Bacterial colonization of *Hydra* hatchlings follows a robust temporal pattern

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Animals are colonized by complex bacterial communities. The processes controlling community membership and influencing the establishment of the microbial ecosystem during development are poorly understood. Here we aimed to explore the assembly of bacterial communities in *Hydra* with the broader goal of elucidating the general rules that determine the temporal progression of bacterial colonization of animal epithelia. We profiled the microbial communities in polyps at various time points after hatching in four replicates. The composition and temporal patterns of the bacterial communities were strikingly similar in all replicates. Distinct features included high diversity of community profiles in the first week, a remarkable but transient adult-like profile 2 weeks after hatching, followed by progressive emergence of a stable adult-like pattern characterized by low species diversity and the preponderance of the Betaproteobacterium *Curvibacter*. Intriguingly, this process displayed important parallels to the assembly of human fecal communities after birth. In addition, a mathematical modeling approach was used to uncover the organizational principles of this colonization process, suggesting that both, local environmental or host-derived factor(s) modulating the colonization rate, as well as frequency-dependent interactions of individual bacterial community members are important aspects in the emergence of a stable bacterial community at the end of development.

**Introduction**

All animals, ranging from simple invertebrates to primates, are host to complex microbial communities (Fraune and Bosch, 2007; Ley et al., 2008; Ochman et al., 2010). Microbial colonization appears to be an essential step in vertebrate ontogeny, contributing to the maturation of the immune system and gut development (Rawls et al., 2004; Mazmanian et al., 2005; Kelly et al., 2007). Resident gut bacteria can cause disease if the balance of the community is disrupted by antibiotics (Ayres et al., 2012). Neonatal recolonization of germ-free mice with microbes prevents enhanced colitis and asthma sensitivity while exposure of adult mice to these conditions is not effective (Olszak et al., 2012). In invertebrates, germ-free *Drosophila* larvae show drastically increased mortality compared with conventional larvae when reared on a nutrient-poor diet (Shin et al., 2011); and in anthozoan cnidarians shifts in the composition of the microbial community are associated with coral bleaching (Mao-Jones et al., 2010). Thus, vertebrates and invertebrates appear not only to tolerate, but to require colonization by beneficial microorganisms for metabolism, immune defense, development, behavior (Sandstrom et al., 2000; Xu et al., 2003; Nyholm and McFall-Ngai, 2004; Rawls et al., 2004; Mazmanian et al., 2005; Chow et al., 2010) and most likely many other not yet identified functions. Any animal with its associated microbes, therefore, can be considered as a metaorganism defined as ‘an ensemble of individuals representing numerous species that coexist and interact in an area or habitat’ (Drake, 1990). Membership in such a community is frequently influenced by interactions among species and properties, which emerge from such interactions (Drake, 1990). In this sense, humans develop into ecological communities after being born with a sterile gastrointestinal tract that is successively colonized with microbial populations until adult-like communities stabilize (Koenig et al., 2013).
The processes, which control community membership in the neonatal gut and influence the colonization pattern during infancy and childhood are poorly understood. A vitally important question to ask, therefore, is what are the factors and rules in a particular host that influence community assembly, composition and diversity. Because a dysregulation of host–microbe homeostasis can have severe impact on the host’s health, controlling and mediating ‘correct’ bacterial colonization in early life would confer fitness advantages to the host. This view has encouraged discussions as to what extent the microbiota is controlled by the host through top–down mechanisms involving the immune system, relative to microbiota intrinsic bottom–up mechanisms (Ley et al., 2006). Despite the importance of understanding the factors that control bacterial colonization in man, however, the inaccessibility of the microbial niches of the human gut, and the restriction of studies in infants to collection of fecal samples (Cilleborg et al., 2012) make it desirable to use animal models for understanding basic principles of colonization processes in detail. Here we aimed to explore the assembly of bacterial communities in the freshwater polyp Hydra. Our goal was to obtain mechanistic information about specific host–bacterial interactions and to elucidate the rules that determine how large and diverse bacterial populations colonize a newly born animal.

