally transmitted bacteria, symbiotic life is facultative: a free-living population serves as the inoculum for the symbiosis. Such free-living populations occur in soil<sup>37-39</sup>, marine shallow waters<sup>40-42</sup> and the deep sea<sup>43</sup>. In some cases the free-living population is replenished by the release of symbionts from the host<sup>44,45</sup>.

## Accumulation and entry

To establish an intimate association, the two free-living partners must physically contact each other, at the right time and in the right place, and must do so anew with each generation. Infection is often restricted to a specific life phase, such as post-settlement larvae in tubeworms, or juveniles in bivalves and squid. For example, the symbiont of the beard worm *Siboglinum poseidoni* can be ingested or taken up through the anus only during the larval phase<sup>46</sup>. By contrast, most microorganisms can access (and associate with) a functional gut throughout the life of the host, and infection of hornwort thalli and legume roots is continuous as long as the plants grow.

In some systems, the time frame for infection can be extended experimentally. In the squid *Euprymna scolopes* the speed of colonization of the light organ depends strongly on the density of the free-living symbiont *Vibrio fischeri*<sup>47</sup>. Similarly, infection of the gill epithelium of the lucinid clam *Codakia orbicularis*, which usually occurs after metamorphosis, can be artificially postponed by several months<sup>48</sup>. Whether such prolonged symbiosis competence is relevant in the natural environment remains unknown.

Infection can also be spatially limited. Stomata-like openings in hornwort thalli or pores on the ciliated appendages of the nascent squid light organ guide the prospective symbionts to defined areas inside the host. Even in the absence of special uptake structures, infection can be restricted to specific areas, such as the trunk region of tubeworm larvae<sup>49</sup> or the gills (and sometimes part of the mantle epithelium) in bivalves<sup>45</sup>. In legumes, the susceptibility to colonization by rhizobia varies even within subsets of root hairs (reviewed in REF. <sup>11</sup>).

One of the most widespread entry sites in animals is the oral opening. By accessing their hosts on ingested particles, microorganisms can colonize the gut epithelia. Freshly hatched fish and nymphs of the broad-headed insect *Riptortus clavatus* acquire their microbiota through the mouth from water<sup>50</sup> or soil<sup>39</sup>, respectively. Once inside the gut, peristaltic movements can help the symbionts to contact the appropriate niche.

Other bacteria actively swim or glide, sometimes following chemical gradients. Legumes and hornworts are sessile and must inhabit a water-saturated environment for their

symbionts to reach them. Hornworts even induce cyanobacteria to become motile by secreting hormogonium-inducing factors (HIFs) (reviewed in REF. <sup>15</sup>). Following the action of HIFs, hormogonia enter stomata-like openings and glide into plant slime cavities. Free-living rhizobia have flagella and can also move towards plant roots by chemoattraction to compounds released by the plant root exudates, including various nutrients and host-specific flavonoids. Flavonoids can also induce rhizobial growth and, most importantly, the expression of symbiotically active genes such as *nod* genes (reviewed in REFS <sup>51,52</sup>). The metagenome of the tubeworm symbiont *Candidatus Endoriftia persephone* displays remarkable dedication to chemoreception and motility, suggesting that the free-living counterparts of these endosymbionts can reach their respective hosts through chemotaxis<sup>53</sup>.

Given the turbulent nature of aquatic environments, it can be hazardous for a host looking for symbionts to rely exclusively on establishing chemoattractant gradients. Moreover, aquatic environments typically have low concentrations of potential symbionts<sup>54,55</sup>. Many aquatic hosts therefore actively pump water along their tissues, enhancing their chances of entrapping potential symbionts. Water currents generated by ingestion and egestion openings in bivalves facilitate encounters between prospective symbionts and the gill epithelium. For example, a squid hatchling captures planktonic *V. fischeri* only if it comes close to the ciliated appendages of the nascent light organ. This is promoted by mantle contractions and by cilia-generated microcurrents (reviewed in REF. <sup>31</sup>).

### Box 1

#### Transmission strategies and symbiont genomes

The sequence of several symbiont genomes or at least their size and GC content is available (Supplementary information S1 (table)). Environmentally acquired symbionts are derived from a large and genetically diverse free-living population. In this case, selection can freely act against mildly deleterious alleles that have arisen by chance in the symbiont genome. The risk that these alleles will accumulate and persist is therefore limited<sup>116,118</sup>. These versatile symbionts must compete with the rest of the microbial community while free-living and also retain the genetic repertoire that is necessary to associate with their hosts. This reduces selection pressure on genome reduction.

By contrast, during strictly vertical transmission there is a reduction in the purifying activity of natural selection<sup>110,117,118</sup>. In this case, the symbionts transferred to the next generation are selected out of a restricted genetic population (this is known as a transmission bottleneck). In aphids, for example, the subpopulation of a single mother's bacteriocyte is transferred to the embryo, and later, on cellularization, all symbionts that have penetrated the embryo are subdivided again into different bacteriocytes<sup>110</sup>. Transmission bottlenecks leave irreparable scars on the genomes of host-relegated symbionts. These genomes, as in asexual populations, irreversibly accumulate harmful mutations (in a process known as Muller's ratchet)<sup>117</sup>: symbionts that cannot exist in a free-living state cannot purge their genetic repertoire. The accumulation of deleterious mutations results in inactivation and loss of genes, such as DNA repair genes, and, finally, in reduced genomes (reviewed in REFS <sup>27,29,30</sup>). Besides high rates of amino acid substitution, the resulting reduced nucleotide base composition does not favour guanidine and cytosine (reviewed in REF. <sup>29</sup>). Another possibility to explain the loss of gene function in strictly vertically transmitted symbionts is that many genes may be unnecessary in the nutrient-rich, safe environment provided by the host<sup>119,120</sup>.

Vertically transmitted symbionts that are either facultative or that only recently became obligate are a special case. Aside from a moderate reduction in size, the genomes of these symbionts (such as those found in mouthless oligochaetes<sup>121</sup> and weevils<sup>122</sup>) have many

pseudogenes and insertion sequences<sup>123</sup>. Increasing disconnection from a free-living pool will eliminate both of these kinds of genetic elements (reviewed in REFS <sup>124,125</sup>).

