Review

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Author for correspondence:

Spencer V. Nyholm

e-mail: spencer.nyholm@uconn.edu

In the beginning: egg–microbe interactions and consequences for animal hosts

Spencer V. Nyholm

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269 USA

SVN, 0000-0001-5597-1945

Microorganisms are associated with the eggs of many animals. For some hosts, the egg serves as the ideal environment for the vertical transmission of beneficial symbionts between generations, while some bacteria use the egg to parasitize their hosts. In a number of animal groups, egg microbiomes often perform other essential functions. The eggs of aquatic and some terrestrial animals are especially susceptible to fouling and disease since they are exposed to high densities of microorganisms. To overcome this challenge, some hosts form beneficial associations with microorganisms, directly incorporating microbes and/or microbial products on or in their eggs to inhibit pathogens and biofouling. Other functional roles for egg-associated microbiomes are hypothesized to involve oxygen and nutrient acquisition. Although some egg-associated microbiomes are correlated with increased host fitness and are essential for successful development, the mechanisms that lead to such outcomes are often not well understood. This review article will discuss different functions of egg microbiomes and how these associations have influenced the biology and evolution of animal hosts.

This article is part of the theme issue ‘The role of the microbiome in host evolution’.

1. Introduction

Microorganisms have had a profound influence on the development and evolution of animals and plants [1]. A number of animal model systems are currently being studied in order to understand how symbiotic associations are established and maintained and the effects of microbial colonization on developmental processes [2]. Microbiota impact their hosts in many ways, including influencing proper development of the digestive and immune systems, supporting nutrition, and providing unique metabolic capabilities [1]. Many studies have focused on how symbioses influence the long-term development and health of animals and the molecular mechanisms by which these associations are established, but for some hosts, microbes can have significant impacts from the earliest stages of development, in the egg (figure 1).

The origin of eggs was a critical step in animal evolution, possibly to facilitate internal self-organizational processes to allow differentiation of cells and tissues [3]. Since the egg serves as such an essential site of embryogenesis, it may seem counterintuitive that some animals form associations with microorganisms in such a critical microenvironment. Although symbiont associations with eggs have been mostly studied in the context of vertical transmission processes between host generations, egg microbiota do serve other roles. This review article will focus on some of the functional roles of egg microbiomes and discuss how studying such associations may reveal ways in which these microbiota have influenced the evolution of animals.

Figure 1. Examples of functions associated with egg microbiomes from different animal groups. Egg-associated microbes are linked to diverse interactions in different hosts. In some associations, the egg facilitates transmission of symbionts between generations. Egg microbiota can provide antimicrobial defence from fouling and/or pathogenic microbes, protection from predation, and detoxification, and play nutritional or other metabolic roles. Some egg microbiomes are correlated with improved host development or fecundity, although the mechanisms are not well understood. In other systems, egg microbiota have been described but functional roles remain unknown.

2. Functional roles of egg microbiomes

(a) Facilitating transmission between generations and developmental effects on hosts

Many animals that foster endosymbionts use vertical transmission via the egg as a mechanism to ensure the transfer of microorganisms that often perform critical functions for the host. This transmission of symbionts occurs either in the egg (transovarial), on or near eggs, or directly associated with larvae that are released into the environment [4,5]. For many intracellular endosymbiotic associations, vertical transmission inside the egg ensures successful transfer of symbionts that provide critical nutritional functions for their hosts. Some examples include the bacteria *Buchnera aphidicola*, which provide essential amino acids for pea aphids [6], the photosynthetic dinoflagellate *Symbiodinium* in some coral species [7,8] and the sulfur-oxidizing bacteria associated with the gills of vesicomyid and solemyid clams at hydrothermal vents and seeps, respectively [9,10]. Other animals transfer symbionts through the colonization of embryos that are deposited with eggs. Adult *Eisenia* earthworms deposit *Verminephrobacter* and a small consortium of other bacteria into egg capsules where they then colonize extracellular spaces of developing nephridia in embryos [11–13].

