# Microbiome reduction and endosymbiont gain from a switch in sea urchin life history

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**Animal gastrointestinal tracts harbor a microbiome that is integral to host function, yet species from diverse phyla have evolved a reduced digestive system or lost it completely. Whether such changes are associated with alterations in the diversity and/or abundance of the microbiome remains an untested hypothesis in evolutionary symbiosis. Here, using the life history transition from planktotrophy (feeding) to lecithotrophy (nonfeeding) in the sea urchin *Heliocidaris*, we demonstrate that the lack of a functional gut corresponds with a reduction in microbial community diversity and abundance as well as the association with a diet-specific microbiome. We also determine that the lecithotroph vertically transmits a Rickettsiales that may complement host nutrition through amino acid biosynthesis and influence host reproduction. Our results indicate that the evolutionary loss of a functional gut correlates with a reduction in the microbiome and the association with an endosymbiont. Symbiotic transitions can therefore accompany life history transitions in the evolution of developmental strategies.**

Heliocidaris | development | animal–microbe | symbiosis | Rickettsiales

Animal gastrointestinal tracts contain microbial communities that are integral to host metabolism, immunity, and development (1, 2). Symbioses between animals and their gut microbiome have deep evolutionary origins (1, 2), often exhibit phylosymbiosis (3), and can serve as a physiological buffer to heterogeneous environments (2). Despite the necessity of the gastrointestinal tract and benefits of the gut microbiome (3), species in various phyla have lost a functional digestive system (4, 5). Loss of a functional gut should, in theory, cascade into a reduction in microbial diversity and the loss of diet-induced shifts in microbiome composition. These nutritional shifts may then provide a niche for functionally important endosymbionts, such as the chemoautotrophic bacteria commonly associated with gutless invertebrates (6, 7).

Major life history transitions are driven by tradeoffs in reproduction and development that, in turn, impact fitness (8). These tradeoffs are particularly evident in benthic marine invertebrates whose developmental stages broadly group into two alternative nutritional strategies (4, 9). The first—planktotrophy—typically includes the production of a high number of small, energy-poor eggs that develop into larvae with feeding structures used to collect and process exogenous resources required to reach metamorphic competency (4, 9). The second—lecithotrophy—involves the production of fewer large, energy-rich eggs and nonfeeding larvae that undergo metamorphosis without the requirement of external nutrients through feeding (4, 9). Life history transitions between these developmental modes have occurred in several major animal lineages, with rapid evolutionary shifts from planktotrophy to lecithotrophy being well documented in echinoderms (4, 5, 10–13). It is thought that an increase in the eggs energetic content relaxes the selective pressure maintaining the feeding structures (e.g., the larval arms and a functional gastrointestinal tract) and that development to metamorphosis is accelerated once these are lost (5).

One of the most comprehensively studied systems for life history transitions among marine invertebrates involves species in the sea urchin genus *Heliocidaris*. A speciation event ~5 Mya resulted in two sister species with alternative life history strategies: *Heliocidaris tuberculata* is planktotrophic while *Heliocidaris erythrogramma* is lecithotrophic (14). Typical of planktotrophs, *H. tuberculata* develops from small eggs into feeding larvae that exhibit morphological plasticity in response to food limitation (15), which is correlated with compositional shifts in the microbiome (16, 17). *H. erythrogramma*, on the other hand, develops from eggs ~53× to 86× the volume of *H. tuberculata* (18), lacks the morphological structures required for feeding, and has a reduced, nonfunctional digestive tract (11). This life history switch and heterochronic shift in development (11) corresponds with a rewiring of the gene regulatory network (19), reorganization of cell fates (20), and modification to gametogenesis (21).

Here, we compare the bacterial communities of these *Heliocidaris* species and test two hypotheses. First, we test whether the loss of gut function coincides with a reduction in microbial

## Significance

**Microbes have a strong impact on the biology of their host, with those living in the gut being essential to immunity, development, and metabolism. A functional gut, however, has been lost several times during animal evolution. Here, using sister sea urchin species, we report that the loss of a functional gut corresponds with a reduced microbial diversity and abundance. Gut loss also coincides with associating with an endosymbiont that complements host nutrition and potentially impacts host reproduction. Therefore, transitions in developmental life histories in animals can accompany shifts in the microbial community.**

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symbiont diversity, and second, by simulating the natural range in food availability, we also test that the loss in gut function coincides with a loss in diet-related shifts in the microbiome. We report major reductions in microbiome diversity and abundance as well as the absence of bacterial communities correlated with food availability for the lecithotrophic *H. erythrogramma*. Moreover, we find that this species vertically transmits a Rickettsiales that encodes pathways for the biosynthesis of essential amino acids, proteins with pivotal roles in host reproduction, and enzymes to metabolize diacylglycerol ethers, the major lipid group responsible for the increase in egg size in *H. erythrogramma* and that is used to fuel growth and development (18, 22).