### Interpartner recognition

The initial physical encounter between partners typically occurs in host-secreted mucus. The mucus can cover plain surfaces (for example, the sticky developing tube surrounding tubeworm larvae<sup>49</sup>) or fill in the spaces among papillae (for example, in the giant rhubarb *Gunnera* spp.; reviewed in REF. <sup>12</sup>), cilia (for example, in bivalves, beard worms and squid; reviewed in REFS <sup>19,31</sup>) and villi (for example, in the guts of vertebrates; reviewed in REFS <sup>34,35</sup>). Mucus quantity and quality can be subject to microbial control. Environmental bacteria stimulate the production of the mucus that covers the appendages of the squid nascent light organ. Similarly, vertebrate gut residents stimulate mucus shedding and modulate the gut's glycan composition.

Bacterial binding to host surfaces often involves proteinaceous surface appendages known as pili (or fimbriae). Loss-of-function mutations in the gene encoding Pila, the major component of type IV pili, affect the colonization competence of *V. fischeri*<sup>56</sup>. *Azoarcus* sp. also requires Pila to adhere to kallar grass (*Leptochloa fusca*), and *Nostoc punctiforme* uses Pila for chemotaxis and probably adherence (reviewed in REFS <sup>10,15</sup>).

In mucus matrices, partners can select and attach to each other using their specific repertoires of surface sugars and sugar-binding proteins. Host surface sugars are present in the form of highly glycosylated proteins, whereas symbiont sugars are mainly exopolysaccharides (EPS) or lipopolysaccharides (LPS). Indeed, *V. fischeri* mutants lacking correctly modified LPS are colonization defective<sup>57</sup>. Examples of bacterial molecules that bind host sugars are rhizobial adhesins (known as ricadhesins) (reviewed in REF. <sup>58</sup>) and the OmpU (*V. fischeri*<sup>59</sup>) and SuscD-like (*Bacteroides thetaiotaomicron*<sup>60</sup>) outer membrane proteins. The host molecules that can bind to symbiont sugars include LPS-binding protein on the surface of the developing light organ in the squid<sup>61</sup>, Mermaid on the surface of marine nematodes<sup>62</sup> and codakine on the gills of lucinids<sup>63</sup>.

Bacterial autoaggregation is often a prerequisite of host attachment, and many of the bacterial surface molecules and structures mentioned above facilitate both processes. The ability of *V. fischeri* to specifically aggregate in the squid mucus depends on signalling by the RscS two-component regulation system, which controls production of EPS I by regulating the *syp* operon<sup>64,66</sup>. Moreover, mutations affecting the AinS–AinR quorum sensing system reduce the colonization speed in *V. fischeri*, probably owing to insufficient flagellar motility (reviewed in REF. <sup>32</sup>). Along the same lines, in *Sinorhizobium meliloti* the *exoS* two-component regulatory system controls EPS I production and flagellum formation (reviewed in REF. <sup>13</sup>), and quorum sensing controls EPS II production and swarming motility<sup>67, 68</sup>.

Although the rule in horizontally transmitted symbioses is that interpartner recognition occurs in the host-secreted mucus, in most plant symbioses recognition starts at a distance. The binding of plant flavonoids to rhizobial nodD transcription factors results primarily in the synthesis and release of specific nod factors (reviewed in REFS <sup>51,69</sup>). A high local concentration of the appropriate nod factor is required for the root hair to curl round a rhizobium. At this stage, ricadhesins mediate bacterial agglutination and binding to root hairs. Concomitantly, plant lectins mediate adhesion to specific rhizobial EPS (reviewed in REF. <sup>70</sup>). These initial weak and reversible sugar–sugar and lectin–sugar interactions are followed by a tighter, irreversible binding that is mediated by bacterial cellulose fibrils

(reviewed in REF. <sup>11</sup>). Notably, nod factors are also required for the formation of *S. meliloti* biofilms<sup>71</sup>.

### The journey to the symbiont housing organ

In digestive tract symbioses and in all marine ectosymbioses, the host mucus is also the final residence for the symbiont. However, the symbiont must migrate to the symbiont housing organ if it differs from the site of initial contact. In this situation, the symbiont must migrate either extracellularly in canals (such as hornwort slime cavities, *Gunnera* spp. stem gland channels and squid ducts), intercellularly (for example, rhizobia entering through cracks), through transcellular tunnels (for example, rhizobia inducing root hair curling) or both intercellularly and intracellularly (such as in tubeworms and kallar grass).

#### Box 2

##### Phylogenetic implications of transmission strategies

Horizontally transmitted symbioses typically show little evidence of phylogenetic congruence or cospeciation. For example, there is no overlap in the tree topologies of mussels and tubeworms and their symbionts<sup>20</sup>. Several lucinid clam species co-occurring in the same habitat are associated with the same symbiont phylotype<sup>126</sup>. *Burkholderia* spp. symbionts of *Riptortus clavatus* and *Leptocorisa chinensis* do not cluster according to host, but intermingle together with free-living strains<sup>39, 127</sup>. Congruence between the topology of seven *Vibrio* spp. strain *gapA* sequences and 14 squid internal transcribed spacer or cytochrome *c* oxidase sequences indicates cospeciation<sup>128</sup>, although the few analysed squid–*Vibrio* spp. Associations and their large geographical distance raised some disagreement<sup>20, 129</sup>.

The phylogenetic trees of symbionts that are exclusively vertically transmitted are congruent with those of their hosts over long evolutionary time. Several obligate insect symbionts are well-known examples (reviewed in REFS <sup>27-30</sup>). Studies on clams showed overlapping symbiont and host genealogies, suggesting cospeciation<sup>130, 131</sup>. However, recent analysis of the northeastern Pacific *Vesicomya* sp. Mt-II lineage indicates additional horizontal transfer of symbionts in this clade<sup>132</sup>. A cospeciation pattern was also found between primate lice and *Candidatus* Riesia spp.<sup>133</sup> and between earthworms and *Verminephrobacter eiseniae*<sup>134</sup>.

Although many symbionts are transmitted vertically, phylogenetic incongruence points to additional horizontal transfer through host switching or the environment. This was the case for insects harbouring *Wolbachia* spp.<sup>135-137</sup>, representatives of four ascidian genera and *Prochloron* spp.<sup>138</sup>, gutless oligochaetes<sup>139</sup>, several bryozoan *Bugula* spp. and *Candidatus* Endobugula spp.<sup>140</sup>, and several solemyid species and their endosymbiotic gamma proteobacteria<sup>141</sup>. In sponges, recent evidence suggests that horizontal acquisition must also be present to account for the global distribution of closely related microbial lineages in geographically disparate host sponges<sup>142</sup>.

