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Eco-evolutionary Dynamics Linked to Horizontal Gene Transfer in Vibrios

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Abstract :

Vibrio is a genus of ubiquitous heterotrophic bacteria found in aquatic environments. Although they are a small percentage of the bacteria in these environments, vibrios can predominate during blooms. Vibrios also play important roles in the degradation of polymeric substances, such as chitin, and in other biogeochemical processes. Vibrios can be found as free-living bacteria, attached to particles, or associated with other organisms in a mutualistic, commensal, or pathogenic relationship. This review focuses on vibrio ecology and genome plasticity, which confers an ability to adapt to new niches and is driven, at least in part, by horizontal gene transfer (HGT). The extent of HGT and its role in pathogen emergence are discussed based on genomic studies of environmental and pathogenic vibrios, mobile genetically encoded virulence factors, and mechanistic studies on the different modes of HGT.

Keywords : genome plasticity, genomics, population structure, mobile genetic elements, natural competence, pathogen emergence

1. Introduction

The advent of large-scale sequencing efforts has changed our ability to study marine samples from around the world. Based on metagenomic data, substantial oceanic microbial diversity has been discovered (114, 127). For example, it was shown that surface ocean prokaryotic picoplankton consists of two primary microbial groups: The first group, which includes *Pelagibacter* (SAR11), *Prochlorococcus*, and *Synechococcus* (~10^5 cells/ml), are smaller-celled, resulting in a reduced biomass per individual that serves to shield them from predators, making their genomes streamlined to correlate with this smaller cell size (138). The second group, while less abundant (~10^3/ml), might be more adaptable to specific environments than their genome-streamlined counterparts. Such organisms, including members of the genus *Vibrio* ("vibrios") that can reach a 100-fold higher biomass per cell compared to for example *Pelagibacter* (18), often engage in associations with plants and animals (Figure 1) in mutualistic, commensal, or pathogenic manners and are strongly affected by eukaryotic predators and their released products (138). Consequently, these bacteria have a higher genetic diversity and often carry a plethora of genes that encode important regulatory and metabolic pathways, provide the ability for cell-to-cell communication (known as quorum sensing), and allow for organismal interactions (99, 138). Such functions might be crucial for adapting to changing environmental conditions. Studying the seasonal dynamics of marine microbial communities showed that vibrios were capable of massive blooms, increasing from a few percent (0-2%) to more than 50% of detectable microbes (44), that often co-occurred with or succeeded plankton blooms. Therefore, the use of remote sensing methods has been suggested to predict and monitor the spread of the causative agent of cholera, *Vibrio cholerae*, around the Bay of Bengal (69, 71), demonstrating the importance of studying the dynamics of these bacteria in their local environments.

The awareness of vibrios is often limited to their pathogenic representatives (Box 1), even though they are common marine bacteria represented by over 110 species (72). Here, we present important examples of genomic diversity among both the pathogenic and non-pathogenic members of the genus

Vibrio to illustrate how horizontal gene transfer (HGT) contributes to niche adaptation and, ultimately, to the emergence of pathogens.

BOX 1: Human pathogenic vibrios

All members of the genus *Vibrio* are Gram-negative γ-proteobacteria, often found in salty or brackish water. The most common human diseases caused by the 11 recognized human pathogenic species of vibrios are gastroenteritis and wound infections (with the risk of developing septicemia), which can ultimately lead to death, especially in immunocompromised individuals. Foodborne disease is predominantly caused by the ingestion of contaminated water or through consuming raw or undercook seafood and shellfish.

Vibrio cholerae

V. cholerae is the causative agent of the severe diarrheal disease cholera. More than 200 serogroups are known, but only O1 and O139 have been reported to cause large-scale cholera outbreaks (23). Upon ingestion, the pathogen colonizes the small intestine of its host. Cholera symptoms appear 12-72 hours later and frequently result in dehydration due to extensive diarrheal fluid loss (up to 1 liter/hour) (23, 89). The World Health Organization estimates that there are around 4.3 million cases of cholera resulting in up to 142,000 deaths every year (133). Severe pathogenesis in *V. cholerae* is determined by two primary factors: cholera toxin (CT), which initiates the secretion of ions and, concomitantly, of water from intestinal cells, and an adhesion appendage known as the toxin-coregulated pilus (TCP). Both of these virulence determinants are encoded on mobile genetic elements (MGE).

Vibrio parahaemolyticus

V. parahaemolyticus is common in tropical and temperate estuarine habitats, though incidents are becoming more frequent due to rising sea surface temperatures (128). Among vibrios, *V. parahaemolyticus* is the most common bacterial cause of seafood-borne gastroenteritis in the USA, with more than 34,000 annual cases (72). Its primary virulence factors are pore-forming hemolysins (thermostable direct hemolysin [TDH] and TDH-related hemolysin [TRH]) and type III secretion systems (T3SS), which inject toxins into eukaryotic cells. Both *tdh* and the T3SS genes are located on a MGE in pathogenic isolates of *V. parahaemolyticus*.

Vibrio vulnificus

V. vulnificus is an emergent pathogen of the aquaculture species, as well as a deadly opportunistic human pathogen (2, 4). It is common in estuarine environments, but the disease incidence is usually low. The virulence mechanisms are poorly understood. Putative virulence determinants include the capsular polysaccharide, the multifunctional-autoprocessing repeats-in-toxins protein, hemolysin, elastase, and siderophores. None of these factors reliably correlate with human disease (91). Several MGEs were identified in the *V. vulnificus* genome, an analysis that revealed significant genome plasticity in this microbe (97).

2. The complex architecture of the *Vibrio* universe

Due to the digestion, excretion, exudation, and lysis of other marine organisms, vibrios have access to heterogeneous microhabitats that are rich in nutrients but often transient (93, 112). As examples, phytoplankton exude the products of photosynthesis to form a surrounding phycosphere rich in dissolved organic matter (109), and the viral infection and lysis of microorganisms can form nutrient patches (14, 115). Imperishable micro-plastics have been added to this universe, forming the plastisphere, which represents a new ecological niche (5). Indeed, a study has shown that microbial diversity in plastic particles is different from the surrounding seawater, with a considerable proportion of vibrios among the colonizers (40, 140).

Within this heterogeneous landscape, competitors and predators also control bacterial populations and shape their evolution. Metabolic competitors exert influence by utilizing needed resources. Among predators, phages constitute the most abundant biological entity in the ocean, and play a significant role in controlling the abundance and composition of microbial communities. Present at ~10^6 to 10^7 particles /ml (101), at least one order of magnitude greater than bacteria, phages are responsible for up to 40% of bacterial mortality (15).