## Results

**Reduction in Microbial Community Diversity.** We qualitatively and quantitatively compared the microbiome of both *Heliocidaris* sister species to test whether lecithotrophic developers associate with a bacterial community that is less diverse and less abundant than planktotrophic developers. Consistent with other echinoderm larvae (16, 23), the bacterial communities of *H. tuberculata* and *H. erythrogramma* are species-specific and are distinct from the seawater microbiome throughout development (permutational multivariate analysis of variance [PERMANOVA], unweighted and weighted UniFrac, $P < 0.001$; *SI Appendix*, Fig. S1 and Table S1).

The bacterial community of *H. tuberculata* was significantly different from and, on average, more than three times as diverse in total bacterial taxa (ANOVA, $P < 0.0001$) and phylogenetic diversity (ANOVA, $P < 0.0001$) than *H. erythrogramma* (Fig. 1*A* and *SI Appendix*, Table S2). Moreover, the bacterial community of *H. tuberculata* had ~13× more copies of the 16S ribosomal RNA (rRNA) gene than that of *H. erythrogramma* (*t* test, $P < 0.0001$; Fig. 1*A* and *SI Appendix*, Table S3). The bacterial community of *H. tuberculata* was also more taxonomically dominant (ANOVA, $P < 0.0001$) and, thus, less even (ANOVA, $P < 0.0001$) than that of *H. erythrogramma* (*SI Appendix*, Fig. S1 and Table S2).

Despite differences in community composition and structure (Fig. 1 and *SI Appendix*, Fig. S1), *H. tuberculata* and *H. erythrogramma* shared 38 amplicon sequence variants (ASVs), with 263 and 55 ASVs being specific to *H. tuberculata* and *H. erythrogramma*, respectively (*SI Appendix*, Fig. S1). A majority of these bacteria were α- and γ-proteobacteria or Bacteroidia, with the α- and γ-proteobacteria being significantly different and inversely abundant between the two *Heliocidaris* species (*t* test, α: $P = 0.014$, γ: $P = 0.051$; *SI Appendix*, Fig. S2 and Table S4).

**Loss of a Diet-Specific Microbiome.** Planktotrophic larvae from a number of echinoderm species are plastic in their morphology (24) and microbiome (16) in response to natural variation in food availability. To test whether the ability to associate with a diet-specific microbiome was lost during the transition to lecithotrophy, we compared the bacterial communities for the *Heliocidaris* species reared in different phytoplankton (i.e., food) environments through development. As previously reported (15), *H. tuberculata* exhibited morphological plasticity in response to food availability (multivariate analysis of variance, $P < 0.0001$), where diet-restricted larvae increased the length of their postoral (feeding) arms relative to the larval body and also reduced their stomach volume (*SI Appendix*, Fig. S3 and Table S5).

The onset and expression of morphological plasticity in response to food availability for *H. tuberculata* also resulted in compositional differences in the bacterial community (PERMANOVA, weighted UniFrac, $P < 0.05$; Fig. 1*B* and *SI Appendix*, Tables S6 and S7). This response, however, was not observed for *H. erythrogramma*, as these developmental stages associated with bacterial communities that were similar between phytoplankton treatments