HIFs attract cyanobacteria to the slime cavities on the ventral side of hornwort thalli, and similar compounds probably attract cyanobacteria to the bottom of *Gunnera* spp. stem glands. *V. fischeri* reaches the deep crypts of the squid nascent light organ by flagellum-mediated chemotaxis towards diverse nutrient sources opposing the current that is generated by the ciliated epithelium lining the ducts. In *Gunnera* spp. the prospective symbionts swim against their positive phototactic behaviour and against the mucilage flow (reviewed in REFS <sup>12, 15, 31</sup>).

Rhizobia that enter through cracks in the epidermis travel to the deep cortical layer in an intercellular infection thread, which is an organized line of infecting rhizobia with associated lignification of adjacent cell walls. The infection thread initiates from an infection pocket, which is generated by host cell programmed cell death (reviewed in REF. <sup>72</sup>). However, migrating rhizobia are contained in a tubular invagination originating from the curled root hair cell (reviewed in REF. <sup>11</sup>). The construction of this transcellular tunnel requires massive rearrangements of the plant cell cytoskeleton. As rhizobia en route to the deep cortical cells lack flagella, it was suggested that invasion progresses by bacterial proliferation at the infection thread tip, coupled to its constant extension<sup>13</sup>. This requires highly specific nod factors and, for indeterminate nodules, specific EPS.

The tubeworm symbiont *Candidatus* endoriftia persephone migrates from the epidermis to the symbiont housing organ, the trophosome, through mesoderm tissue, probably by entering and exiting all the cells it encounters (in small, infected larvae, symbionts have been found both intracellularly and intercellularly). The migrating symbionts are free in the cytoplasm, whereas later, in the trophosome, they are enclosed in vacuoles<sup>49</sup>. Also *Azoarcus* sp. can infect kallar grass by moving intracellularly and intercellularly, but it can only enter and colonize decaying or dead cells (reviewed in REF. <sup>10</sup>).

In addition to physical obstacles, travelling symbionts must withstand the host humoral and cellular immune responses. *Candidatus* Endoriftia persephone possesses a wide repertoire of defence-associated genes, suggesting that it can withstand a vigorous host immune response<sup>53</sup>. Invading rhizobia are protected from oxidative stress (reactive oxygen and nitrogen species) in the infection thread by producing catalases, and can attenuate the plant immune response by producing LPS (reviewed in REF. <sup>12</sup>). Furthermore, rhizobia are thought to avoid phagocytosis by immune cells using proteins secreted by a type III secretion system (T3SS)<sup>73</sup>. *V. fischeri* requires periplasmic catalase to survive oxidative stress while travelling from the pores to the light organ deep crypts<sup>74</sup> and ompu to avoid phagocytosis by haemocytes<sup>59</sup>.

Once the rhizobia have reached the root cortex, the cyanobacteria have arrived at the bottom of the *Gunnera* spp. gland channels and *Candidatus* Endoriftia persephone has reached the prospective trophosome, they must accomplish a final task: internalization by host cells. In indeterminate nodules, each bacterium is endocytosed by a cortical cell in an individual unwallled membrane compartment that is derived from the infection thread. Each cortical cell can be infected by the single rhizobium that first entered the infection thread. Among the numerous bacterial factors that can affect symbiosome formation are LPS composition and tolerance to osmotic stress. The plant factors required for endocytosis include the *Medicago truncatula* NIP gene and wild-type expression levels of the leucine rich repeat domain-containing receptor DMI2 (also known as NORK). It remains unclear how cyanobacteria penetrate the thin walls of small dividing cells lining the gland channels, although mucilage pectolytic or cellulolytic activities might be involved (reviewed in REFS <sup>12, 13</sup>).

In conclusion, all horizontally transmitted symbioses require sophisticated molecular machineries to select specific symbionts from the environment. Most, if not all, involve sugar–lectin interactions and cellular surface structures, which can mediate symbiont–symbiont and host–symbiont attachment, and sometimes even help the partners to meet. Moreover, at each generation, the host and symbiont need to get accustomed to each other: the symbionts must combat the host immune response and, in turn, the host must not succumb to its symbionts.

## Vertical transmission

In many vertically transmitted symbioses, there is no aposymbiotic phase and so the symbiotic association is permanent (BOXES 1,2; FIGS 3,4) (Supplementary information S1 (table)). Uptake occurs in developing or fertilized eggs in some sponges, the gutless oligochaetes, bivalves, bryozoans, several insects and during sporulation in the water fern *Azolla* spp.. In some cases a long symbiotic phase follows a short aposymbiotic phase. This begins with the egg and may extend to various embryonic stages (in some sponges, *Eisenia foetida* earthworms, *Trididemnum miniatum* ascidians, and *Acrytosiphon pisum* and *Brevicoryne brassicae* aphids), to larvae still associated with the parent (in several ascidians and *Glossina morsitans* tsetse flies) or to juveniles developing in or outside the mother (*Heterorhabditis bacteriophora* nematodes and medical leeches (*Hirudo* spp.), respectively). Although all these associations are obligate for the host, the entomopathogenic nematode *Steinernema carpocapsae* can accomplish its entire life cycle aposymbiotically and only facultatively associates with its symbiont, *Xenorhabdus nematophila*, during the juvenile stage.

Vertically acquired symbionts are often transmitted through the female germ line (through the transovarial route) in animals with separate sexes (for example, in sponges) and hermaphrodites (for example, gutless oligochaetes). An example of biparental transfer is found in *A. pisum* and its facultative symbionts *Candidatus Hamiltonella defensa*, *Candidatus Serratia symbiotica* and *Candidatus Regiella insecticola*<sup>75</sup>. Furthermore, *Candidatus Synechococcus spongiorum* is present in 2% of the sperm of the sponge *Chondrilla australiensis*<sup>76</sup>. Although the *Wolbachia* spp. symbionts of *Drosophila melanogaster* (reviewed in REF. <sup>77</sup>) and *Anabaena azollae* symbionts of the water fern<sup>78</sup> also occur in the male germ line, there is no evidence of paternal transfer.

Vertical transmission is rarely the only means of symbiont transfer into the next generation. More often, phylogenetic evidence indicates that vertical acquisition is dominant, with occasional horizontal transfer within or between species or from the environment (BOX 2). For example, *A. pisum* females often mate with more than one male, so the progeny can acquire the symbionts from the ejaculate of several different males<sup>75</sup>. Intraspecific transfer was also proven among the parasitoid wasps *Nasonia vitripennis*<sup>79</sup> and *Trichogramma kaykai*<sup>80</sup>. The rarer interspecific transfer naturally occurs between *Trichogramma* spp.<sup>81</sup> and between sweet potato whiteflies and their parasitic wasps<sup>82</sup>. The examples below describe transmission of both exclusively and non-exclusively vertically transmitted symbionts.