Living and proliferating in such a landscape requires special abilities for vibrios, such as those needed to seek nutritional sources and move in a low-Reynolds-number medium (like a human swimming in molasses) (96). Beyond random encounters, vibrios can use chemotaxis and motility to actively access nutrients (42, 109, 112). Membrane-spanning chemoreceptors detect attractants (e.g., nutrients) and repellents (e.g., waste products) and transduce the signal to the controllers of their locomotion appendix, the flagellum (24). The rotating flagellum is a complex machine requiring approximately fifty genes and a high-energy expenditure, necessitating a strict control of its synthesis and function (73). Remarkably, the sodium-driven flagellum of marine vibrios can spin at up to 1,700 Hz (100,000 r.p.m.; (74)), which is much faster than the ~300 Hz of the proton-driven flagellum of *Salmonella* (85).

Most vibrios can sense and chemotact along nutrient gradients, and can specialize in metabolizing specific nutrient sources. As heterotrophic bacteria, they can use dissolved or particulate organic matter as a carbon source. Chitin, a homopolymer of β-1,4 linked N-acetyl-D-glucosamine (GlcNAc), is the most abundant biopolymer in the aquatic environment with more than 10^{11} tons produced annually by marine zooplankton (Figure 1), and a majority of vibrios can degrade this as carbon and nitrogen source (45, 60). In contrast to chitin, only a small proportion of vibrios can metabolize algal and plant polysaccharides, suggesting metabolic peculiarities as sources of adaptation to certain niches (49, 53, 118). For instance, *V. breoganii* exploit alginate, a sugar from brown algae cell walls, but cannot use chitin as carbon source (49).

The phenotypic diversity of vibrios is therefore indicative of adaptive processes resulting from a genomic evolution required to adjust to various transient microhabitats. Hence, deletions, duplications, or HGT events led to genetic innovations, which were subsequently selected for by environmental factors. It is therefore important to study both the chemical makeup of their environment as well as their potential interaction partners (Figure 1) to better understand the link between the ecology and evolution of vibrios and their genome plasticity.

3. *Vibrio* population dynamics genomically linked to ecological functions

The population structure in vibrios was explored in a series of studies that associated differential genotypes with potential microhabitats (e.g., free-living, particle- or zooplankton-associated) or animal tissues (e.g., guts, gills) (52, 94, 95, 116). Unlike many other marine microbes, a major advantage of vibrios is that most of them can be cultured, allowing multilocus or whole genome sequencing to reveal populations as fine-scale genotypic clusters with differential environmental distributions (Figure 2). Subsequent work showed that these populations also represented gene flow (49, 110), social (25, 26), and behavioral units (136).

The genome comparison of two closely related populations of *V. cyclitrophicus* occurring in the

large (L) or small (S) size fraction of filtered seawater revealed single nucleotide polymorphisms (SNPs) that clustered in few genomic loci and coincided with the L and S ecology (ecoSNPs). The variability of these loci within populations seemed lower than the rest of the genome, suggesting they arrived recently by homologous recombination and spread through the entire population before accumulating polymorphisms (i.e., gene selective sweep). Recombination events involving both core genes, shared by both populations, and flexible, population-specific genes, were shown to be higher within populations than between populations, and HGT largely shaped the flexible genome. The L and S populations pursued different nutritional-seeking strategies (136). The L population specialized in interacting with living organisms and colonizing particles by attaching and growing biofilms (for which exopolysaccharide biosynthetic genes (*syp*) are important (137)), while the S population rapidly detected and swam towards short-lived particles patches (136). These results showed that few genes implicated in behavioral adaptation and host association could trigger ecological population differentiation and that these two populations, L and S, are on independent evolutionary trajectories, which may, ultimately, lead to distinct taxonomic *Vibrio* species.

Ecological populations also form socially cohesive units (25, 26, 50). The ability of social interactions to mediate competition effects at the intra- and inter-population level was investigated by establishing a network of antibiotic-mediated antagonistic interactions among strains (26). The results were consistent with competition between populations but not within populations. Genetic analyses showed that within populations, broad-range antibiotics were produced by a few genotypes (the super-killers), whereas all other strains were resistant, suggesting cooperation. Genome comparison of closely related strains with contrasting phenotypes (super-killers versus resistant) showed that a recent HGT event mediated the acquisition of the antimicrobial gene cluster in *V. ordalii.* As the resistance factor seems to not be encoded by the same region, it has been suggested that antimicrobial genes can only transfer in pre-adapted (resistant) individuals within the population (26).

The idea that "public good" (i.e., secreted molecules that exert their function outside the cell and benefit the whole population) dynamics drive the evolution of ecological populations is based on iron-acquisition strategies (25). Within ecological populations of vibrios, only some genotypes were shown to produce iron-chelating compounds called siderophores, whose production was explored by comparing the genome sequences of siderophore producers and non-producers ("cheaters") within populations. In the producers, siderophore biosynthesis genes and receptors were co-localized in a single operon, while in non-producers, the biosynthesis genes but not the receptors, had been excised or replaced. This frequent loss of biosynthesis genes was observed in different species (*V. splendidus, V. crassostreae, V. tasmaniensis,* and *V. cyclitrophicus*), suggesting that producers and cheaters coexist in a dynamic equilibrium because public good production is stable at the population level. Moreover, the study found that the frequency of cheaters increases with large-particles association, suggesting that these environments can harbor communities stable enough for dependents to evolve (25).

The ability of vibrio populations to degrade the algal glycan alginate led to the study of the evolutionary history of ecophysiological specialization strategies and resource partitioning (49). The authors showed that the acquisition of alginate degradation genes by HGT initiated metabolic pathway diversification followed by recent extensive gene flow events that created several ecophysiological populations adapted to different forms of alginate (49).

4. Association of vibrios with larger organisms

Living organisms, such as plants, algae, zooplankton, and other animals (99, 117, 121) (Figure 1) also constitute niches for vibrios, allowing for their proliferation and dispersion, but a formal demonstration of an intimate host-bacterial relationship is often lacking. The presence of bacteria, instead, can be the chance result of different feeding modes (41, 94) rather than the specific colonization of live animals. Among the specific ecological associations suspected, diazotrophy

(fixation of atmospheric nitrogen into ammonia) in vibrios inhabiting in the anaerobic rhizosphere of marine plants or in coral larvae illustrates the advantage of being a facultative anaerobe (3, 66). Detailed knowledge about these associations and the underlying molecular mechanisms are, however, still largely unknown.