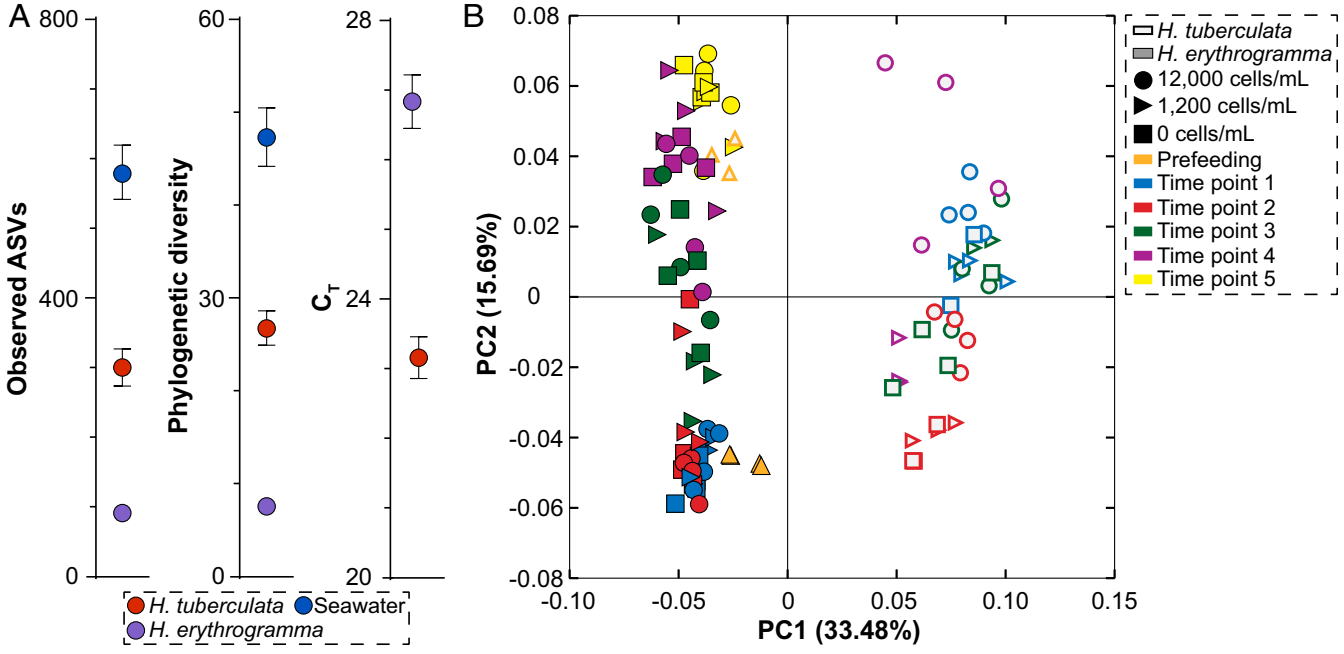


**Fig. 1.** Differential structuring of the microbiome between life histories. (*A*) Enumeration of ASVs, phylogenetic diversity of those ASVs, and $C_T$ values estimating the abundance of 16S rRNA gene copies between *Heliocidaris tuberculata* (dark red), *H. erythrogramma* (purple), and the seawater (blue) microbiome. Both alpha diversity indices ($n = 40$ for *H. tuberculata* and $n = 64$ for *H. erythrogramma*; i.e., all samples across treatments and time points) and $C_T$ ($n = 40$ per species) represent average values (±SE). (*B*) Community similarity of the larval-associated microbiome, as estimated by weighted UniFrac for composition, for both *Heliocidaris* species based on food availability (12,000, 1,200, and 0 cells per milliliter of a phytoplankton represented by a circle, rightward triangle, and square, respectively) and over several time points (times 1, 2, 3, 4, and 5 were represented by blue, red, green, purple, and yellow colorings).

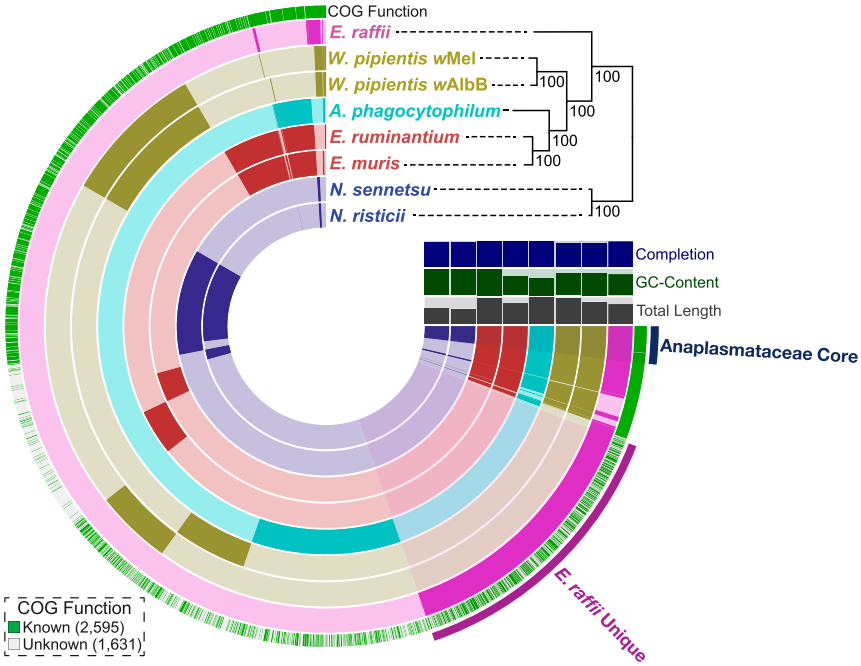


**Fig. 2.** Gene content and phylogeny of *Echinorickettsia raffii* within Rickettsiales. Full genome comparison of *E. raffii* to several members of the Anaplasmataceae with statistics for total length of the genome, guanine-cytosine content, and completion percentage. This comparison identified 51 core gene clusters ("Anaplasmataceae Core") and 799 gene clusters unique to *E. raffii* ("*E. raffii* Unique"). This Anaplasmataceae Core served as the basis for a phylogenetic analysis that placed *E. raffii* in the clade encompassing *Wolbachia*, *Ehrlichia*, and *Anaplasma*.



(PERMANOVA, unweighted UniFrac, $P > 0.05$; PERMANOVA, weighted UniFrac, $P > 0.05$; Fig. 1*B* and *SI Appendix*, Fig. S4 and Tables S6 and S7).

Microbial community structure varied across developmental time for both *Heliocidaris* species (ANOVA, total ASVs, and phylogenetic diversity: $P < 0.001$; *SI Appendix*, Fig. S5 and Table S8), with each species undergoing different temporal successions. The bacterial communities of *H. tuberculata* were taxonomically and phylogenetically rich in early stages and fluctuated over developmental time, while *H. erythrogramma* eggs were dominated by a single ASV (representing 95.1% of the community) and then associated with a more diverse bacterial community later in development (*SI Appendix*, Fig. S5 and Table S8).

The richness of the bacterial community associated with sea urchin eggs was inversely proportional to egg size (linear regression, $P < 0.0001$; $R^2 = 0.808$; *SI Appendix*, Fig. S6 and Table S9). Therefore, the diversity of *H. tuberculata* was similar to the eggs of previously studied planktotrophic sea urchins, while the dominance by a single ASV in *H. erythrogramma* eggs was unique (*SI Appendix*, Fig. S6). This ASV associated with *H. erythrogramma* throughout development and was identified as a member of the Rickettsiales, a bacterial order rich with endosymbionts that influence animal biology across the tree of life (25). It was not present on *H. tuberculata* eggs but was present at low abundance (<0.27%) in *H. tuberculata* larvae and the surrounding seawater.