### Hanging onto the germ line

No symbiont translocation is needed when the symbiont is permanently present in the female germline stem cells, for example *Wolbachia* spp. (reviewed in REF. <sup>77</sup>). This is achieved through an unknown mechanism that equally partitions *Wolbachia* spp. between the self-renewing stem cells and the differentiating daughter cells, which give rise to the oocytes. In early oogenesis, the prospective oocyte undergoes mitosis with incomplete cytokinesis, yielding 16 cells that are interconnected by cytoplasmic bridges. Fifteen of these cells give rise to the nurse cells, and the most posterior cell gives rise to the oocyte; *Wolbachia* spp. are segregated to the oocyte during this process by minus end-directed motors travelling on microtubules. An alternative infection route is from the heavily infected follicle cells that encase the developing oocyte. Later in oogenesis, the oocyte cytoskeleton is rearranged so that plus end-directed motors transport *Wolbachia* spp. to the oocyte's posterior side, guaranteeing symbiont integration into the future germ line. The cellularization of the embryo occurs after multiplication of the zygote nucleus into hundreds of nuclei and after these have migrated to the periphery. Because nuclei at the posterior pole are the first to be surrounded by plasma membranes, the first embryonic cells are

*Wolbachia*-containing germ cells. Although posterior localization of *Wolbachia* spp. also occurs in mosquito and hymenopteran oocytes, some strains remain homogeneously distributed throughout oogenesis. In these cases, *Wolbachia* spp. associate with the nuclei so that the small proportion that eventually migrates towards the posterior pole will be incorporated in the future germ line. An insect cell line infected by *Wolbachia* spp. upregulates the expression of antioxidant proteins. Although this indicates that cultured host cells mount an oxidative stress response against *Wolbachia* spp., it is unclear how this endosymbiont combats this response<sup>83</sup>.

If the symbiont population is divided into a germ line and somatic population during early development, no transfer from the symbiont housing organ to the female germ line is necessary (reviewed in REF. <sup>84</sup>). This is the case for the obligate symbionts of *Sitophilus zeamais* weevils, which reside in a bacteriome in the caecum and the ovary. When the fertilized eggs of *S. zeamais* start to differentiate, some symbionts populate the female gonad and others the bacteriome. When the symbionts must invade new bacteriomes, components of the T3SS are strongly overexpressed by the *S. zeamais* principal endosymbionts<sup>85</sup>.

### The journey to the reproductive organ

Symbiont transfer from parent to progeny can vary depending on several parameters: which partner migrates (both partners, only the parent or only the symbiont); at which ontogenetic stage of the parent (embryo or adult) and progeny (developing and fertilized egg, embryo, larva or juvenile) development does migration occur; the distance from the symbiont housing organ to the reproductive organ; the mode of translocation (within bacteriocytes or free following release); and the site of egg infection (posterior pole or apolar).

The distance from the symbiont housing organ to the progeny can be covered by either or both migrating partners. The descent of eggs down the reproductive tract can be followed by external smearing with symbionts (in gutless oligochaetes<sup>86</sup> and some insects; reviewed in REF. <sup>26</sup>). In insects, this also requires symbiont transfer to pouches that are connected with the female genital openings. In addition, hatching larvae must feed on the symbionts. In some ascidians, instead of the eggs, it is the embryos or larvae (depending on the species) that migrate from the ovary through the tunic and are infected when entering the cloacal cavity, which is lined with extracellular immobile symbionts (*Prochloron didemni*)<sup>87-90</sup>.

Many cases of symbiont transfer from parent to progeny are known. In sponges, both symbionts and eggs are located in the extracellular mesohyl, which shortens the distance to cover. Which partner is responsible for getting together remains unknown. However, either the egg is infected before fertilization<sup>91</sup> (and in one case the sperm is also infected<sup>76</sup>) or infection occurs during embryogenesis<sup>92,93</sup>. In aphids, bacteriocytes housing *Buchnera aphidicola* can also be found in the haemocoel, close to the ovaries, and are transferred from an adult to a sexual or parthenogenetic egg (reviewed in REF. <sup>26</sup>).

Although transfer from the symbiont housing organ to the reproductive organ usually takes place in the adult host, it can also occur while the parent is still developing. In embryonic cockroaches, intact bacteriocytes migrate from the fat body to the closely opposed oocytes as soon as these have developed microvilli. Symbionts hang outside the oocytes, entrapped in the microvilli, until the cockroaches develop into adults and undergo ovulation. Only then are symbionts endocytosed by the oocytes<sup>26</sup>.

In other examples, the symbionts undertake a longer journey. In *Bugula neritina* bryozoans the extracellular endosymbiont is distributed in a network of channels that reach into zooids, which are specialized cells in which the embryos and larvae brood<sup>94</sup>. In shipworms and



clams, endosymbionts must migrate from the gills to the ovary to infect the eggs; *Solemya reidi* symbionts have been detected in the clam ovary, encapsulated eggs and brooded larvae<sup>95</sup>. The eggs of the clam *Calyplogena* spp. are apparently infected through follicle cells, but the mechanism of symbiont transfer from the gills to the ovary remains enigmatic<sup>96,97</sup>.

A spectacular journey is undertaken inside the human louse, which harbours the intracellular *Candidatus Riesia pediculicola* in a gut-derived bacteriome<sup>98,100</sup>. During female development, symbionts exit the bacteriocytes through a hole and migrate directly to the lateral oviducts by moving posteriorly on the ventral surface of the gut. Because the symbionts lack flagella, they probably move by twitching or gliding. Subsequently, they enter the lateral oviduct cells by penetrating a fibrous mesh, which does not coat other oviduct regions. Haemocytes are also attracted to the lateral oviduct following moulting and are thought to repair the tissue damage caused by massive symbiont penetration; up to 200 symbionts can be transmitted in lice<sup>25</sup>.

Instead of the egg or embryo stage, it is the larvae of tsetse flies and the juveniles of entomopathogenic nematodes that are infected. Tsetse fly symbionts are transmitted to the larvae through infected milk secretions. Although *Sodalis glossinidius* resides in multiple fly tissues, including the milk gland, it is unknown how the symbiont *Wigglesworthia glossinidia* is transferred from the midgut mycetome to the lumen of the milk glands. *S. glossinidius* uses its T3SS to invade insect cells *in vitro*, as in its absence, *S. glossinidius* cannot be vertically transmitted, even when microinjected into the thorax of an adult fly<sup>85</sup>.