The *V. fischeri*-squid symbiosis

Among well-studied host-*Vibrio* associations is the interaction of the bioluminescent bacterium *V. fischeri* and the bobtail squid *Euprymna scolopes* (76, 82). In this symbiosis, a specific organ of the squid, the light organ, is colonized by the symbiont *V. fischeri* shortly after hatching, and all captured squid harbor this bacteria (82). This interaction is essential for the squid, as the light produced by the luminescent bacteria allows the animal to avoid predators through a counterillumination strategy, mimicking of downwelling moon-/starlight. The host's acquisition of the specific bacterial partner is a highly complex process, especially given the abundance of other bacteria in the aquatic environment and that *V. fischeri* does not strictly depend on the squid but can also thrive in the open water. In this context, it was shown that a fish symbiotic *V. fischeri* strain could not robustly colonize the squid. This ability was, however, conveyed to this strain through the introduction of the *rscS* gene from the squid native isolate ES114. Notably, this squid symbiont-specific gene encodes a biofilm regulator that was acquired through HGT (77).

Population structures in vibrios associated with coastal marine invertebrates

It is important to address to what extent a host organism mirrors the *Vibrio* population structure in the water or if certain populations reproduce more specifically in the host. An initial analysis revealed zooplankton-associated populations showed a higher degree of host specificity than those found on larger animals, such as mussels and crabs (94). Within the species *V. splendidus*, populations that were adapted to various aqueous environments coexisted in animals without evidence of specialization (52), therefore suggesting that dispersal-colonization dynamics (especially through

filtering/scavenging) dominate population assembly in these animals. A more recent study showed that although oyster-colonizing vibrios resembled those from the surrounding seawater, there was a selective enrichment of some populations, suggesting that oysters represented a permissive habitat for these specific populations (16). For example, *V. crassostreae* was found to be particularly abundant in diseased oysters, reaching up to ~50% of the vibrio colonizers, underlining the importance of accounting for environmentally dependent physiological responses of the host (65).

Populations as the unit of pathogenesis in oysters

The onset of disease in oysters was shown to be associated with the progressive replacement of diverse benign vibrio colonizers by members of a phylogenetically coherent virulent population, *V. crassostreae* (39, 67). Although the virulent population was genetically diverse, the majority of its members were disease-causing (16). Comparative genomics across virulent and non-virulent populations identified population-specific genes clustered in genomic patches and on a large mobilizable plasmid. Genetic analyses revealed that a cluster of two chromosomal genes encoding an exported protein of unknown function (R5-7/8) as well as the plasmid pGV were necessary for the infection of oysters (16, 67).

While *V. crassostreae* is abundant in all diseased oysters, it always co-occurs with diverse populations across individuals, and it has been shown that the resultant population delineation correlates with virulence potential ((52) and Bruto *et al*., in preparation). In accordance with the hypothesis that co-infections could provide variable sets of virulence traits that may be synergistic, experimental infections have demonstrated that some strains are moderately virulent when injected into animals individually and display heightened virulence in mixed infections (43, 67). Hence, oyster disease may result from microbial interactions within and between populations and should be evaluated as potentially polymicrobial (Figure 2).

Pathogen transition through HGT

V. nigripulchritudo is an emergent pathogen of farmed shrimp in New Caledonia and other regions in the Indo-Pacific. Phylogenomic analysis revealed the recent emergence of three pathogen-containing lineages: two from diseased shrimp in New Caledonia in summer (A) or winter (B), and one from septicemic shrimp in Madagascar (M) (46). Contemporary lineages are comprised of nearly identical strains with contrasted virulence. Genetic elements specific to virulent strains and acquired by HGT were evidenced in comparative genomic analyses (Figure 2). Notably, a large mobilizable plasmid (pA_X) present in all virulent strains encoded a new toxin, the nigritoxin (63). The sequence comparison of the pA_X plasmid (160 to 260 kb) revealed modules that differentiated the plasmids by geography, clade, and pathogenicity, suggesting that these plasmids are extensively circulating among the *V. nigripulchritudo* strains and convey some sort of selective advantage to their hosts. Within each lineage, other MGEs acquired by virulent strains were also identified. In lineage A, a small plasmid of 11 kb (100) was shown to contribute to virulence, and interactions between factors encoded on the two plasmids have been suggested (64). In lineage B, two large genomic islands, a prophage, and an integrative conjugative element (ICE), were specifically found in virulent strains (46) (Figure 2).

5. Evolutionary reconstructions based on genomic analyses of human pathogenic vibrios

Genomics of *Vibrio cholerae* isolates

Numerous bacterial pathogens have emerged from environmental populations through the acquisition of MGEs that encode, for example, host colonization factors and toxins followed by their clonal expansion. The species *V. cholerae,* for instance, contains genetically diverse strains that interact with a plethora of aquatic inhabitants (Figure 1) as well as with humans (1, 89). The genome sequence of strain N16961, an O1 El Tor *V. cholerae* strain and representative of the ongoing seventh pandemic of cholera, was first reported by Heidelberg and colleagues in 2000 (51). This study confirmed the

presence of two previously suggested chromosomes (122), a large chromosome 1 (2.96 Mbp) and a smaller chromosome 2 (1.07 Mbp), which might have originated from an ancient megaplasmid (51). This bipartite genome structure is conserved among all vibrios (Figure 3). Notably, the genome size of strain N16961 still represents well the average genome size of other *V. cholerae* isolates for which closed genomes are available nowadays (Figure 3). The sequencing of the *V. cholerae* genome then allowed for the design of N16961-specific microarrays for comparative genomic hybridization experiments. These experiments, performed by the Mekalanos group, showed the absence of certain genes and gene clusters in diverse pathogenic or environmental isolates of *V. cholerae*, thereby providing evidence for gene acquisition via HGT (32, 33). Some of the acquired gene regions, such as the *Vibrio* pathogenicity islands and seventh pandemic islands (see below), were speculated to encode gain-of-function traits, possibly contributing to the successful displacement of ancestral *V. cholerae* strains by the current pandemic O1 El Tor strains (32). These comparative hybridization studies also confirmed a previous hypothesis that *V. cholerae* O139 strains, which are the only serogroup apart from O1 to have caused large-scale cholera outbreaks, evolved from O1 El Tor strains through horizontal gene exchange of the O-antigen cluster (32). Microcosm experiments by Blokesch and Schoolnik demonstrated that such serogroup conversions occur frequently by natural competence for transformation (Box 2) and clonally expand through O-antigen-specific bacteriophage predation (9).

In 2009, Colwell and collaborators performed a comparative genomics study in which they compared whole genome sequences of 23 *V. cholerae* strains isolated over the past 98 years from clinics or the environment. A genome-based phylogeny of these 23 strains revealed several distinct lineages, including one lineage (denoted as the phylocore genome clade or as the pandemic group [PG]) that comprised the pandemic *V. cholerae* isolates, and a total of 73 horizontally moving genomic islands among these strains (20). The authors therefore concluded that "genetic diversity of *V. cholerae* derives most significantly from lateral gene transfer" and that "O serogroup conversion occurs frequently in nature" (20), consistent with the experimental data mentioned above (9).