For some associations, the symbiont may influence the developmental process in the host to specifically facilitate transmission. For example, stinkbugs use a number of strategies to transfer gut symbionts vertically between generations. In *Megacopta punctatissima*, the behaviour of nymphs can be influenced by the numbers of midgut symbionts ('*Candidatus* Ishikawaella capsulata') that are successfully transmitted from capsules that are associated with egg masses [14,15]. Nymphs that have sufficient numbers of symbionts enter a quiescent stage while those with low numbers or no symbionts are active and 'wander', presumably as an adaptation to facilitate colonization. Cynid stinkbugs of the genus *Adomerus* engage in maternal care where females smear symbiont-containing secretions onto

eggs that help transfer symbionts. Aposymbiotic stinkbugs generated by surface sterilization of eggs resulted in animals with several fitness defects [5]. A study of pentaomid stinkbugs from Costa Rica found that in *Sibaria englemanni*, midgut symbionts are transferred via egg smearing of the chorion during oviposition, and nymphs from surface-sterilized eggs had delayed developmental responses in growth and caeca morphology [16]. The authors also proposed that egg-smearing strategies in insects might allow the introduction of other bacteria or symbiont replacement from the environment.

The water flea *Daphnia magna* has served as a model crustacean for ecology and more recently has been studied in the context of its microbiome. *Daphnia* are capable of generating eggs via parthenogenesis that can then be sampled and are amenable to experimental manipulation. *Daphnia* eggs may also have a resting state where they are covered in a thick case called an ephippium that allows them to remain in the environment for extended periods of time (up to years) and under stressful conditions (e.g. extreme desiccation and anoxia). A study that manipulated surface bacteria from *Daphnia* eggs using chemical or antibiotic treatment led to decreased fecundity in animals raised bacteria-free [17]. *Daphnia* often contain bacteria within their eggs, but surprisingly, environmental epibiotic bacteria associated with the surface of the egg appear necessary for proper development and reproduction of the host [18,19]. How these environmental bacteria positively influence *Daphnia* is unknown but it has been proposed that they may benefit the host during variable environmental conditions.

Among vertebrates, vertical transmission of symbionts may also be important in some cases. A recent study of four bird species and a lizard species used culture-independent techniques to detect *in ovo* microbiota that the authors hypothesize may serve as a reservoir for the vertical transmission of gut symbionts in some oviparous vertebrates [20], while a study of woodlarks and skylarks did not find evidence for transmission of gut bacteria from egg

shell surfaces [21]. A number of fish species are known to engage in egg–bacteria interactions during embryogenesis. Spawning brown trout have a core microbiome dominated by *Flavobacterium* and host genetics appears to influence composition of the egg microbiota [22,23]. Although a function for the egg microbiome has not been demonstrated, a more diverse microbiota was correlated with longer developmental times [23]. In coho salmon, epibiotic bacteria associated with eggs may be involved with later gastrointestinal colonization after hatching [24]. Syngathid fishes (pipefish and sea horses) are known for evolving male pregnancy where eggs are transferred to males and are carried in paternal brood pouches. Analyses of the microbiome of the maternal ovaries, eggs and paternal brood pouch of the pipefish *Syngnathus typhle* identified distinct microbiomes in ovaries and eggs that shifted in late stage embryos in the paternal pouch [25]. The authors suggest that immune priming may help shape the brood pouch microbiome and have long-term developmental consequences in preventing infection by pathogens. In other species, pathogens may also cause a number of developmental abnormalities or kill animal eggs, but in the red-eyed tree frog, fungal infections can accelerate development, leading to early hatching of and better survival outcomes for tadpoles [26].

Some endosymbiotic bacteria are also known as reproductive parasites of arthropods and use vertical transmission to ensure successful infection of animal hosts [27]. Among the best studied of these are *Wolbachia* bacteria, which are transmitted in the egg and can have profound developmental effects on their hosts [28]. *Wolbachia* spp. use different strategies *in ovo* to promote successful maternal inheritance between generations and manipulate host biology. These strategies include feminization, parthenogenesis, male killing, and cytoplasmic incompatibility [29]. Other types of bacteria in this category are members of the *Spiroplasma* spp., which are vertically transmitted and can lead to both positive and negative outcomes in different insect hosts [30]. Like other reproductive parasites, some strains cause male killing in their hosts [31,32]. The development of robust experimental host models such as *Drosophila melanogaster* has led to a number of key advances in recent years that have shed light on the molecular mechanisms of cytoplasmic incompatibility [33] and male killing [34] along with ways in which *Wolbachia* and *Spiroplasma* use host cellular machinery to facilitate transmission through oocytes (e.g. manipulating host actin, microtubules and yolk uptake machinery) [35–37].