**Identification of a Rickettsiales Lineage.** In consideration of the functional relevance of Rickettsiales to host reproduction in other animals (25), we sequenced the metagenome of *H. erythrogramma* eggs to generate a bacterial genome for this dominate ASV. Following the trimming and filtering of host sequences based on an *H. erythrogramma* draft genome, a total of ~1.13 million paired-end reads (*SI Appendix*, Fig. S7) were coassembled into five scaffolds that totaled ~1.11 million base pairs with 460× read coverage. This draft genome was nearly complete at 97.2% of required single copy genes (*SI Appendix*, Fig. S8) and had a guanine-cytosine content (33.3%) typical of endosymbionts (see *SI Appendix*, Table S10 for full assembly statistics) (26).

One full-length 16S rRNA gene was identified within these scaffolds. We compared this to full-length 16S rRNA sequences of other Rickettsiales as well as the 20 most similar 16S rRNA sequences in the National Center for Biotechnology Information (NCBI) database (*SI Appendix*, Fig. S9). This sequence grouped within the Anaplasmataceae family and had an ~80 to 85% nucleotide sequence similarity to other Anaplasmataceae from a wide range of nematode and arthropod hosts (*SI Appendix*, Fig. S9). Because of the divergence of this lineage, we propose the genus "*Candidatus* Echinorickettsia" and the species name "*Candidatus* E. raffii" after the late Rudy Raff, who was an early pioneer of evolutionary developmental biology in *Heliocidaris*.

We identified 1,206 protein coding sequences for *E. raffii* and, of these, 324 protein coding regions (26.9%) were classified as hypothetical proteins. We compared the *E. raffii* genome with seven complete genomes of closely related Rickettsiales genera to identify potentially unique features of this bacterium (Fig. 2). A total of 51 gene clusters were shared across all eight isolates and were identified as the Anaplasmataceae core (Fig. 2 and Dataset S1*A*). Using these concatenated core genes, we performed a phylogenetic analysis to better resolve the branching pattern of this bacterium and its relationship to other Anaplasmataceae. This verified the position of *E. raffii* within the Anaplasmataceae and placed it within a clade encompassing *Wolbachia*, *Ehrlichia*, and *Anaplasma* (Fig. 2).

A total of 799 gene clusters in *E. raffii* were not observed in other Anaplasmataceae and 21 gene clusters were shared with its closest relative (i.e., *Wolbachia*; Fig. 2 and Dataset S1*B*). Gene clusters unique to *E. raffii* included a complete set of flagellar biosynthesis genes, which have not been observed in other Anaplasmataceae and suggests that *E. raffii* may have a free-living phase outside of *H. erythrogramma* (Fig. 3). The *E. raffii*