The cnidarian Hydra is an established animal model for studying host–microbe interactions. It is inexpensive, easily handled, has a transcriptome and genome that more closely resembles humans than those of flies and worms (Chapman et al., 2010; Hemmrich et al., 2012), and is colonized by stable and species-specific bacterial communities (Fraune and Bosch, 2007). Unlike most animal systems, it is possible to examine temporal bacterial profiles of clonal lineages without being limited to fecal samples, as Hydra grows clonally under constant laboratory conditions. Hydra’s tube-like body (Figure 1a) resembles in several aspects the anatomy of the vertebrate intestine with the endodermal epithelium lining the gastric cavity and the ectodermal epithelium providing a permanent protective barrier to the environment (Bosch, 2012). Changes in Hydra’s epithelial homeostasis cause significant changes in the microbial community (Fraune et al., 2009), implying a direct interaction between epithelia and microbiota. Multifunctional epithelial cells recognize microbial-associated molecular patterns with the help of the Toll-like receptor signaling pathway (Bosch et al., 2009; Franzenburg et al., 2012). Intracellular recognition of bacteria in Hydra is supposed to be mediated by a large number of nucleotide oligomerization domain-like receptors (Lange et al., 2011). Prominent effector molecules downstream of the conserved Toll-like receptor cascade are antimicrobial peptides.

Following fertilization, Hydra oocytes develop by a radial cleavage pattern outside the female polyp (Figure 1a) (Martin et al., 1997). Gastrulation is followed by a cuticle stage, which is characterized

Figure 1  Hydra morphology and experimental design. (a) Female polyp of Hydra vulgaris (AEP) with a developing embryo. (b) Hydra hatchling eclosing from the cuticle. (c) Experimental design: First batch of hatchlings was used for immediate DNA extraction. Second batch of hatchlings was used for the establishment of clonal cultures, from which one polyp was removed for DNA extraction every week until week 15. Subsequently, the bacterial communities of these samples were determined by 454 sequencing.
by a thick protective outer layer (Martin et al., 1997). We have shown previously (Fraune et al., 2010) that early embryonic stages before the cuticle stage are colonized by a limited number of microbes, which are clearly distinct from later developmental stages. Interestingly, the differential colonization is reflected in differences in antimicrobial activity (Fraune et al., 2010). Young Hydra polyps directly hatch from the cuticle stage (Figure 1b) (Martin et al., 1997). To elucidate how bacterial communities assemble in newly hatched Hydra polyps, we profiled the composition of the microbiota in polyps over the first 15 weeks after hatching (Figure 1c) using culture-independent pyrosequencing of bacterial DNA. The surprisingly dynamic, but robust bacterial succession pattern prompted us to include a mathematical modeling approach to infer organizational principles that may influence community assembly and diversity. To this end, we identified the minimally required principles to model the observed bacterial succession pattern during colonization mathematically.

**Materials and methods**

**Animals**

Experiments were carried out using *Hydra vulgaris* (strain AEP) (Hemmrich et al., 2007). Female polyps were induced to sexual reproduction by reduced feeding (Wittlieb et al., 2006). Detached eggs were collected and separated into single wells. Eggs were screened daily for hatched polyps. After 2 weeks, first eclosed polyps were stored at −20°C. Next, clonal cultures were established using four hatchlings eclosed on the same day. All animals were cultured under constant, identical environmental conditions including culture medium, food (first-instar larvae of *Artemia salina*, fed three times per week) and temperature according to standard procedures (Lenhoff and Brown, 1970). One week post hatching, the first bud detached from the founder polyp and was frozen for later sequencing. Subsequently, one polyp of each clonally aging culture was removed and frozen every week until 15 weeks post eclosion.