(b) Defensive symbioses

In some animals, symbionts also serve important defensive roles in the egg in order to protect developing embryos. This can be especially important for animals that lay eggs in environments where microorganisms are abundant (e.g. 10^6 bacteria ml^{-1} in coastal seawater to 10^{10} bacteria g^{-1} in terrestrial soil and marine sediments [38]). One of the challenges that many animals that lay their eggs in microbe-rich environments face is that successful embryogenesis depends on minimizing fouling by microorganisms and/or infection by pathogens [39]. Given that embryogenesis can take days to weeks, mechanisms must exist to prevent biofoulers and pathogens that may harm developing embryos. To overcome

these threats, some animals use the help of beneficial microorganisms that are associated with eggs [39–43].

In terrestrial environments, a number of insect species use defensive symbioses to provide protection of eggs from potential pathogenic microorganisms associated with soil deposition [43]. *Lagria* beetles have accessory glands containing *Burkholderia* bacteria that are deposited on the surfaces of eggs, where they produce antimicrobial compounds that protect larvae from fungal infections [44,45]. The European beewolf digger wasp, *Philanthus triangulum*, incorporates a cocktail of antimicrobial compounds derived from *Streptomyces* bacteria into its cocoon to protect larvae from fungal and bacterial infections during a long developmental period that can take up to 9 months [46–48]. Bacteria associated with the surface of eggs of the housefly (*Musca domestica*) can suppress growth of fungi that may outcompete housefly embryos for nutrition in dung piles [49,50]. Bacteria can have antipredatory effects on insect eggs as well. In *Paederus* sp. beetles, *Pseudomonas* bacteria produce a toxin (the polyketide pederin) that prevents predation from wolf spiders. A study using fluorescence *in situ* hybridization showed that female *Paederus riparius* smear a biofilm of the symbiont around the egg during oviposition [51]. Larvae that hatch from eggs that lack pederin are more susceptible to predation [52,53]. Although not extensively studied, egg microbiomes have also been described for aquatic insects of the Chironomidae (chironomids) where bacteria associated with egg masses may protect larvae from heavy metals [54] while also serving as a probiotic for emerging larvae, which feed on the egg jelly after hatching [55].

Many crustaceans brood their young, and females can carry eggs for extended periods of time during embryogenesis. A few studies have analysed these eggs for associated microbiota and a functional role in egg defence. In the American lobster, *Homarus americanus*, gravid females brood their eggs on their exteriors for an extended period of 9–16 months. With such a prolonged embryogenesis, females may lose 30–50% of their egg clutch to fouling and disease, including to the fungal pathogen *Lagenidium callinectes* [56]. The eggs of *H. americanus* are thought to be partially protected by the production of 4-hydroxyphenethyl alcohol (tyrosol) from unidentified bacteria associated with the eggs [57]. The eggs of the brooding shrimp *Palaemon macrodactylus* are protected from fungal infection by *Alteromonas* bacteria through the production of 2,3-indolinedione (isatin), which inhibits *L. callinectes* [42], while epibiotic bacteria associated with the eggs of the sand shrimp *Crangon septemspinosa* protect against a different fungus, *Lagenidium myophilium* [58].

Among cephalopods, some squid and cuttlefish species harbour a bacterial consortium in the accessory nidamental gland (ANG), part of the female reproductive system [59–62]. The bacterial consortium is deposited into the jelly coat of egg cases before being laid in the environment, where embryos develop for weeks without parental care. Although the ANG symbiosis was first described over a hundred years ago [63], few studies have addressed the function of the association. Work in some species suggests that the symbionts may protect developing embryos from pathogens and fouling microorganisms [41,64]. The Hawaiian bobtail squid, *Euprymna scolopes*, is a model animal host for studying interactions with beneficial bacteria [65]. Numerous studies have focused on understanding the mechanisms that confer specificity during the environmental transmission of the