The presence of several genomic islands among *V. cholerae* isolates was later also confirmed in a comparative study of 154 whole genome sequences (87) and two recent follow-up reports that investigated 252 and 1070 isolates from the Americas and Africa, respectively (31, 131). The purpose of these studies was to elucidate transmission routes among seventh pandemic strains, meaning that HGT was not a primary emphasis, though the introduction of genomic islands, including those that conferred resistance to antimicrobials, into pandemic *V. cholerae* strains was nonetheless highlighted (31). Interestingly, these data separate the presence of diverse local *V. cholerae* linages that trigger sporadic outbreaks across Latin America from the inter-continental seventh pandemics strains, most likely transferred through carriers or patients from endemic regions in Asia, responsible for the historical cholera epidemics in these areas (31).

Based on these and previous data that showed the Asian origin of all pandemic isolates, the importance of eliminating the pathogen's natural reservoirs in this part of the world was underscored (62). It was also suggested that "the ecology there is almost certainly different from elsewhere", emphasizing our need for a better understanding of the pathoecology of *V. cholerae* in this part of the world. However, this also raises the question as to why a limited number of pathogenic clones have emerged from environmental populations of *V. cholerae* in modern history. Trying to answer this, Shapiro and colleagues recently showed that, prior to the emergence of the PG clones, specific genomic features named "virulence adaptive polymorphisms" (VAP) conferring beneficial properties for virulence had to be present in the environmental population (111). Only strains with the "right combination" of VAPs were therefore able to cause pandemic disease after the horizontal acquisition of the major virulence factors.

Bacteriophage blooms can also control the abundance of *V. cholerae* strains (35-37, 55, 89). As phage expansion occurs in the intestines of patients, stools are a major source of phage delivery back into the environment, thus causing cholera epidemics to be self-limiting in nature. Phage-resistant *V. cholerae* strains have emerged by exchanging, mutating, or altering the expression of bacterial-surface phage-receptor appendages, such as the O antigen (9, 103, 139). Such adaptations, however,

can interfere with the bacterium's virulence potential and do not sustain ongoing epidemics (103, 105, 139). To defend against frequent phage attacks, several *V. cholerae* strains have horizontally acquired a phage-defense system known as phage-inducible chromosomal island-like elements (90). However, certain phages carry their own CRISPR/Cas system that counteracts these islands (104), highlighting the constant evolutionary arms race between phages and their vibrio hosts, which is frequently accomplished by HGT.

Genomic analyses of *V. parahaemolyticus*

While the most recent and still ongoing *V. cholerae* pandemic started more than 50 years ago, *V. parahaemolyticus* emergence and transcontinental spread is a recent phenomenon that provides an opportunity to study the evolutionary process of these pathogens. Classified by its somatic/O-antigen (O) and capsular antigen (K), the most prominent clone, serovar O3:K6, emerged in India in 1996 and spread worldwide over the last two decades (17, 72). The evolutionary history of pandemic and pre-pandemic isolates has suggested that the founder clone was an O3:K6 non-pathogenic strain that initially horizontally acquired membrane-bound virulence regulators through the *toxRS* operon followed by at least seven novel genomic islands (12, 28, 34), the origin of which remain elusive. This horizontal movement also included the 80-kb pathogenicity island Vp-PAI, carrying *tdh* and T3SS-encoding genes (75). Other genomic islands differentially distributed among isolates encode colonization factors, chemotaxis proteins, surface structures, and putative colicins (17). Serogroup-converted derivatives of the pandemic O3:K6 strains emerged through altered O and K antigens (such as serovariant O4:K68) with their biosynthetic genes carried on a variable gene cluster (54). Based on comparative genomic studies, it was therefore concluded that the acquisition of novel genetic material by HGT played a pivotal role in shaping the pathogen's genome (17).

6. Molecular characterization of HGT mechanisms and MGEs in *V. cholerae*

The molecular mechanisms driving the high genome plasticity, revealed through next generation sequencing and comparative genomics in several *Vibrio* species (Figure 3), are often poorly understood. Over the last two decades, it has been demonstrated that all three primary modes of HGT do occur in vibrios (Box 2), and ongoing research aims to elucidate these molecular mechanisms and their impact on the ecology and evolvability of vibrios. Today, *V. cholerae* serves as a model organism for HGT not least due to important studies on its lysogenic conversion by a cholera toxin-carrying phage (129), its self-transmissible ICE (130), the discovery of superintegrons (22, 81), and its chitin-inducible competence state (83).

BOX 2: Primary modes of HGT

While recent research has highlighted the ability of few bacteria to move DNA horizontally through non-canonical mechanisms, the three most abundant and best-studied modes of HGT are natural competence for transformation, conjugation, and transduction.

Natural competence for transformation

Natural competence describes the ability of a microbe to take up free DNA from its surroundings. If sufficiently homologous, this incoming DNA can recombine into the competent bacterium's genome, thereby transforming it. Natural transformation can contribute to genome repair and to the acquisition of new genetic information. The uptake of genetic material is accomplished by dedicated DNA-uptake nanomachines, which are only synthesized in the competence state (78).

Conjugation

Conjugation or mating describes the transfer of genetic material from a donor bacterium to a recipient. Transferred genetic material is based on the replication of circularized DNA that originates from plasmids or excised ICEs. Conjugation requires direct cell-to-cell contact and the synthesis of a multi-protein "mating pilus" between the two cells. Conjugation is the primary mode of HGT for plasmid-borne antibiotic resistance genes.

Transduction

Transduction leads to the transfer of genetic material through the aid of bacteriophages as shuffling agents. Generalized transduction occurs due to the low fidelity of DNA packing by phages so that pieces of host DNA can end up in a new viral particle and be transferred from one cell to another. No direct contact between the cells is required for transduction to occur.

Horizontally acquired genomic regions in *V. cholerae*

A plethora of horizontally acquired regions have been identified in *V. cholerae* through comparative genomics and a recent study even identified HGT events within patients, despite the short duration of acute cholera in humans (68). The most prominent example of HGT is the cholera toxin-encoding filamentous phage CTXϕ that resides as a prophage in pathogenic/pandemic *V. cholerae* genomes (129). This phage can excise itself and infect new strains that carry and express the genes for TCP, a major intestinal colonization factor (119) that also serves as a CTXϕ receptor (129). This vastly limits the number of potential phage recipients, as most environmental isolates of *V. cholerae* are TCP-minus. However, it was recently shown that alternative HGT mechanisms, such as generalized transduction and natural competence for transformation, can lead to prophage spread, even to strains lacking TCP (13, 123).