**DNA extraction and sequencing of 16S rRNA genes**

For total DNA extraction, single polyps were subjected to the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) after being washed three times with sterile filtered culture medium. Extraction was performed following the manufacturer’s protocol, except that DNA was eluted in 50 μl. For sequencing of the bacterial 16S rRNA genes, the variable regions 1 and 2 (V1V2) were amplified using the universal forward primer V2_B_Pyro_27F (5'-CTATTCGCTTCCTGAGTGCTCAGAGTT TGATTCGAGTGCTCAG-3') which consists of the 454 FLX Amplicon primer B, a two base linker and the universal 16S primer 27F and the barcoded reverse primer V2_A_338R (5'-CGTATCGCTTCCTGAGGCA TCAGNNNNNNNCCATGTGCTCCTCCCGTAGGA GT-3') which contains the 454 FLX Amplicon primer A, a sample-specific 10-mer barcode (N's), a two base linker and the universal 16S primer 338R. Twenty-five microlitre PCR reactions were performed using the Phusion Hot-Start II DNA polymerase (Finnzymes, Espoo, Finland) following the manufacturer’s instructions. PCR conditions consisted of an initial denaturation step (98°C, 30 s) followed by 30 cycles of denaturation (98°C, 9 s), annealing (55°C, 30 s) and elongation (72°C, 20 s). PCR was terminated by a final elongation of 72°C for 10 min. All reactions were performed in duplicates, which were combined after PCR. PCR products were extracted from agarose gels with the Qiagen MinElute Gel Extraction Kit (Hilden, Germany) and quantified with the Quant-iT dsDNA BR Assay Kit (Invitrogen, Calsbad, CA, USA) on a NanoDrop 3300 Fluorometer. Equimolar amounts of purified PCR product were pooled and further purified using Ampure Beads (Agencourt, Brea, CA, USA). A sample of each library was run on an Agilent (Santa Clara, CA, USA) Bioanalyzer before emulsion PCR and sequencing as recommended by Roche (Penzberg, Germany). Amplicon libraries were subsequently sequenced on a 454 GS-FLX (Roche) using Titanium sequencing chemistry.

**16S rRNA 454 analysis**

16S rRNA amplicon sequence analysis was conducted using the Qiime 1.3.0 package (Caporaso et al., 2010). Using the sequence fasta-file, a quality file and a mapping file which assigned the 10-nucleotide barcodes to the corresponding sample as input, the sequences were analyzed using the following parameters: length between 300 and 400 bp, no ambiguous bases and no mismatch to the primer sequence. Chimeric sequences were identified and removed using Chimera Slayer (Haas et al.). Resulting high-quality reads ranged from 2110 to 9845 per sample. Sequences were normalized to 2000 sequences per sample, grouped into operational taxonomic units (OTUs) at a ≥ 97% sequence identity threshold and classified by ribosomal database project (RDP) classifier. Alpha diversity was calculated using the Chao1 metric implemented in Qiime. Beta diversity was assessed using Pearson’s distance (1000 replicates). A total of 454 data are deposited at MG-RAST (id: 1706).

**Quantification of bacterial 16S genes by quantitative reverse transcriptase-PCR**

Total bacterial quantification was performed with the original DNA used for 454 sequencing using the QuantiTect Probe RT-PCR Kit (Qiagen) and a 7300 real-time PCR system (ABI, Foster City, CA, USA). Template amounts were equilibrated for the
**Results**