Shortly after colonizing an insect, entomopathogenic nematodes release their symbionts into the haemolymph, causing the death of their prey. As the progeny of the invading nematode feed on the dead insect, they ingest prospective symbionts. After entering and migrating through a specific group of gut cells, the symbiont of the nematode *Heterorhabditis bacteriophora*, *Photorhabdus luminescens*, infects and replicates within the neighbouring rectal gland cells. The eggs hatch internally, so that the mother is consumed by the developing infective juveniles. During the development of infective juveniles, the infected rectal glands of the adult break open and release symbionts into the mother's body cavity. Each infective juvenile is then colonized by a single bacterium that attaches to the pre-intestinal valve cell<sup>101</sup>. *P. luminescens* can use a T3SS to secrete YopT, a homologue of a *Yersinia pestis* protein that has antiphagocytic activity, but it is unclear whether this secretion system is necessary for nematode colonization<sup>102</sup>. Moreover, a correctly modified LPS on the surface of *P. luminescens* might confer resistance to the nematode humoral defences (reviewed in REF. 22). In *S. carpocapsae* (reviewed in REF. 21), after a few rounds of aposymbiotic reproduction in the insect carcass, one or two *X. nematophila* symbionts colonize the lumen at the anterior end of the infected juvenile gut between two epithelial cells (known as the vesicle). The vesicle contains a group of untethered spheres that probably evolved to optimize presentation of sugar-rich mucus<sup>103</sup>.

*Aeromonas veronii*, the main symbiont of the digestive tract of the medical leech, must be released to be taken up by the cocoon within 24 hours of deposition<sup>104</sup>. Because symbionts are undetectable in the reproductive organs and on the body surface but are found in the oral opening, the cocoon fluid is probably infected by oral smearing<sup>105</sup>. Proteins secreted by a T3SS have been implicated in the avoidance of phagocytosis by leech-associated *A. veronii*, and its LPS seems to confer resistance to the complement system that is present in the blood meal<sup>106</sup>.

At the end of their journey, vertically transmitted intracellular symbionts are internalized by the host cells by endocytosis (for example, in the sponge *Halisarca dujardini*<sup>93</sup>), by fusion of

symbiont housing nurse cells with eggs (for example, in the sponge *Chondrilla australiensis*<sup>107</sup>) or by the formation of transient collagen-like connections between nurse cells and eggs (for example, in the sponge *Hippospongia lachine*<sup>92</sup>). In the sweet potato whitefly *Bemisia tabaci*, however, intact bacteriocytes are directly enclosed in the egg<sup>108</sup>. *B. aphidicola* is the best-described example of this internalization<sup>109</sup>. Invasion of the *A. pisum* aphid embryo by *B. aphidicola* varies depending on its sexual or parthenogenetic origin. If the embryo originates from sexual reproduction, the posterior follicle cells lengthen along their apical–basal axis just before symbiont invasion. The bacteria are released by the bacteriocytes and probably enter the embryo through multiple, filamentous actin-reinforced channels in the posterior follicular epithelium. At this point, the prospective bacteriocyte nuclei migrate to the bacterial mass and form a bacteriome. By contrast, to enter a parthenogenetic aphid embryo, symbionts must penetrate enlarged posterior follicle cells. A channel appears between the posterior follicle cells, allowing bacteria to flow into the posterior of the embryo. Bacterial transmission apparently involves the fusion of a membrane-bound bacterial package with the posterior follicular epithelium. Because the most posterior nuclei are the only embryonic nuclei, they can associate with the bacteria and give rise, on cellularization, to symbiont-containing bacteriocytes. The number of *B. aphidicola* transmitted to each egg varies between 850 and 8,000 bacteria among different aphid taxa<sup>110</sup>.

### Interpartner recognition

As all symbionts that are strictly vertically transmitted are obligate symbionts and do not need to be selected from an environmental population, surface molecules for specific interpartner recognition are not mandatory. Given the pivotal role of lectins in horizontally transmitted symbioses, it is remarkable that the *Azolla* spp.–*Anabaena* spp. system is the only vertically transmitted symbiosis in which lectins are known to be involved (reviewed in REFS<sup>12,15</sup>). Nevertheless, facultative symbionts (for example, *Wolbachia* spp. and *P. luminescens*) and symbionts that confront environmental bacteria (for example, *Verminephrobacter eiseniae* and *X. nematophila*) display surface structures and molecules that are similar to those identified in horizontally transmitted symbionts.

*Wolbachia* spp. outer membrane proteins (Wsp) exhibit great variability between strains and might function in mediating infection of specific host cells, which can, in turn, infect the oocytes. Alternatively, Wsp might inhibit apoptosis of host cells by binding Toll-like receptor 2 (reviewed in REF.<sup>77</sup>).

The *P. luminescens* *pbgPE* operon is required for correct LPS modification and successful nematode colonization. As the *P. luminescens* genome is predicted to encode different fimbriae, these could allow attachment to the surface of different digestive tract cells. Because fimbria-encoding loci vary among *P. luminescens* isolates, the molecular signature of certain fimbriae might also mediate acquisition of specific isolates (reviewed in REF.<sup>22</sup>).

The *X. nematophila* *nilABC* genes are genetically linked, coordinately regulated and encode membrane proteins that are essential for colonizing the nematode host<sup>111</sup>. These three proteins function together to mediate attachment to the sugar-rich host mucus. Intriguingly, *nilB* homologues are present in pathogens that colonize mucosa (reviewed in REF.<sup>21</sup>). In addition to *nil* genes, *mrxA*, which encodes the structural subunit of type I fimbriae, is also required for successful nematode colonization<sup>112</sup>.

Although the role of quorum sensing in vertically transmitted symbioses remains elusive, successful *X. nematophila*, and probably *P. luminescens*, colonization requires a global transcriptional change in response to environmental signals (reviewed in REF.<sup>21</sup>).

## The journey to the symbiont housing organ

As intricate as the journey to the reproductive organ can be, some vertically transmitted symbionts must migrate further, to the symbiont housing organ of the progeny. After complete cellularization, the *A. pisum* embryo undergoes a series of morphogenetic movements that include germ band extension, twisting, flipping and retraction. Although these movements are so radical that the dorsal–ventral and the anterior–posterior axes are inverted twice, the symbiont-containing bacteriocytes remain tightly associated with the germ cells in the dorsal abdominal region, close to the ovaries<sup>109</sup>.