In addition to the CTX prophage and the O-antigen cluster mentioned above, the two *Vibrio* pathogenicity islands, VPI-1 (41 kbp) and VPI-2 (57.3 kbp), as well as the *Vibrio* seventh pandemic islands, VSP-I (16 kbp) and VSP-II (27 kbp), bear hallmarks of recent acquisition by HGT (11, 32, 56, 58, 86). Research over the last 20 years on VPI-1, which encodes several virulence regulators and TCP (58), and on the other islands has been reviewed by Boyd and colleagues (11). However, a recent genomic characterization study on VSP-I showed that one of its encoded proteins was a new dinucleotide cyclase (DncV). DncV synthesizes cyclic AMP-GMP and down-regulates chemotaxis genes, making it necessary for efficient intestinal colonization (30). Hence, it was concluded that this island classifies as a *bona fide* pathogenicity island, which is still questionable for VSP-II. Recent West African–South American (WASA) *V. cholerae* isolates contained a different version of the VSP-II genomic island in which a part of the genes were replaced through homologues recombination (31). These isolates also contained an additional genomic island named WASA-1 (87), though the function of this horizontally acquired genetic material hasn't yet been experimentally tested. It is important to mention that a previous study claimed to identify VPI-1 as the genome of another filamentous phage (59). However, it is now widely accepted that VPI-1 does not support the

production of phage particles that might transmit the pathogenicity island to other bacteria (38), and the mechanistic aspects of how this and the other genomic islands are transferred to new strains is therefore still unknown. Interestingly, most of these islands can excise and form circular intermediates, which was suggested as a first step in their horizontal transfer (86, 98).

ICE share features of prophages and of conjugative plasmids, as they reside in bacterial chromosomes but maintain the ability to excise themselves and spread to neighboring cells through their encoded conjugation machinery (135). The first ICE to be described in the γ-proteobacteria was SXT of the *V. cholerae* serogroup O139 strain MO10 (130), which was later, together with other members of the SXT/R391 family (134), also detected in several O1 El Tor pandemic isolates (87). SXT (~100 kbp) carries, apart from the conjugal system, several antibiotic resistance genes (130).

Many vibrios also contain superintegron (SI) islands (Figure 3). Integrons are gene-capturing platforms that can incorporate exogenous genetic material by site-specific recombination. They share three specific features: an integrase-encoding gene, a primary recombination site, and a single promoter that drives expression of the captured open reading frames or cassettes that reside downstream of this promoter (80). The first SI was described in *V. cholerae* (22, 81) and distinguishes itself from well-characterized antibiotic-resistance integrons mainly by its enormous size, almost 130 kbp or 3% of the genome, and by its chromosomal location that by itself doesn't seem to be mobile. The SI of *V. cholerae* strain N16961 has acquired 175 cassettes mostly encoding proteins of unknown function, which can be rearranged or deleted. To date, it is clear that SIs represent evolutionary hotspots in vibrios (20) (Figure 3) and that they can reach very high fluxes of incoming and outgoing gene cassettes, which ultimately contributes to environmental adaptation (61).

Apart from these larger genomic islands, smaller horizontally moving DNA regions have also been identified, including those related to the type VI secretion system (T6SS) of *V. cholerae* (124). T6SSs are macromolecular complexes that transport effector proteins into adjacent cells through the T6SS-puncturing device (21). Hence, the production of T6SS results in the intoxication of neighboring cells, except for clonal siblings that are protected through the production of suitable immunity proteins.

The effector- and immunity-encoding genes are frequently clustered in effector/immunity (E/I) cassettes and show clear signatures of HGT (124). Such horizontal movement of E/I cassettes was also suggested based on the genomic comparison of non-cholera vibrios, such as *V. parahaemolyticus*, *V. alginolyticus*, and *V. campbellii* (102). When transferred into *V. parahaemolyticus*, an E/I cluster from *V. alginolyticus* could be shown to be functional, demonstrating high compatibility between these E/I cassettes in different vibrios (102). Functionality of exchanged E/I cassettes among two different *V. cholerae* isolates was also confirmed (120). Salomon *et al*. therefore concluded that T6SS E/I cassettes might be shared among vibrios in their environmental reservoirs (102).

Chitin-induced natural competence for transformation

Many bacterial species are naturally competent for transformation (Box 2), including *V. cholerae*, which was shown to enter competence upon growth on chitin (83) (Figure 4). Within the last decade, studies on the regulatory circuit of competence induction in *V. cholerae* established the contribution of three environmental cues (recently reviewed by (84)): chitin/chitinous oligosaccharides, high cell density, and the absence of catabolite-repressing sugars (7, 70, 113) (Figure 4). When all these signals merge, the bacterium enters competence and produces a sophisticated DNA-uptake complex, which includes a type IV pilus structure (106) and a periplasmic DNA-binding protein (107, 108), that pulls DNA into the cells for homologous recombination (78).

DNA-uptake gene conservation was shown from a comparison of diverse *Vibrio* genomes (106), which was consistent with the experimental validation of chitin-induced competence in several vibrios (e.g., *V. fischeri, V. parahaemolyticus*, and *V. vulnificus*) (19, 88, 92). As most quorum sensing-proficient *V. cholerae* strains maintain their natural transformability (Blokesch, personal observation), except for those strains that carry transformation counter-acting MGEs (8, 27, 29), this mode of HGT could explain the dispensable genome diversification of vibrios, especially for those regions that do not bear hallmarks of other modes of HGT (such as prophages or ICE).

Enhancement of HGT by environmental cues

In addition to inducing natural competence for transformation, chitin also serves as an environmental signal to induce the T6SS of *V. cholerae* (10). The Blokesch group showed that the chitin-induced T6SS killing of neighboring non-kin cells led to the release of their genetic content followed by the immediate absorption of this DNA by the attacking bacterium, concluding that the T6SS enhances HGT in *V. cholerae* (10) (Figure 4). It will be interesting to see whether such neighbor predation followed by DNA incorporation can contribute to the spread of large DNA regions, including genomic islands, and, potentially, whole chromosomes. Indeed, based on the population study mentioned above, Shapiro and colleagues suggested that the small chromosome of *V. cyclitrophicus* might spread horizontally, leading to the question: "how often and by what mechanism are entire chromosomes mobilized" (110).

Apart from chitin, other cues that enhance HGT have been identified. This includes environmental factors and antibiotics that trigger the SOS response in *V. cholerae*. This response alleviates the repression of the conjugal transfer genes of ICEs, such as SXT, therefore fostering their spread (6). Similarly, the integrase gene of the *V. cholerae* SI was shown to be highly activated by SOS-inducing compounds, leading to integron cassette recombination and the transfer a previously silenced cassette to the first position, which is the primary integrase-mediated integration site (48). Hence, SOS/stress-induced induction of the integrase gene occurs when innovation is needed and leads to integron recombination and HGT (48).