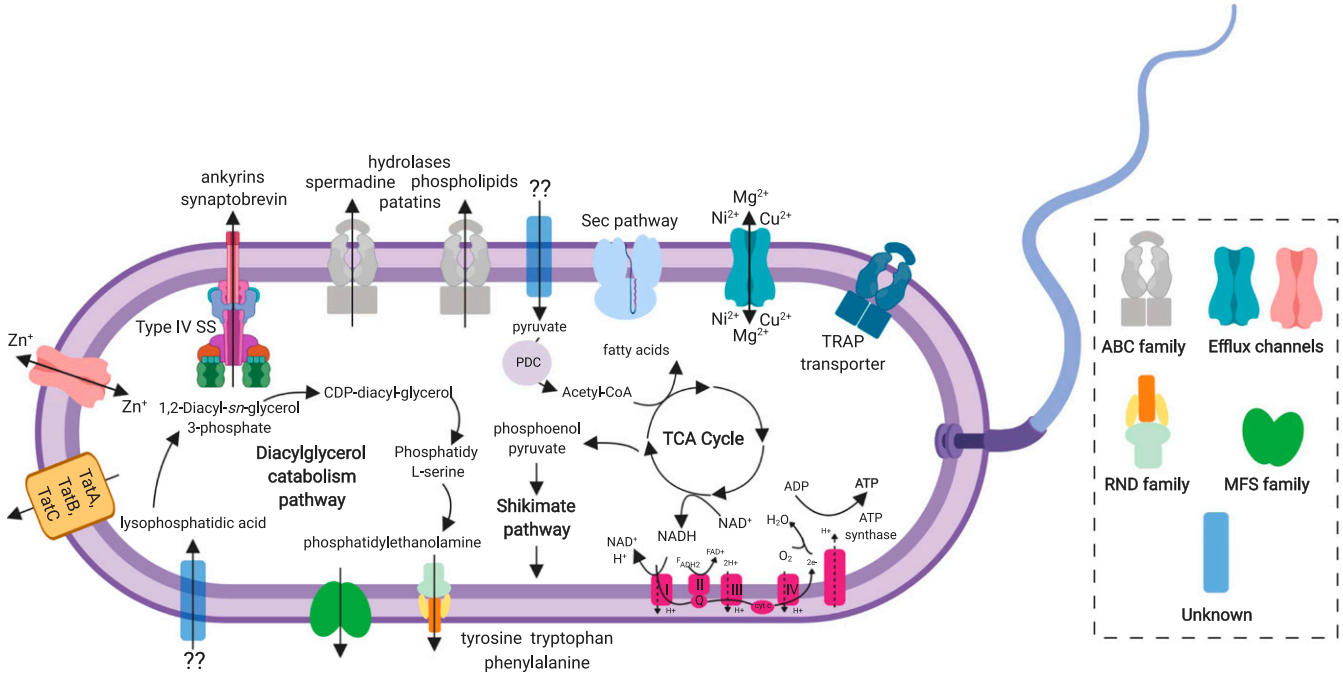


**Fig. 3.** Proteins and inferred pathways of *Echinorickettsia raffii*. The major enzymatic pathways (e.g., complete shikimate pathway for essential amino acid biosynthesis and catabolism of diacylglycerol ethers), transport systems (e.g., efflux channels and ATP-binding cassette transporters), and secretion systems (e.g., a Type IV secretion system) in the *E. raffii* genome. In this, predicted effector proteins are illustrated outside of the cell from their predicted secretion system.

genome was also unique in that it encoded gene clusters encompassing a complete set of enzymes required for the biosynthesis of essential amino acids via the shikimate pathway (Fig. 3). These essential amino acids (i.e., phenylalanine, tyrosine, and tryptophan) cannot be synthesized de novo by *H. erythrogramma* or other echinoderms. Instead, they are acquired exogenously through feeding or taken directly from the seawater during development (27, 28). Interestingly, similar essential amino acids are symbiont derived in the lecithotrophic larvae of the sponge *Amphimedon queenslandica* and regulate metamorphosis (29), suggesting that the evolution of lecithotrophy may favor the establishment of symbiotic relationships that provide the host with essential amino acids.

The *E. raffii* genome also included a suite of transport and secretion systems, including a Type IV secretion system predicted to translocate transfer DNA among other effectors (Fig. 3). A total of 84 effector proteins from *E. raffii* were predicted to be secreted from Type IV secretion systems, including three that maintained eukaryotic-like domains (Dataset S1 *B–D*). Two of these eukaryotic-like effectors were ankyrins, a diverse protein family known to interact with eukaryotic ligands (30) and to be enriched in endosymbionts (31). One of these ankyrin proteins maintained a domain with distant homology to the male killing gene of *Spiroplasma poulsonii* that is involved in the sex determination pathway (32). The third was a hypothetical protein with a synaptobrevin domain, a protein necessary in the all-or-nothing acrosomal reaction of sea urchin sperm during fertilization (33). The presence of these predicted secretory proteins suggests a potential involvement of *E. raffii* in host reproduction.

Nearly one-third of the protein coding sequences for *E. raffii* had Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs related to metabolism. Many of the metabolic modules necessary for carbon metabolism were incomplete (*SI Appendix*, Fig. S10), a feature characteristic of Rickettsiales that acquire essential resources from their host (34). Notably, *E. raffii* does not have a pyruvate kinase, the enzyme required for converting the intermediate phosphoenolpyruvate into pyruvate in the tricarboxylic acid cycle (Fig. 3). This intermediate, however, is used in the first step of the shikimate pathway for the production of essential amino acids (Fig. 3). Additionally, the most abundant nutritional reserve in the developmental stages of *H. erythrogramma* and the primary maternally derived lipid group acquired in the transition to lecithotrophy are diacylglycerol ethers (22, 35, 36). Like other Rickettsiales, *E. raffii* encoded numerous enzymes within the glycerolphospholipid metabolism pathway, including enzymes that are required for the metabolism of diacylglycerol ethers (Fig. 3 and *SI Appendix*, Fig. S11). The ability to catabolize diacylglycerol ethers and produce essential amino acids suggests a nutritional interaction between *E. raffii* and *H. erythrogramma*.

**Abundance and Localization of *E. raffii*.** We observed a decline in the relative abundance of *E. raffii* from 95.1% of egg-associated bacteria to 8.0% in 1-d–old embryos (ANOVA, $P < 0.0001$; *SI Appendix*, Fig. S12 and Table S11). This difference equated to an ~104× decline in *E. raffii* abundance, from ~33,822 cells per egg to ~324 cells per embryo (*t* test, $P = 0.002$; *SI Appendix*, Fig. S13). Using fluorescent in situ hybridization, we found that this bacterium was located in the cytoplasm and between yolk granules throughout the egg (*SI Appendix*, Fig. S14). This observation confirms that this bacterium is intracellular like all other members of the Anaplasmataceae family and suggests that it is vertically transmitted. Using PCR, we also found the full-length 16S rRNA sequence of *E. raffii* in *H. erythrogramma* ovaries ($n = 3$; *SI Appendix*, Fig. S15), indicating that this bacterium is inherited via transovarian (vertical) transmission.