**Bacterial population profiles in newly hatched Hydra polyps**

To follow the assembly of the microbiota in newly hatched polyps, we established four clonal cultures (that is, four replicates) of single hatchlings and examined them for up to 15 weeks (Figure 1c). The composition of the microbiota was determined by pyrosequencing of the variable regions 1 and 2 (V1V2) of the bacterial 16S rRNA gene, amplified from total DNA extractions of single polyps. Pyrosequencing resulted in 1 77 862 high-quality reads ranging from 2 110 to 9 845 reads per sample. For inter-sample comparisons, the number of reads was normalized to 2000 reads per sample. In all four replicates, comprising bacterial profiles of a total of 36 polyps, bacterial species estimation using the Chao1 metric showed the highest bacterial diversity immediately after hatching (black line, Figure 2). About 350 different OTUs (97% sequence similarity) were estimated in polyps just emerging from the cuticle stage. In all four replicates, bacterial diversity decreased in the following 2–3 weeks to about 150 OTUs. Thereafter, 4 weeks post hatching, a transient but distinct increase in bacterial diversity to 200–300 OTUs per polyp was observed (Figure 2), followed by a decrease in bacterial diversity in all four replicates to ~100 OTUs per polyp 15 weeks post hatching. Thus, the diversity of the bacterial community in *Hydra* polyps at various time points after hatching is negatively correlated with developmental age (see also Supplementary Figure S1A).

To examine whether the changes in bacterial diversity are accompanied by changes in the overall density of bacteria, bacterial abundance was quantified using a broad-range bacterial primer pair. As shown in Figure 2 (red line), fluctuation in bacterial load across replicates and between time points was observed. For example, in replicate 1, bacterial abundance was highest immediately after hatching whereas in replicate 2 hatchlings had a rather low bacterial abundance. Thus, bacterial abundance is not correlated with bacterial diversity, nor with developmental age.

We next addressed the question of which bacterial species are members of the microbiota and whether...
membership changes during development. Sequence analysis uncovered remarkable differences between the bacterial communities in newly hatched polyps compared with adult (that is 15 weeks post hatching) animals (Figure 3a). Hatchlings were characterized by a high abundance of bacteria of the Bacteroidetes (Flavobacteria, Sphingobacteria) group, which represented about 50% of the total OTUs (Figure 3a). In addition, there was also a large number of Betaproteobacteria present in hatchlings. Other members of the bacterial community include Actinobacteria, Alphaproteobacteria and Gammaproteobacteria (Figure 3a). Interestingly, the bacterial community in polyps 15 weeks post hatching differed greatly from the composition of the community characteristic for hatchlings (Figure 3a) and resembled the previously described (Fraune et al., 2010) bacterial community in clonally growing *Hydra vulgaris* (AEP) polyps.

Figure 3b shows the temporal assembly of this bacterial community (resolved at the OTU level) in four replicates at various time points after hatching. Only OTUs exceeding a relative abundance of 10% in at least one time point are shown. The assembly pattern is characterized by three distinct features. First, the bacterial composition immediately after hatching was similar in all four replicates and was characterized by a large number of *Flavobacterium sp.* (OTU 1217). Other members of the ‘young’ bacterial community belong to a variety of different bacterial phylotypes and include *Curvibacter* (OTU 942) (Supplementary Figure S2). Second, the first 3 weeks of assembly were characterized by a high degree of fluctuation in community composition with the presence of Burkholderiales (OTU 424) and *Acidovorax sp.* (OTU 1608) bacteria at week 1, *Curvibacter* (OTU 942) at week 2 and *Methylophilus sp.* (OTU 12), Comamonadaceae (OTU 1605) and

**Figure 2** Dynamics of bacterial diversity and bacterial load. Estimated number of OTUs (97% sequence identity, Chao1 metric, black line) was plotted against time post hatching. Red lines indicate the relative bacterial (EUB) abundance per host cell as analyzed by quantitative PCR for the bacterial 16S gene (equilibration to *Hydra* actin, reference value 15 weeks post hatching).