7. Perspective

Recent advances in next generation sequencing provided "big data", allowing researchers to propose interesting scenarios of bacterial population structures and the role that HGT plays in the ecology and

evolution of prokaryotes. Future studies should, however, move from the descriptive nature of some of these big data studies towards the testing of these novel hypotheses in experimental settings. Indeed, while evidence for the enormous amount of HGT that occurs among most bacteria is overwhelming, the questions on 'why' and 'how' should be a priority in the upcoming years. These days, mechanistic aspects of the 'how' can be tackled through better-suited cultivation strategies, improved genetic tools, and advanced microscopy techniques. Moreover, downsizing biological complexity through the establishment of nature-inspired microcosm settings, which include vibrios together with predatory cells or well-studied model organisms (79, 126), will allow us to test important hypothesis such as the role that virulence factors might play, if any, in environmental settings (57, 125, 132). This knowledge will be important for understanding why horizontally moving genes and genomic islands are maintained and selected for by environmental pressures.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Almagro-Moreno S, Taylor RK. 2013. Cholera: Environmental Reservoirs and Impact on Disease Transmission. *Microbiol. Spectr.* 1
2. Amaro C, Sanjuan E, Fouz B, Pajuelo D, Lee CT, et al. 2015. The Fish Pathogen *Vibrio vulnificus* Biotype 2: Epidemiology, Phylogeny, and Virulence Factors Involved in Warm-Water Vibriosis. *Microbiol. Spectr.* 3
3. Bagwell CE, Rocque JR, Smith GW, Polson SW, Friez MJ, et al. 2002. Molecular diversity of diazotrophs in oligotrophic tropical seagrass bed communities. *FEMS Microbiol. Ecol.* 39:113-9
4. Baker-Austin C, Oliver JD. 2017. *Vibrio vulnificus* - new insights into a deadly opportunistic pathogen. *Environ. Microbiol.*
5. Barnes DK, Galgani F, Thompson RC, Barlaz M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364:1985-98
6. Beaber JW, Hochhut B, Waldor MK. 2004. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 427:72-4
7. Blokesch M. 2012. Chitin colonization, chitin degradation and chitin-induced natural competence of *Vibrio cholerae* are subject to catabolite repression. *Environ. Microbiol.* 14:1898-912
8. Blokesch M. 2017. In and out-contribution of natural transformation to the shuffling of large genomic regions. *Curr. Opin. Microbiol.* 38:22-9
9. Blokesch M, Schoolnik GK. 2007. Serogroup conversion of *Vibrio cholerae* in aquatic reservoirs. *PLoS Pathog.* 3:e81
10. Borgeaud S, Metzger LC, Scrignari T, Blokesch M. 2015. The type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer. *Science* 347:63-7
11. Boyd EF, Carpenter MR, Chowdhury N, Cohen AL, Haines-Menges BL, et al. 2015. Post-Genomic Analysis of Members of the Family Vibrionaceae. *Microbiol. Spectr.* 3
12. Boyd EF, Cohen AL, Naughton LM, Ussery DW, Binnewies TT, et al. 2008. Molecular analysis of the emergence of pandemic *Vibrio parahaemolyticus*. *BMC Microbiol.* 8:110
13. Boyd EF, Waldor MK. 1999. Alternative mechanism of cholera toxin acquisition by Vibrio cholerae: generalized transduction of CTXPhi by bacteriophage CP-T1. *Infect Immun* 67:5898-905
14. Brum JR, Hurwitz BL, Schofield O, Ducklow HW, Sullivan MB. 2016. Seasonal time bombs: dominant temperate viruses affect Southern Ocean microbial dynamics. *ISME J.* 10:437-49
15. Brum JR, Sullivan MB. 2015. Rising to the challenge: accelerated pace of discovery transforms marine virology. *Nat. Rev. Microbiol.* 13:147-59
16. Bruto M, James A, Petton B, Labreuche Y, Chenivesse S, et al. 2017. *Vibrio crassostreae*, a benign oyster colonizer turned into a pathogen after plasmid acquisition. *ISME J.* 11:1043-52
17. Ceccarelli D, Hasan NA, Huq A, Colwell RR. 2013. Distribution and dynamics of epidemic and pandemic *Vibrio parahaemolyticus* virulence factors. *Front. Cell Infect. Microbiol.* 3:97
18. Cermak N, Becker JW, Knudsen SM, Chisholm SW, Manalis SR, Polz MF. 2017. Direct single-cell biomass estimates for marine bacteria via Archimedes' principle. *ISME J.* 11:825-8
19. Chen Y, Dai J, Morris JG, Jr., Johnson JA. 2010. Genetic analysis of the capsule polysaccharide (K antigen) and exopolysaccharide genes in pandemic *Vibrio parahaemolyticus* O3:K6. *BMC Microbiol.* 10:274
20. Chun J, Grim CJ, Hasan NA, Lee JH, Choi SY, et al. 2009. Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic *Vibrio cholerae*. *Proc. Natl. Acad. Sci. USA* 106:15442-7
21. Cianfanelli FR, Monlezun L, Coulthurst SJ. 2015. Aim, Load, Fire: The Type VI Secretion System, a Bacterial Nanoweapon. *Trends Microbiol.*
22. Clark CA, Purins L, Kaewrakon P, Manning PA. 1997. VCR repetitive sequence elements in the *Vibrio cholerae* chromosome constitute a mega-integron. *Mol. Microbiol.* 26:1137-8