**Female-Dominant *H. erythrogramma* Population.** The *E. raffii* genome had several homologous genes known to interfere with

host reproduction. These are hypothesized to favor maternal transmission like other Anaplasmataceae (Fig. 3 and *SI Appendix*, Fig. S15). If *E. raffii* interferes with host reproduction, then we would expect that *H. erythrogramma* populations are female dominant. We sampled *H. erythrogramma* in Sydney Harbor and found that ~67% of the sampled individuals were female ($\chi^2$: $P < 0.001$; $n = 314$). The youngest reproductive individuals in this population did not deviate from a 1:1 female-to-male ratio ($\chi^2$: $P = 0.796$; $n = 15$), while the largest individuals had a 4:1 female to male ratio ($\chi^2$: $P < 0.020$; $n = 15$; *SI Appendix*, Fig. S16). The female dominance of *H. erythrogramma* is not unique among echinoderms; 18.6% of recorded sex ratios were female dominant, while 14.7% were male dominant, and 66.7% did not deviate from a 1:1 ratio ($n = 130$, 110 species; *SI Appendix*, Fig. S17 and Table S12 and Dataset S1*E*). The *H. erythrogramma* population where *E. raffii* is present is female dominant, but whether this is the result of *E. raffii* requires future research.

## Discussion

The gastrointestinal tract is a principal site for microbial colonization in animals and is primarily responsible for the taxonomic and functional diversity of animal–microbe symbioses. Coevolution between host and microbiome has shaped metabolic codependency and mediated niche expansion of the host (1–3, 37). This interpretation is primarily based on animals that are dependent on a gastrointestinal tract, but species in various phyla have lost a functional digestive system and have evolved obligate nutritional mutualisms with endosymbionts (6, 7). We, however, lack a fundamental understanding of how host–microbe relationships are altered during transitions in nutrition and life histories as has occurred between planktotrophy and lecithotrophy (4, 10, 13).

Comparison of the bacterial communities associated with the planktotrophic and lecithotrophic development of these *Heliocidaris* species supports three primary findings. First, the planktotrophic species responds to and associates with a bacterial community specific to food availability, while the lecithotrophic species does not. Second, the bacterial community associated with the lecithotroph, as compared to the planktotrophic congener, is markedly reduced in diversity and abundance. Third, *H. erythrogramma* acquired an endosymbiotic Rickettsiales that is transmitted from parent to offspring and is enriched with gene clusters for flagellar biosynthesis as well as the production of essential amino acids. This bacterium also encodes proteins with homologous domains known to interact with eukaryotic hosts, modulate sperm, and metabolize diacylglycerol ethers.

The planktotrophic larvae of several echinoderm species associate with bacterial communities that are taxonomically and compositionally specific to food availability (16, 23). When diet restriction is prolonged and morphological plasticity is expressed, planktotrophic larvae exhibit shifts in their bacterial community that precede and then correlate with the expression of morphological plasticity (16, 17). Like other species with feeding larvae, *H. tuberculata* associated with a bacterial community that correlated with food availability. This response was not observed in *H. erythrogramma*, suggesting that the mechanisms used to cope with environmental (food) variability may have been lost during the transition from planktotrophy to lecithotrophy (16).

Following a simplification in morphology, the transition toward lecithotrophy leads to the loss of a functional gastrointestinal tract (4, 10, 13). For *H. erythrogramma*, gastrulation is abbreviated, the blastopore is closed shortly after invagination, and this reduced gut remains inactive (13, 20, 38, 39). The taxonomic and phylogenetic diversity of the bacterial community associated with *H. erythrogramma* was approximately a third of *H. tuberculata*, with a bacterial abundance that was ~13× less. This suggests that the transition from planktotrophy to lecithotrophy and the evolutionary loss of a functional gut is correlated with a significant reduction in the diversity and abundance of the microbiome (13, 20, 38, 39). This, in principle, is consistent with the expectation and overall consensus that the evolution of the gut gave rise to animal-associated microbial communities that are taxonomically and phylogenetically diverse with a high cell density (1–3, 37).

Arguably, the most notable modification associated with the transition toward lecithotrophy is to oogenesis (4, 13), where maternal investment is altered to provide the offspring with a surplus of diacylglycerol ethers and other nutrients required to sustain nonfeeding larvae through metamorphosis (22, 35, 36). This biochemical transition in echinoid eggs is correlated with a shift toward a low-diversity bacterial community and the evolution of vertical transmission. For *H. erythrogramma*, this includes the association with *E. raffii*, a bacterium with genes involved in diacylglycerol ether catabolism. These lipids in *H. erythrogramma* are partially utilized during development but are primarily reserved for juvenile growth (18, 22). We therefore hypothesize that diacylglycerol ethers are a primary metabolic resource for *E. raffii* throughout the biphasic life cycle of the host and, in particular, are used to maintain symbiont titer in the benthic adults.