Aphid bacteriocytes are at the mercy of harsh developmental movements, whereas *V. eiseniae* acts on its way to earthworm nephridia: it enters the embryo only when segmentation has already started and recruitment canals are available. After gathering on the dorsal side of a subepithelial canal, it migrates down to the ventral side and reaches the nephridiopore, where it remains for some time before entering the nephridium and proceeding to its definitive residence, the ampulla. Although other, less abundant bacterial phylotypes are present in the canal, they do not migrate into the open nephridial pore. Because both nephridial development and symbiont colonization proceed segment after segment from anterior to posterior, one hypothesis is that as the recruitment canals sequentially mature, they chemo-attract prospective symbionts<sup>113</sup>. Although the leech bladder symbiont *Ochrobactrum* sp. was detected in the cocoons immediately following deposition, it is not known how it is directly transmitted to the embryo<sup>104</sup>.

An unusual strategy is used by the symbionts of *S. reidi* to migrate to the progeny's gills. Bacteria located in test cells surrounding the embryo are ingested and transported into the foregut, and degradation in the digestive system positions them in the perivisceral cavity<sup>114, 115</sup>. It is not clear how they then reach the developing gills.

In summary, hosts that can pass on their symbionts by vertical transmission have evolved strategies to deeply integrate the symbionts in their reproductive and developmental biology. Nevertheless, the molecular mechanisms underpinning this integration are not merely host directed. Instead, components of bacterial origin, such as the T3SS, are emerging as conserved requirements.

## Conclusions

This Review illustrates how some symbiotic plants and animals have retained the ancestral, horizontal transmission mode throughout evolution, whereas others have opted for the vertical route and have become capable of integrating the symbionts, usually into the female germ line. However, some hosts have kept one foot in both camps: environmental acquisition has been maintained or host-switching mechanisms have evolved, so the mode of transmission is mixed. More rarely — and unquestionably demonstrated only for some insects — partners have ‘unlearned’ all the transfer strategies except for vertical transmission.

The integration of microbial symbionts into the reproductive and developmental processes of their hosts has many manifestations. Regardless of the kingdom to which the host belongs, each transmission strategy involves costs that are difficult to calculate. This complicates predicting how the progeny will inherit microbial partners. Although symbiont acquisition in a hornwort and a squid are strikingly similar, the horizontal and vertical transmission modes, strictly speaking, share few features. The transmission of *V. fischeri* and *B. aphidicola* clearly rely on antithetic molecular and anatomical adaptations. However, both paths can lead to specific, obligate and long-lived symbioses.

Horizontal acquisition typically takes place after progeny dispersal or dormancy associated with major developmental morphological changes (for example, after metamorphosis). A mucus interface often facilitates symbiont attraction, accumulation and recognition. It can also provide the settings for initial symbiont selection. Once the symbionts have gained entry and reached their final residence, the selection stringency often increases. By contrast, strict vertical transmission occurs before the progeny leaves the mother. In a sterile host, heritable symbionts face no competition with environmental bacteria. Although recognition and selection represent ancillary issues here, two symbiont translocations must be finely choreographed: from the symbiont housing organ of the parent to the transmission site, and from this site to the symbiont housing organ in the progeny.

Whether beneficial or pathogenic, environmentally acquired microorganisms attach to host-secreted mucus and exploit similar invasion mechanisms, without succumbing to host immune responses. Oxidative stress is a common immune response in hosts with horizontally transmitted symbioses. In some cases, such a defence strategy can even promote symbiosis establishment. Bacterial strategies to resist host immune defences include secretion systems and appropriately modified LPS. Remarkably, symbionts exposed to a non-sterile environment, whether horizontally or vertically transmitted, display similar surface structures to contact their hosts.

Symbionts that are exclusively transmitted through the vertical route resemble organelles more than beneficial symbionts or pathogens acquired from the environment. They lack genes for motility and environmental stress responses and, because they are enslaved by their hosts, they only retain genes that supply them with essential dietary requirements.

Our understanding of transmission paths does not yet extend to their causes. Theoretical evolutionary models could direct our attention to crucial factors linked with, or restricted to, one or the other mode of transmission. Moreover, they could help to identify future directions for symbiosis research. At the same time, we will greatly profit from integrating our knowledge on the immense diversity of symbioses and their countless transmission strategies into an ecological framework.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

<b>Pelagic dispersal</b>	The spreading of an organism, usually its larval stage, in the water column.
<b>Sporophyte</b>	The diploid generation in the life cycle of the hornwort, which develops from the zygote and produces spores.

<b>Thallus</b>	Undifferentiated vegetative tissue in hornworts that has a circular or ribbon-like arrangement.
<b>Hormogonium</b>	A short, motile filament that lacks heterocysts. Hormogonia provide a means of dispersal for otherwise immotile cyanobacteria.
<b>Flavonoid</b>	A 2-phenyl-1,4-benzopyrone derivative, produced by plants, that serves as a defence and signalling compound.
<b>Swarming motility</b>	Rapid and coordinated movement of a bacterial population across solid or semi-solid surfaces that is powered by numerous flagella.
<b>Indeterminate nodule</b>	A nodule formed by plants of some clades of legumes that develops a continuously growing nodule meristem at the distal end and has zones of tissue at different stages of development.
<b>Trophosome</b>	Symbiont-housing organ in siboglinid tubeworms, of mesodermal origin in Vestimentifera, and endodermal origin in Frenulata.
<b>Symbiosome</b>	Host membrane surrounding the symbionts
<b>Nurse cell</b>	A polytenic germline cell in insects that contributes to the development of the oocyte, producing the bulk of its cytoplasm and multiple nuclei.
<b>Bacteriome</b>	A specialized organ containing host cells (bacteriocytes), which house endosymbiotic bacteria.
<b>Ontogenetic stage</b>	Life cycle phases in the development from the fertilized egg to the adult.
<b>Mesohyl</b>	A proteinaceous gelatinous matrix between the epidermis and gastrodermis of cnidarians.
<b>Haemocoel</b>	The space between the organs through which haemolymph circulates in arthropods.
<b>Parthenogenetic egg</b>	An unfertilized egg that develops into a new individual.