23. Clemens JD, Nair GB, Ahmed T, Qadri F, Holmgren J. 2017. Cholera. *Lancet* 390:1539-49
24. Colin R, Sourjik V. 2017. Emergent properties of bacterial chemotaxis pathway. *Curr. Opin. Microbiol.* 39:24-33
25. Cordero OX, Ventouras LA, DeLong EF, Polz MF. 2012. Public good dynamics drive evolution of iron acquisition strategies in natural bacterioplankton populations. *Proc. Natl. Acad. Sci. USA* 109:20059-64
26. Cordero OX, Wildschutte H, Kirkup B, Proehl S, Ngo L, et al. 2012. Ecological populations of bacteria act as socially cohesive units of antibiotic production and resistance. *Science* 337:1228-31
27. Croucher NJ, Mostowy R, Wymant C, Turner P, Bentley SD, Fraser C. 2016. Horizontal DNA Transfer Mechanisms of Bacteria as Weapons of Intragenomic Conflict. *PLoS Biol.* 14:e1002394
28. Cui Y, Yang X, Didelot X, Guo C, Li D, et al. 2015. Epidemic Clones, Oceanic Gene Pools, and Eco-LD in the Free Living Marine Pathogen *Vibrio parahaemolyticus*. *Mol. Biol. Evol.* 32:1396-410
29. Dalia AB, Seed KD, Calderwood SB, Camilli A. 2015. A globally distributed mobile genetic element inhibits natural transformation of *Vibrio cholerae*. *Proc. Natl. Acad. Sci. USA* 112:10485-90
30. Davies BW, Bogard RW, Young TS, Mekalanos JJ. 2012. Coordinated regulation of accessory genetic elements produces cyclic di-nucleotides for *V. cholerae* virulence. *Cell* 149:358-70
31. Domman D, Quilici ML, Dorman MJ, Njamkepo E, Mutreja A, et al. 2017. Integrated view of *Vibrio cholerae* in the Americas. *Science* 358:789-93
32. Dziejman M, Balon E, Boyd D, Fraser CM, Heidelberg JF, Mekalanos JJ. 2002. Comparative genomic analysis of *Vibrio cholerae*: genes that correlate with cholera endemic and pandemic disease. *Proc. Natl. Acad. Sci. USA* 99:1556-61
33. Dziejman M, Serruto D, Tam VC, Sturtevant D, Diraphat P, et al. 2005. Genomic characterization of non-O1, non-O139 *Vibrio cholerae* reveals genes for a type III secretion system. *Proc. Natl. Acad. Sci. USA* 102:3465-70
34. Espejo RT, Garcia K, Plaza N. 2017. Insight Into the Origin and Evolution of the V*ibrio parahaemolyticus* Pandemic Strain. *Front. Microbiol.* 8:1397
35. Faruque SM. 2014. Role of phages in the epidemiology of cholera. *Curr. Top. Microbiol. Immuno.* 379:165-80
36. Faruque SM, Mekalanos JJ. 2003. Pathogenicity islands and phages in *Vibrio cholerae* evolution. *Trends Microbiol.* 11:505-10
37. Faruque SM, Naser IB, Islam MJ, Faruque AS, Ghosh AN, et al. 2005. Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. *Proc. Natl. Acad. Sci. USA* 102:1702-7
38. Faruque SM, Zhu J, Asadulghani, Kamruzzaman M, Mekalanos JJ. 2003. Examination of diverse toxin-coregulated pilus-positive *Vibrio cholerae* strains fails to demonstrate evidence for *Vibrio* pathogenicity island phage. *Infect. Immun.* 71:2993-9
39. Faury N, Saulnier D, Thompson FL, Gay M, Swings J, Le Roux F. 2004. *Vibrio crassostreae* sp. nov., isolated from the haemolymph of oysters (*Crassostrea gigas*). *Int. J. Syst. Evol. Microbiol.* 54:2137-40
40. Foulon V, Le Roux F, Lambert C, Huvet A, Soudant P, Paul-Pont I. 2016. Colonization of Polystyrene Microparticles by *Vibrio crassostreae*: Light and Electron Microscopic Investigation. *Environ. Sci. Technol.* 50:10988–96
41. Froelich BA, Williams TC, Noble RT, Oliver JD. 2012. Apparent loss of *Vibrio vulnificus* from North Carolina oysters coincides with a drought-induced increase in salinity. *Appl. Environ. Microbiol.* 78:3885-9
42. Garren M, Son K, Raina JB, Rusconi R, Menolascina F, et al. 2014. A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. *ISME J.* 8:999-1007

43. Gay M, Renault T, Pons AM, Le Roux F. 2004. Two *Vibrio splendidus* related strains collaborate to kill *Crassostrea gigas*: taxonomy and host alterations. *Dis. Aquat. Organ.* 62:65-74
44. Gilbert JA, Steele JA, Caporaso JG, Steinbruck L, Reeder J, et al. 2012. Defining seasonal marine microbial community dynamics. *ISME J* 6:298-308
45. Gooday GW. 1990. Physiology of microbial degradation of chitin and chitosan. *Biodegradation* 1:177-90
46. Goudenege D, Labreuche Y, Krin E, Ansquer D, Mangenot S, et al. 2013. Comparative genomics of pathogenic lineages of *Vibrio nigripulchritudo* identifies virulence-associated traits. *ISME J.* 7:1985-96
47. Goudenege D, Travers MA, Lemire A, Petton B, Haffner P, et al. 2015. A single regulatory gene is sufficient to alter *Vibrio aestuarianus* pathogenicity in oysters. *Environ. Microbiol.* 17:4189-99
48. Guerin E, Cambray G, Sanchez-Alberola N, Campoy S, Erill I, et al. 2009. The SOS response controls integron recombination. *Science* 324:1034
49. Hehemann JH, Arevalo P, Datta MS, Yu X, Corzett CH, et al. 2016. Adaptive radiation by waves of gene transfer leads to fine-scale resource partitioning in marine microbes. *Nat. Commun.* 7:12860
50. Hehemann JH, Truong LV, Unfried F, Welsch N, Kabisch J, et al. 2017. Aquatic adaptation of a laterally acquired pectin degradation pathway in marine gammaproteobacteria. *Environ. Microbiol.* 19:2320-33
51. Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, et al. 2000. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* 406:477-83
52. Hunt DE, David LA, Gevers D, Preheim SP, Alm EJ, Polz MF. 2008. Resource partitioning and sympatric differentiation among closely related bacterioplankton. *Science* 320:1081-5
53. Hunt DE, Gevers D, Vahora NM, Polz MF. 2008. Conservation of the chitin utilization pathway in the *Vibrionaceae. Appl. Environ. Microbiol.* 74:44-51
54. Izutsu K, Kurokawa K, Tashiro K, Kuhara S, Hayashi T, et al. 2008. Comparative genomic analysis using microarray demonstrates a strong correlation between the presence of the 80-kilobase pathogenicity island and pathogenicity in Kanagawa phenomenon-positive *Vibrio parahaemolyticus* strains. *Infect. Immun.* 76:1016-23
55. Jensen MA, Faruque SM, Mekalanos JJ, Levin BR. 2006. Modeling the role of bacteriophage in the control of cholera outbreaks. *Proc. Natl. Acad. Sci. USA* 103:4652-7
56. Jermyn WS, Boyd EF. 2002. Characterization of a novel Vibrio pathogenicity island (VPI-2) encoding neuraminidase (nanH) among toxigenic *Vibrio cholerae* isolates. *Microbiology* 148:3681-93
57. Kamareddine L, Wong ACN, Vanhove AS, Hang S, Purdy AE, et al. 2017. Activation of *Vibrio cholerae* quorum sensing promotes survival of an arthropod host. *Nat. Microbiol.*
58. Karaolis DKR, Johnson JA, Bailey CC, Boedeker EC, Kaper JB, Reeves PR. 1998. A *Vibrio cholerae* pathogenicity island associated with epidemic and pandemic strains. *Proc. Natl. Acad. Sci. USA* 95:3134-9
59. Karaolis DKR, Somara S, Maneval DRJ, Johnson JA, Kaper JB. 1999. A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature* 399:375-9
60. Keyhani N, O., Roseman S. 1999. Physiological aspects of chitin catabolism in marine bacteria. *Biochim. Biophys. Acta.* 1473:108-22
61. Koenig JE, Bourne DG, Curtis B, Dlutek M, Stokes HW, et al. 2011. Coral-mucus-associated *Vibrio* integrons in the Great Barrier Reef: genomic hotspots for environmental adaptation. *ISME J.* 5:962-72
62. Kupferschmidt K. 2017. Genomes rewrite cholera's global story. *Science* 358:706-7
63. Labreuche Y, Chenivesse S, Jeudy A, Le Panse S, Boulo V, et al. 2017. Nigritoxin is a bacterial toxin for crustaceans and insects. *Nat. Commun.* 8:1248