Metabolism of diacylglycerol ethers is common for bacteria within Anaplasmataceae while the production of essential amino acids using the shikimate pathway is not. This pathway contains genes to synthesize amino acids that animal hosts cannot (40). Symbiont-derived essential amino acids can regulate development (29) and were previously hypothesized to be acquired by larval feeding or from dissolved organic matter in the seawater (28, 41). This argument, however, was based on the assumption that echinoid gametes are devoid of bacteria (28), a notion that was recently disproven with the observation that planktotrophs inherit diverse bacterial communities (42) and here with the vertical transmission of *E. raffii*. Endosymbiont-derived nutrients are beneficial and widely observed in gutless invertebrates in coastal and deep-sea habitats, including in worms, corals, and molluscs (6, 7). Associating with an endosymbiont with the potential for a nutritional interaction and to complement the host lifestyle may, therefore, be common when a functional gut is lost.

The phylogenetic position and genomic content of *E. raffii* suggests that this bacterium could influence host reproduction (25) and we suspect it has for three reasons. First, these *Heliocidaris* species are sympatric in Sydney Harbor (13) and their gametes co-occur in the water column, as the onset of spawning by *H. erythrogramma* coincides with gamete release for *H. tuberculata* (39, 43). Second, hybrid incompatibility is unidirectional, whereby *H. tuberculata* sperm bind to but cannot fertilize *H. erythrogramma* eggs unless the jelly coat is removed (14, 44), allowing for a fertilization efficiency (~85%) equal to the reciprocal cross (14). Third, *H. erythrogramma* populations are disproportionately female in Sydney Harbor (67.2%) and Tasmania (56.9%) (45). This would be expected to be deleterious for benthic marine invertebrates that commonly live in a sperm-limited environment (46). We therefore hypothesize that *E. raffii* influenced the reproductive and population biology of *H. erythrogramma*.

A dual role as a mutualist in host nutrition and parasite to host reproduction is common for Rickettsiales that are most closely related to *E. raffii* (25). These multifaceted symbioses first arise through a mutualism (e.g., the exchange of essential amino acids) or parasitism (e.g., cytoplasmic incompatibility or feminization), and the mechanisms for the opposing interaction evolve later (25, 47, 48). Host–microbe evolution with these types of microbes has, for example, mediated speciation events in insects (49). We hypothesize that a similar stepwise symbiosis occurred between *Heliocidaris* and *E. raffii* and that this influenced the evolutionary transition in developmental mode. Alternatively, the reproduction and population biology of *H. erythrogramma*

could be the result of adaptive protandry, an accelerated female growth, and/or differential male mortality (50).

While the impact of *E. raffii* on *Heliocidaris* reproduction is unknown, a complete set of flagellar biosynthesis genes would suggest that *E. raffii* is a facultative endosymbiont. This bacterium is located within *H. erythrogramma* eggs, and the majority of these cells are lost once the embryo hatches (39). A few hundred *E. raffii* cells remain associated with *H. erythrogramma* throughout development, and this bacterium can later be found in the adult ovaries. This suggests that *E. raffii* is not lost upon metamorphosis and that this bacterium locates the ovary upon sexual maturity, where it may influence sex determination and subsequently enter the oocytes during gametogenesis. We suspect that *E. raffii* are incorporated during the second phase of oogenesis, which evolved as a result of this life history transition and is where the lipogenic program is largely responsible for the increase in egg size (21). The mechanisms and dialogue between *H. erythrogramma* and *E. raffii* to elicit vertical transmission during gametogenesis remains unresolved.

A remaining unknown is when during life history transitions may a bacterium such as *E. raffii* be acquired. The transition from planktotrophy to lecithotrophy in sea urchins includes two primary intermediate stages: facultative planktotrophy, where larvae may feed but are not required to, and facultative lecithotrophy, where larvae cannot feed but have reduced feeding structures (Fig. 4) (4, 5, 10, 51). We hypothesize that environment-elicited shifts in the microbiome and total microbial diversity is reduced and/or lost as the dependence on maternally derived nutrients increase. This nutritional shift allows for an endosymbiont akin to *E. raffii* to be established and support development by providing a nutritional contribution (e.g., the production of essential amino acids) that would otherwise be accrued through larval feeding. This symbiosis is then sustained between host generations and a parasitism that overrides host reproduction subsequently evolves, further accelerating the life history transition (Fig. 4). Despite these unknowns, we find that the microbiome is a dynamic character in animal evolution and that this character may be modified during life history transitions (52).