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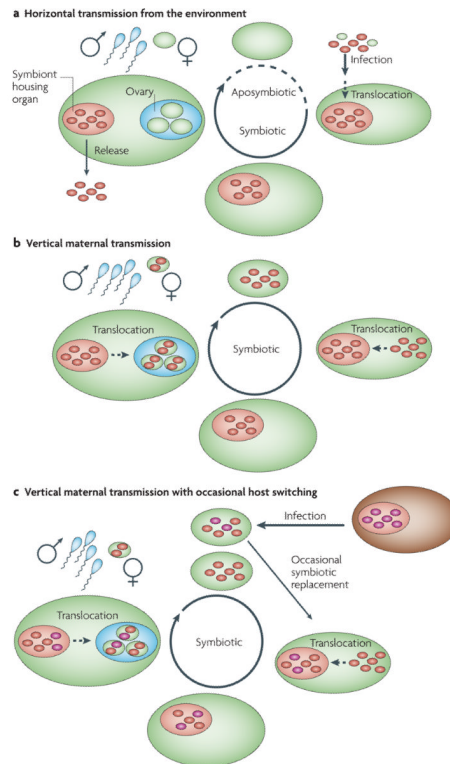


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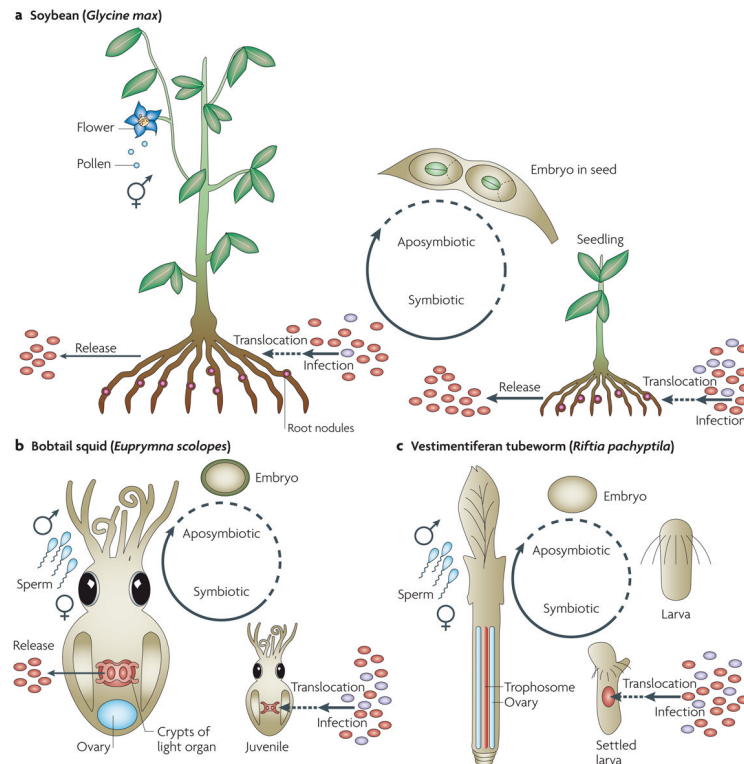
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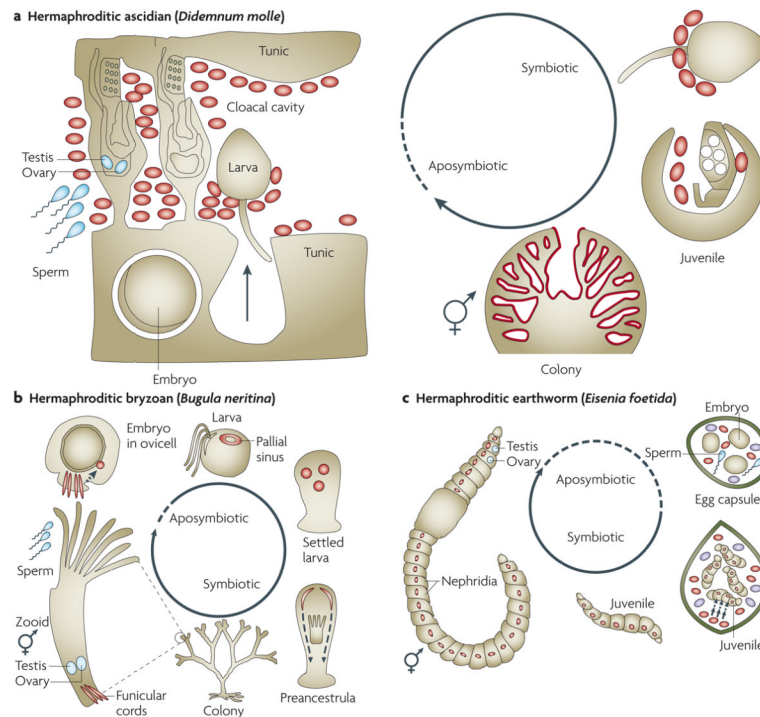
### Figure 1. Symbiont transmission pathways

**a** | Horizontal transmission from the environment. Host reproduction leads to aposymbiotic descendants, which at a certain life stage are infected with symbionts from the environment; often symbionts are translocated from the initial site of host contact to a putative symbiont housing organ; sometimes the environmental pool is replenished by symbiont release. **b** | Vertical transmission through the female germ line. Prior to host reproduction, symbionts are typically translocated from the symbiont housing organ to the female gonad, resulting in symbiotic descendants; often symbionts are then translocated from the colonization site to the symbiont housing organ. **c** | Mixed mode of transmission. Vertical transmission and occasional horizontal transmission can occur through host switching. Besides vertical transmission, occasional horizontal transfer of new symbionts (magenta) from a host population within the same species, but not the parent (intraspecific host switching; not shown), from a different host species (interspecific host switching; shown in brown) or from a free-living population (not shown) occurs.