light organ symbiont *Vibrio fischeri* [66,67]. Recent work has also investigated the ANG consortium, describing the bacterial community and its function [41,61,68–70]. In *E. scolopes*, the ANG contains mainly Alphaproteobacteria and Verrucomicrobia, along with members of the Gammaproteobacteria and Flavobacteriia [61,69]. Bacteria from the ANG are deposited into multiple egg jelly layers, which are separated by a membrane-like structure [61,69]. Symbiont community analysis revealed that the ANG bacterial consortium of a given female bobtail squid reflects the eggs of its clutches, adding evidence that bacteria found in eggs come from the ANG, although females must acquire symbionts from the environment each generation [69]. A recent study also demonstrated that the ANG consortium protects eggs from fungi in *E. scolopes* [41]. Eggs treated with antibiotics were fouled by the fungus *Fusarium keratoplasticum*, and cultured ANG strains and chemical extracts were able to inhibit three different strains of *F. keratoplasticum* as well as the yeast *Candida albicans*. Molecular network analysis also identified antimicrobial compounds that may be involved with egg defence, including lincomycin, mycinamycin-like compounds and a suite of glycerophosphocholines. In addition to fungi, ANG bacteria can also inhibit other bacteria. *Leisingera* sp. JC1, a symbiotic strain isolated from the eggs of *E. scolopes*, produces a compound, indigoidine, and has antimicrobial activity against a number of marine vibrios [71]. Antibacterial activity has also been described from ANG symbionts of the eastern longfin inshore squid, *Doryteuthis pealei* [72].

Among vertebrates, the defensive role of egg microbiomes has been studied in some bird and fish species. In hoopoe birds, bacteria from the uropygial gland are transferred in a secretion to specialized crypts on the surfaces of eggs during oviposition, where they are hypothesized to protect embryos from infection [73,74]. In lake sturgeon, predictable bacterial communities colonize the surfaces of eggs and have the ability to inhibit other bacteria, including the formation of biofilms [75,76]. Infection of fish eggs by pathogens is of growing concern in aquaculture. An analysis of eggs from the Atlantic salmon at fish farms in Scotland showed that the presence of actinobacterial species on salmon eggs decreased attachment of the oomycete fungal-like pathogen *Saprolegnia*, and actinobacterial isolates also inhibited the pathogen *in vitro* [77].

(c) Nutritional and metabolic roles

Even though many symbionts that are important for the nutritional requirements of adult host stages are passed through the egg to the next generation (see above), data from a few systems suggest egg microbiomes may also contribute to have nutritional or metabolic roles embryos. For example, bacteria associated with the eggs of *Drosophila melanogaster* are responsible for producing the fermentation product acetoin, which can then have behavioural effects on larval food preference [78]. An analysis of the microbiome of different body sites and developmental stages of the deep-sea yeti crab, *Kiwa puravida*, found a diverse bacterial community associated with eggs, some members of which were also found in juvenile and adult stages, with a developmental shift from Gammaproteobacteria to Epsilonproteobacteria [79]. Examination of eggs associated with the hydrothermal vent shrimp *Rimicaris exoculata* also

showed a similar shift in the microbiota [80]. In *R. exoculata*, bacterial abundance increased on egg surfaces during embryogenesis, and environmental recruitment of symbiotic lineages of bacteria associated with adult stages has been described [81]. For both the yeti crab and vent shrimp, the egg microbiota include methanotrophic bacteria, although whether there are metabolic or other functional roles for members of these egg communities is still unclear.

The yellow-spotted salamander, *Ambystoma maculatum*, forms a symbiotic association with the photosynthetic alga *Oophila amblystomatis*. Female salamanders deposit their eggs during the springtime in parts of North America in shallow pools. Within hours after deposition, eggs are colonized by *O. amblystomatis*, which enters the jelly layers of the eggs. These intracapsular algae quickly divide in the egg and are capable of providing oxygen to the embryos, where diffusion is limited [82]. Eggs with more algae promoted quicker embryo growth and development, and it has been proposed that fixed carbon is transported to the embryos [83,84]. If algae are removed from the eggs, embryo development is negatively impacted [85]. The algae engaged in this ectosymbiosis appear to benefit from nitrogenous compounds excreted by the host [83,86]. Some of these algae are also capable of invading embryonic tissues, the only known example of intracellular algae colonizing a vertebrate [87]. A transcriptomic study of host cells with and without the algae suggests that these intracellular algae are not engaged in photosynthesis and instead are undergoing fermentation [88,89]. An analysis of innate immune gene expression also suggests that some aspects of the host immune response may be attenuated in cells that contain *O. amblystomatis*, perhaps to help facilitate tolerance/maintenance of these endosymbionts or to prime the immune system. Although there is evidence that some vertical transmission may occur between *O. amblystomatis* and *A. maculatum* [87], it is unclear how frequently these events happen.