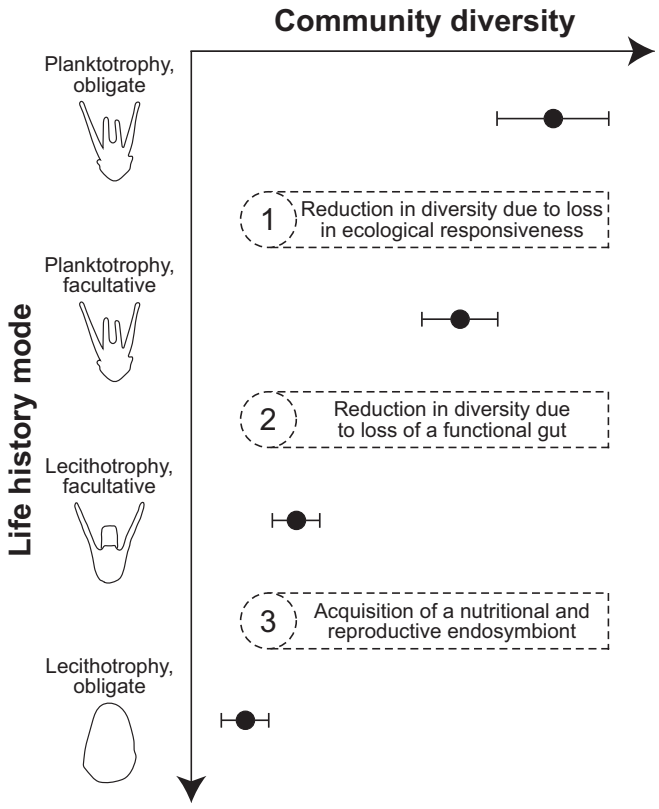


**Fig. 4.** Microbiome during life history transitions. The hypothetical model for the evolution of lecithotrophy presented by Wray (5) and revised by Smith et al. (53) is expanded here. This includes three additional components related to larval-associated bacterial communities: 1) a reduction in community diversity during the transition from obligate to facultative planktotrophy as the dependence on exogenous nutrients and response to the feeding environment is reduced, 2) a reduction in community diversity during the transition from facultative planktotrophy to lecithotrophy while a functional gut is lost, and 3) acquisition of a bacterial symbiont with a nutritional interaction that may be sustained between host generations by complementing host metabolism. This bacterium may then evolve parasitic activities that override host reproduction and further accelerate the life history transition.

## Materials and Methods

**Specimen Collection and Experimental Feeding.** Adult *Heliocidaris* were collected from populations in Sydney Harbor in March and April 2018. Within 2 d of collections, three males and three females were spawned to generate a heterogenous batch of embryos that would represent a population of reproductive adults as occurs in the field (*SI Appendix, Materials and Methods*). Pooled *Heliocidaris* embryos were divided into 12 replicate jars and provided an experimental feeding treatment of either 12,000 (ad libitum), 1,200 (diet restricted), or 0 (starved) cells per milliliter of *Isochrysis galbana* ($n = 4$ per treatment) (15). *H. tuberculata* ($n = 100$) was then sampled every 5 d for 20 d (i.e., until developmental stasis) while *H. erythrogramma* ($n = 50$) was sampled daily for 5 d (i.e., through metamorphosis) (*SI Appendix, Materials and Methods*). Complementary to sampling these sea urchins, the environmental microbiome was sampled at each time point for both species, whereby ~0.5 L of seawater ($n = 3$) was filtering onto a 0.22 μm Millipore filter (*SI Appendix, Materials and Methods*).

**Assessment of the Bacterial Community.** Total DNA was extracted from all samples. The bacteria were profiled using primers for the V3/V4 regions of the 16S rRNA gene and sequenced on an Illumina MiSeq (*SI Appendix, Materials and Methods*). Raw reads along with quality information were then imported into and analyzing using a standard QIIME 2 pipeline at a rarefaction depth of 4,091 sequences (*SI Appendix, Materials and Methods*).

Using the total DNA extracted from *Heliocidaris* samples, the relative abundance of 16S copies was quantified using qPCR. This was performed with "universal" primers for the 16S rRNA gene and the Luna Universal qPCR Master Mix (*SI Appendix, Materials and Methods*).

**Metagenomics.** Metagenomes were sequenced on an Illumina MiSeq using the total DNA extracted from two *H. erythrogramma* egg samples. Raw reads were trimmed and quality filtered, and host sequences were removed. All unmapped reads were coassembled into contigs and checked for genome completeness. Coding regions, conserved domains, and putative secreted effectors were then determined. A gene tree and phylogeny were then constructed using the full-length 16S rRNA gene and a concatenated "core" set of Anaplasmataceae genes, respectively.

**Localization and Abundance of *E. raffii*.** *E. raffii* was visualized within *H. erythrogramma* eggs using a standard fluorescent in situ hybridization protocol and imaged using an Olympus FV1000 inverted confocal microscope (*SI Appendix, Materials and Methods*). *E. raffii* was observed in *H. erythrogramma* ovaries using PCR with custom *E. raffii* primers (*SI Appendix, Materials and Methods*). Lastly, *E. raffii* abundance was quantified in eggs and embryos using qPCR. This was performed with custom primers designed to amplify a unique portion of the 16S rRNA gene of *E. raffii* and the Luna Universal qPCR Master Mix (*SI Appendix, Materials and Methods*).

**Echinoderm Sex Ratios.** Adult *H. erythrogramma* ($n = 314$) were collected from Sydney Harbor between February 1989 and January 1990 and sexed using a scalpel and compound microscope. A randomized subset of individuals ($n = 75$ total, $n = 15$ per group) were used to determine if sex ratio shifted with size (a proxy for age; *SI Appendix, Materials and Methods*). $\chi^2$ tests were used for both comparisons. The sex ratio of echinoderms, in general, was estimated using 130 cases from 110 species found in the literature (*SI Appendix, Materials and Methods*).

**Data Availability.** Reads for 16S rRNA and metagenomic sequencing are publicly accessible on the Dryad Digital Repository (doi: 10.5061/dryad.7h44j0zqt) (54) and the *E. raffii* genome is publicly accessible on NCBI (PRJNA635939).

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