**Figure 3** Dynamics in the microbiota composition. (a) Pie diagrams representing the bacterial community of *Hydra* polyps directly after hatching and 15 weeks post hatching, respectively. Bacterial OTUs are summarized on class level. (b) Relative abundances of bacterial OTUs (97% similarity) over the first 15 weeks post hatching. Only OTUs that exceed 10% in at least one sample were plotted. Note that OTU 942 (*Curvibacter sp.*) is the most abundant at week 2 in all replicates, declines again and becomes the dominating species in adult polyps.
Hydrogenophaga (OTU 1948) bacteria at week 3 in all four replicates. Third, the phase of high variability in the first weeks post hatching was replaced by the establishment of a robust bacterial composition dominated by Curvibacter (OTU 942) (Figure 3b). Interestingly, in all four replicates the occurrence of Curvibacter showed a distinct temporal pattern during assembly. Although first present at low abundance in newly hatched polyps, Curvibacter became the dominant member of the bacterial community for a short period of time 2 weeks post hatching. Thereafter, abundance of Curvibacter decreases markedly again. From week 4 on, the Betaproteobacterium Curvibacter became the dominant member of the bacterial community (Figure 3b; Supplementary Figure S2), which is correlated with a decrease in total bacterial diversity (Supplementary Figure S1B). As there is no general increase in bacterial load over developmental time (Figure 2), the relative increase of Curvibacter is because of relative changes in the community. Therefore, the host seems to offer a certain amount of niches for bacterial colonizers and over time Curvibacter is occupying the niches formerly used by other bacteria.

To further analyze the temporal progression of the bacterial community toward an adult-like bacterial profile, we conducted a principle coordinate analysis (Figure 4a) and calculated the average Pearson’s correlation of each assembly time point (Figure 4b). As reference we used the bacterial profiles of four long-term cultures of Hydra vulgaris (AEP). Figure 4A shows that the four hatchling samples cluster together, indicating a bacterial composition that substantially differs from all other samples. Principle coordinate analysis also indicates that polyps examined 1 week after hatching and three weeks after hatching have distinct bacterial communities different from those found in polyps immediately after hatching as well as from adult polyps (15 weeks post hatching). According to the principle coordinate analysis, the microbial community of polyps analyzed 4–15 weeks after hatching resembles the adult microbiota found in polyps of long-term cultures (Figure 4a). This view is supported by Pearson’s correlation analysis (Figure 4b), which shows a low value immediately after hatching and in 1-week-old polyps. Two weeks post hatching, the correlation was high, indicating striking but transient resemblance to the adult-like microbial pattern. After this transient adult-like pattern the correlation drops to values similar to those found in newly hatched polyps. At 4 weeks after hatching (Figure 4b) the correlation increased and remained high in all later time points. These observations indicate that the progressive development of the adult-like microbial profile in Hydra encompasses a remarkable transient occurrence of the generic adult-like profile 2 weeks post hatching and a preponderance of Curvibacter 4 weeks after hatching.

Figure 4 Temporal profile of the progression to an adult microbiota. (a) Principal coordinate analysis of Pearson’s distances between Hydra microbiota at different time points post hatching. The percent variation explained by the plotted principal coordinates is indicated on the axes. The microbiota of later time points (10 weeks, 15 weeks, adult) show high similarity such that single symbols may be overlaid. (b) Mean Pearson’s correlation between the microbiota (OTU level 97%) of each time point compared with an adult profile retrieved from four independent adult polyps (clonal propagation for 1 year). Note the peak of the Pearson’s correlation at the 2-week time point.

Computing the microbial assembly pattern
Mathematical modeling is a powerful approach to understand the complexity of biological systems (Murray, 2002). Therefore, to uncover principle rules controlling the microbial assembly process in the Hydra metaorganism (Figure 5a), a replicator–colonizer approach was applied (Hofbauer and Sigmund, 1998). This models the temporal evolution of an interacting bacterial community in a competitive environment deterministically. This
allowed us to qualitatively simulate the colonization dynamics based on complex interactions among bacteria and between bacteria and host. As microbial communities in Hydra may contain tens to hundreds of microbial species, we reduced this obviously incomprehensible complexity of community member behavior by assuming that the bacterial community only consists of four distinct members (Figure 5a). Our main assumption was that the progressive assembly of the bacterial population is restricted to pairwise interactions. The notations used in the mathematical model are defined in Figure 5b.