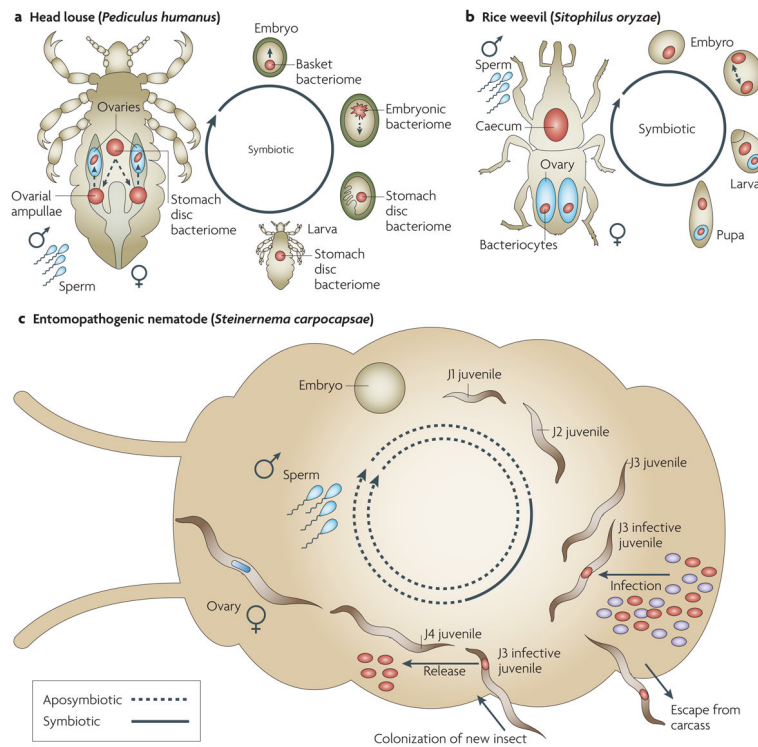


**Figure 2. Simplified host life cycles of horizontally transmitted symbionts**

**a** | Legumes and intracellular rhizobia in root nodules (the example shown is the soybean *Glycine max* and *Bradyrhizobium* spp.). Aposymbiotic germ cells are produced by the flower; internal fertilization leads to aposymbiotic seeds in which the embryo develops. After germination, symbiont uptake occurs through infection threads in the roots of seedlings, as long as the plant grows (reviewed in REF. <sup>11</sup>). **b** | The bobtail squid *Euprymna scolopes* and its extracellular endosymbiont *Vibrio fischeri* in the light organ crypts. Male and female hosts copulate, egg clutches are laid in the environment and they develop into aposymbiotic juveniles. Free-living *V. fischeri* is selectively taken up from the environment, and colonization of the light organ is completed within 12 hours after hatching (reviewed in REF. <sup>31</sup>). **c** | The vestimentiferan tubeworm *Riftia pachyptila* harbours endosymbiotic *Candidatus Endoriftia persephone* in the trophosome. Sperm are released from males and migrate to females, where eggs are fertilized internally; zygotes are released into the water column, disperse and develop to larvae that settle, and metamorphosis is initiated. Symbionts infect the larval skin, migrate to the mesoderm surrounding the gut, and the trophosome develops<sup>49</sup>. Environmental bacteria are shown in purple.



**Figure 3. Simplified host life cycles of vertically transmitted extracellular endosymbionts**  
**a** | In the colonial, hermaphroditic ascidian *Didemnum molle* the extracellular endosymbiont *Prochloron didemni* covers the tunic surface of the cloacal cavity. Sperm uptake into each zooid is followed by internal fertilization and embryonic development. The embryos migrate into the tunic and develop into larvae. When the larvae hatch into the cloacal cavity, they pick up the symbiont onto the posterior part of the body; larvae are then released from the cavity into the water column, settle after dispersal and undergo metamorphosis, during which the symbiont finally ends up on the surface of the cloacal cavity again<sup>88</sup>. **b** | The colonial, hermaphroditic bryozoan *Bugula neritina* harbours the extracellular endosymbiont *Candidatus Endobugula sertula* in the funicular cords of adult colonies and the pallial sinus of larvae. Sperm uptake and internal fertilization result in transfer of the zygote into the ovicell, in which the endosymbiont is most likely taken up through the funicular cords during embryonic development (not shown). The embryo develops into larvae which are then released and can disperse and settle. Following metamorphosis into preancestrula and ancestrula stages (first zooid), the symbionts migrate and end up in the funicular cords of the developing colony again<sup>94</sup>. **c** | The hermaphroditic earthworm *Eisenia foetida* harbours the extracellular endosymbiont *Verminephrobacter eiseniae* in the lumen of nephridial ampullae. During copulation and release of eggs and sperm, symbionts and albumin are released into the egg capsule, which is concurrently also infected with environmental bacteria (shown in purple). The egg is externally fertilized and develops into a juvenile in the egg capsule. During this time the symbionts are selected and taken up through a pore, they migrate through a canal to the ventral pore that leads into the nephridia, where they establish in the ampullae. The juveniles then hatch and develop into adults<sup>113, 143</sup>.



**Figure 4. Simplified host life cycles of vertically or pseudoverteally transmitted intracellular endosymbionts**

**a** | The head louse *Pediculus humanus* harbours the intracellular *Candidatus* *Riesia pediculicola* in the stomach disc and ovarial ampullae. Male and female lice reproduce through copulation and internal fertilization. The symbionts colonize the eggs through hydrophyles in eggshells and reside extracellularly in the periplasm. Individual eggs are laid in which embryonic development proceeds until the first instar nymphs hatch. During this development the bacteriome goes through three maturation stages (embryonic basket bacteriome with extracellular symbionts, embryonic bacteriome with intracellular symbionts and stomach disc bacteriome). After hatching and the development of two further instar nymph stages, in females symbionts are released and migrate to the oviducts to build a new bacteriome in the ovarial ampullae; from there, oocytes are infected and the male stomach disc bacteriome degenerates<sup>98,99,144</sup>. **b** | The rice weevil *Sitophilus oryzae* principal symbiont (SOPE) resides in the caeca and ovaries in females and in the caeca in males. Males and females reproduce through copulation and internal fertilization. Eggs containing endosymbionts are laid and sealed with a gelatinous plug. During early embryonic development, the SOPE population is split into two parts, one becoming associated with the primordial germ cells, which later constitute the intraovarian bacteriocytes that infect the next generation oocytes, and the other associates with the future bacteriome. Larvae develop, moult four times, pupate, and adults hatch; in young females symbionts in the caeca are reduced by unknown mechanisms so that in older females symbionts are found only in the ovaries<sup>145</sup>. **c** | Pseudoverteal transmission of the entomopathogenic nematode *Steinernema carpocapsae* and its symbiont, *Xenorhabdus nematophila*. The male and female reproduce through copulation and internal fertilization, and the embryo develops indirectly, with four juvenile stages. Some juveniles that develop into the infective stage are colonized by symbionts entering the mouth and migrating through the pharynx to the vesicle (the gut lumen between two most anterior intestinal cells). Infective juveniles infected by the bacteria leave the insect cadaver and find a new host. Symbionts are released from the



vesicle into a new insect host by passing through the intestine into the insect blood, where they reproduce and kill the insect. Symbionts within an insect cadaver originate from the infected nematode population; it is unknown how many nematodes infect a single insect (reviewed in REFS <sup>21, 146</sup>). This transmission mode has been termed pseudovertical because more than one nematode can be found within a single insect, and therefore the symbionts transmitted to the nematode progeny could come from the parent (vertical) or from the co-occurring nematodes in the insect (host switching). Environmental bacteria are shown in purple.