3. Future questions

Although many strides have been made in understanding how microbes influence the biology of animals during development and contribute to health and disease, less is understood about the functions that microbes play in eggs during embryogenesis. Several exciting areas of research may come from studying egg microbiomes. For example, in many systems it is unclear to what extent embryo exposure to microbes or microbial products influences later developmental processes and host outcomes, although these mechanisms are better understood in the reproductive parasites of insects [33,34,90]. Interactions between eukaryotic hosts and microorganisms are often mediated via pattern recognition receptors that interact with microbe-associated molecular patterns (MAMPs) [91]. Host interactions with MAMPs can have a major influence on developmental processes. For example, in the squid–vibrio symbiosis, exposure of the host to a derivative of bacterial peptidoglycan (tracheal cytotoxin) along with lipopolysaccharide (LPS) leads to apoptosis and regression of a superficial ciliated epithelium that is involved with recruitment of the light organ symbiont *V. fischeri* [92,93]. In many animal eggs, embryos have a chorion that may prevent microbes from directly interacting with host cells. However, MAMPs, small microbial metabolites and viruses may still be able to reach the

embryo. There are several examples of MAMPs and bacterial metabolites entering eukaryotic cells and tissues and influencing organs or cells that are removed from the primary location of those microbes (e.g. short chain fatty acids produced by bacteria in the mammalian gut [94]). In humans, a number of pathogens are capable of causing congenital disease by breaching the placental barrier, including Zika virus, HIV, *Toxoplasmosis gondii* and *Listeria monocytogenes* [95]. Although not egg-associated, these examples from mammalian pathogens demonstrate that microbes are able to breach host embryonic barriers that normally would protect developmental processes.

Another open question is how do eggs/hosts select for functional symbionts while preventing non-specific microbes from colonization? For animals that have dedicated organs for housing symbionts that contribute to egg deposition, the mechanisms may be more easily characterized, especially in systems in which the partners can be manipulated, e.g. in *Lagria* beetles and cephalopod ANGs. For many associations, recruitment of bacterial symbionts from the environment relies on specific host–symbiont signalling, as with *Rhizobia* bacteria and leguminous plants [96]. In other associations, instead of a single ‘lock and key’ mechanism, multifactorial host and symbiont factors help ensure specificity. In the well-studied squid–vibrio association, mechanisms that lead to successful colonization of the light organ include a combination of symbiont factors (e.g. biofilm formation, motility, chemotaxis, responses to oxidative stress) and host processes (e.g. mucus secretion, immune system interactions, biochemical and biomechanical processes) [66,67]. Understanding the molecular mechanisms that contribute to specificity of symbionts in eggs may also reveal why egg microbiomes are more prevalent in some animal lineages. For example, endosymbiosis is a widely adopted strategy among insects and has contributed to the evolutionary success of this group [97]. Successful transfer of symbionts from somatic cells to the germline is critical for the host. Studying how endosymbionts are transferred from somatic cells such as bacteriocytes to oocytes has revealed different mechanisms among insects, including symbiont exocytosis into the oocyte [98] as well as whole bacteriocyte transfer [99], the latter of which includes dramatic changes in cellular behaviour and gene expression patterns [100]. Do similar

mechanisms exist in other animal lineages that have endosymbiotic bacteria and vertical transmission, for example, in corals and chemosynthetic invertebrates? Development of experimental model systems in diverse hosts will hopefully allow such comparisons. For eggs with epibionts where the symbionts are recruited directly from the environment, the mechanisms of egg colonization are less clear. Perhaps, as has been suggested for the egg microbiome of *Daphnia*, colonization by environmental microbes instead of vertical transmission from mother to egg provides *Daphnia* more flexibility to select from a diverse reservoir of bacteria, which allows the host to adapt to variable environmental conditions [18,19].

Many of the associations discussed here demonstrate how selection pressures on both hosts and symbionts have likely led to egg microbiomes that produce antimicrobial products or provide other benefits for their hosts. The wide distribution of defensive symbioses associated with eggs suggests that this is a function that has evolved multiple times in animals. As more defensive symbioses are described from terrestrial and aquatic environments, it is likely that a broader diversity of microbes and compounds that are used for egg defence will be revealed. However, there may be a number of other functions for egg microbiomes that have yet to be discovered. In associations where transfer of beneficial symbionts to the juvenile and adult stages improves developmental outcomes for the host (e.g. greater growth, fecundity, and/or disease resistance), the mechanisms by which these symbionts confer these traits are often poorly understood. In recent years, technological advances have allowed the characterization of host-associated microbiomes (e.g. 16S rRNA community analysis and multi-omics techniques). Applying such methods coupled with the development of more diverse experimentally tractable model associations will help reveal the range of egg–microbiome interactions and the influence of these symbioses on animal biology and evolution.

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