The mathematical analysis focused on three possible patterns of interactions within this idealized metaorganism. First, we assumed constant type-specific fitness values (constant growth and colonization rates) with competition not being dependent on the distribution of relative abundances. Here, the type with the highest growth rate wins the competition if the colonization rate is small enough. As shown in Figure 5c, the predicted colonization pattern does not agree with the qualitative pattern of the experimental data (see Figure 3b). An intermediate decline in the abundance of the final winner of the competition is not present. Next, fitness of the bacterial members of the microbiota was modeled as abundance-dependent. In this case, competition can be such that a bacterium has an advantage when it is rare, but a disadvantage when it is abundant in the community (frequency dependence). This can give rise to more complex patterns over time. The final distribution depends on the initial distribution. The first as well as the second approach were examined by using a constant colonization rate \( \lambda(t) \) (Figure 5b). As shown in Figure 5d, this can lead to an oscillatory assembly pattern resembling the observed (see Figure 3b) colonization dynamics. However, it does not capture the stable adult bacterial colonization profile of the host by a dominant bacterial species. In a third modeling step, the colonization rate was assumed to be time-dependent, that is assuming that it decays over time as the organism approaches its adult state, \( \lambda(t) = \lambda_0 e^{-t/\tau} \), where \( \tau \) is an environmentally or host-derived specific decay rate. In Figure 5e we show that such an environment/host-controlled colonization rate modulates the abundance-dependent competition such that an initially cyclic competition ends up in a final state dominated by a single bacterial type. Following an initial phase of oscillatory behavior, there is a transition period that sensitively depends on initial condition and on the decay rate. According to this model (see Figure 5b), the eventual winner of the microbial competition is present transiently at relatively high abundance at an earlier stage, long before a stable community with reduced bacterial diversity is reached. This modeling approach qualitatively resembles the experimental data, indicating that both inter-microbial interactions (frequency-dependent fitness) as well as environmental or host-derived factors controlling the bacterial colonization rate (\( \lambda \)) are important in dictating bacterial community assembly in Hydra polyps. Note that we do not fit any parameters to the experimental data. Instead, we infer the minimal
Thus, frequency-dependent bacteria–bacteria interaction process by mediating the colonization rate are responsible for controlling the distinct colonization process. This suggests that host factors stimuli were reduced to a minimum during the highly conserved in our experimental setup, external conditions including food, temperature and medium were bacterial population. Because environmental conditions affected by this dynamic mucus layer adherent to all epithelial cells that can serve as both a physicochemical sensor and barrier network across animal species (Moran et al., 2011). Interestingly, the genome of Curvibacter contains a large number of ABC-transporters for sugar uptake compared with other Comamonadaceae (Chapman et al., 2010). This increased potential of using the host’s glycocalyx components as source of sugar for nutrition might explain the high potential of Curvibacter to out-compete other bacteria for the colonization of niches offered by the host.

Interestingly, similar trends were observed recently in a study of the human infant intestinal microbiota (Palmer et al., 2007). There, profiling the postnatal colonization of 14 human infants by fecal analyses showed that the intestinal microbiota is variable in infants and converges to an adult-like profile with time (Palmer et al., 2007). Moreover, the Pearson’s correlation shows (Palmer et al., 2007) that progression toward the adult-like microbiota contains a transient state around day 5, which remarkably resembles the stable communities found in older children. Thus, in both Hydra and human the progressive development of the adult-like microbial profile seems to require a transient occurrence of the generic adult-like profile.

The factors and processes controlling the bacterial community assembly during ontogeny are not known. In a study comparing the microbiota of Hydra embryos a few hours post fertilization with adult polyps, we have previously shown that the differential colonization is reflected in differences in antimicrobial activity (Fraune et al., 2010). Thus, antimicrobial peptides are capable of mediating host–microbe homeostasis (Vaishnava et al., 2008; Fraune et al., 2010; Salzman et al., 2010; Login et al., 2011) and therefore are candidates for mediating conserved progressions of certain bacteria during the ontogenetic establishment. Future efforts will be directed toward examining whether a spatially and temporally controlled expression of antimicrobial peptides contributes to the stepwise microbiota assembly after hatching from the cuticle stage. To colonize epithelial surfaces, bacteria commonly interact with glycan structures of the host glycocalyx (Hooper and Gordon, 2001). Thus, bacterial colonization of the newly hatched polyp may also be affected by this dynamic mucus layer adherent to all epithelial cells that can serve as both a physicochemical sensor and barrier network across animal species (Moran et al., 2011). Interestingly, the genome of Curvibacter contains a large number of ABC-transporters for sugar uptake compared with other Comamonadaceae (Chapman et al., 2010). This increased potential of using the host’s glycocalyx components as source of sugar for nutrition might explain the high potential of Curvibacter to out-compete other bacteria for the colonization of niches offered by the host.

Discussion

How does Hydra assemble its specific set of microbes? Animals from Hydra to human are colonized by a complex and species-specific microbiota (Fraune and Bosch, 2007; Ley et al., 2008). In the same way that microbial communities are expected to change in different parts of a body, they are also dynamic over time. For a first understanding of the processes that control bacterial community membership in newly hatched Hydra polyps, we profiled the assembly of the microbiota up to 15 weeks post-hatching. Our observations in four independent replicates and profiles of 36 individual polyps indicate that the adult-like microbiota arises in three stages: first the high variability and presence of numerous species, then the transient preponderance of the bacterial species, which later dominate the adult microbiota, and finally the drastic decrease of diversity and assembly to an adult-like pattern from week 4 post hatching on. Owing to the complexity of the interactions and large number of components involved, it is almost impossible to intuitively understand processes such as the assembly of the generic adult-like microbiota. We therefore complemented the in vivo profiling by adding a mathematical model to infer the general principles of the assembly process. One of the mathematical models (Figure 5e) not only resembles key features of the experimental data, but also makes two interesting and falsifiable predictions. First, assembly of a stable microbiota seems to require the transient preponderance of an initial member of the bacterial community, which after a characteristic decay finally becomes the stable and most abundant component of the community. Second, external (environmental or host-derived) factors given by a decaying colonization rate λ appear to be necessary to restrict oscillatory/strongly fluctuating dynamics in the bacterial population. Because environmental conditions including food, temperature and medium were highly conserved in our experimental setup, external stimuli were reduced to a minimum during the colonization process. This suggests that host factors are responsible for controlling the distinct colonization process by mediating the colonization rate λ. Thus, frequency-dependent bacteria–bacteria interactions and host control through temporal modulation of the colonization rate appear to be key features involved in dictating how the bacterial community is assembled in Hydra after hatching.

Conclusion and perspectives

Over the last decade remarkable technical advancements have led to a better understanding of the complexity of organisms as metaorganisms and to a general picture of the interactions involved in the freshwater polyp Hydra (Bosch and McFall-Ngai, 2011). Precisely, how bacteria assemble in their animal host and how they contribute to the metaorganism is an exciting area of current research. We have shown here that in newly hatched Hydra, the microbial assembly is under complex control.
mechanisms. With these insights we can now focus on identifying the underlying mechanistic processes and address questions such as: Is the remarkable variability at the onset of the assembly process host-controlled and ontogenetically fixed or a stochastic event? Does the assembly process vary between related host species? Genome-scale screens and gene-targeting experiments will have an increasing role in answering these questions and identifying the underlying molecular mechanisms. On the other hand, mathematical models of this dynamic process can lead to testable hypotheses. Collectively, the observations reported here contribute to create an integrated view of the interactions within the Hydra metaorganism. With regard to strikingly similar observations in human infants, it seems likely that this distinct colonization pattern described here is a common mechanistic process contributing to the composition of animal microbiota.

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