64. Le Roux F, Davis BM, Waldor MK. 2011. Conserved small RNAs govern replication and incompatibility of a diverse new plasmid family from marine bacteria. *Nucleic Acids Res.* 39:1004-13
65. Le Roux F, Wegner KM, Polz MF. 2016. Oysters and Vibrios as a Model for Disease Dynamics in Wild Animals. *Trends in microbiology* 24:568-80
66. Lema KA, Clode PL, Kilburn MR, Thornton R, Willis BL, Bourne DG. 2016. Imaging the uptake of nitrogen-fixing bacteria into larvae of the coral *Acropora millepora*. *ISME J.* 10:1804-8
67. Lemire A, Goudenege D, Versigny T, Petton B, Calteau A, et al. 2015. Populations, not clones, are the unit of vibrio pathogenesis in naturally infected oysters. *ISME J.* 9:1523-31
68. Levade I, Terrat Y, Leducq JB, Weil AA, Mayo-Smith LM, et al. 2017. *Vibrio cholerae* genomic diversity within and between patients. *Microb. Genom.* 3
69. Lipp EK, Huq A, Colwell RR. 2002. Effects of global climate on infectious disease: the cholera model. *Clin. Microbiol. Rev.* 15:757-70
70. Lo Scrudato M, Blokesch M. 2012. The regulatory network of natural competence and transformation of *Vibrio cholerae*. *PLoS Genet.* 8:e1002778
71. Lobitz B, Beck L, Huq A, Wood B, Fuchs G, et al. 2000. Climate and infectious disease: use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. *Proc. Natl. Acad. Sci. USA* 97:1438-43
72. Lovell CR. 2017. Ecological fitness and virulence features of *Vibrio parahaemolyticus i*n estuarine environments. *Appl. Microbiol. Biotechnol.* 101:1781-94
73. Macnab RM. 1996. Flagella and motility. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, ed. FC Neidhardt, 1:123-45. Washington, DC.: American Society for Microbiology Press.
74. Magariyama Y, Sugiyama S, Muramoto K, Maekawa Y, Kawagishi I, et al. 1994. Very fast flagellar rotation. *Nature* 371:752
75. Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, et al. 2003. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V cholerae*. *Lancet* 361:743-9
76. Mandel MJ, Dunn AK. 2016. Impact and Influence of the Natural *Vibrio*-Squid Symbiosis in Understanding Bacterial-Animal Interactions. *Front. Microbiol.* 7:1982
77. Mandel MJ, Wollenberg MS, Stabb EV, Visick KL, Ruby EG. 2009. A single regulatory gene is sufficient to alter bacterial host range. *Nature* 458:215-8
78. Matthey N, Blokesch M. 2016. The DNA-Uptake Process of Naturally Competent *Vibrio cholerae*. *Trends Microbiol.* 24:98-110
79. Matz C, McDougald D, Moreno AM, Yung PY, Yildiz FH, Kjelleberg S. 2005. Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae*. *Proc. Natl. Acad. Sci. U S A* 102:16819-24
80. Mazel D. 2006. Integrons: agents of bacterial evolution. *Nat. Rev. Microbiol.* 4:608-20
81. Mazel D, Dychinco B, Webb VA, Davies J. 1998. A distinctive class of integron in the *Vibrio cholerae* genome. *Science* 280:605-8
82. McFall-Ngai MJ. 2014. The importance of microbes in animal development: lessons from the squid-vibrio symbiosis. *Annu. Rev. Microbiol.* 68:177-94
83. Meibom KL, Blokesch M, Dolganov NA, Wu C-Y, Schoolnik GK. 2005. Chitin induces natural competence in *Vibrio cholerae*. *Science* 310:1824-7
84. Metzger LC, Blokesch M. 2016. Regulation of competence-mediated horizontal gene transfer in the natural habitat of *Vibrio cholerae*. *Curr. Opin. Microbiol.* 30:1-7
85. Minamino T, Imada K. 2015. The bacterial flagellar motor and its structural diversity. *Trends in microbiology* 23:267-74
86. Murphy RA, Boyd EF. 2008. Three pathogenicity islands of *Vibrio cholerae* can excise from the chromosome and form circular intermediates. *J. Bacteriol.* 190:636-47

87. Mutreja A, Kim DW, Thomson NR, Connor TR, Lee JH, et al. 2011. Evidence for several waves of global transmission in the seventh cholera pandemic. *Nature* 477:462-5
88. Neiman J, Guo Y, Rowe-Magnus DA. 2011. Chitin-induced carbotype conversion in *Vibrio vulnificus*. *Infect. Immun.* 79:3195-203
89. Nelson EJ, Harris JB, Morris JG, Jr., Calderwood SB, Camilli A. 2009. Cholera transmission: the host, pathogen and bacteriophage dynamic. *Nat. Rev. Microbiol.* 7:693-702
90. O'Hara BJ, Barth ZK, McKitterick AC, Seed KD. 2017. A highly specific phage defense system is a conserved feature of the *Vibrio cholerae* mobilome. *PLoS Genet.* 13:e1006838
91. Phillips KE, Satchell KJ. 2017. *Vibrio vulnificus*: From Oyster Colonist to Human Pathogen. *PLoS Pathog.* 13:e1006053
92. Pollack-Berti A, Wollenberg MS, Ruby EG. 2010. Natural transformation of *Vibrio fischeri* requires *tfoX* and *tfoY*. *Environ. Microbiol.* 12:2302–11
93. Polz MF, Hunt DE, Preheim SP, Weinreich DM. 2006. Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 361:2009-21
94. Preheim SP, Boucher Y, Wildschutte H, David LA, Veneziano D, et al. 2011. Metapopulation structure of Vibrionaceae among coastal marine invertebrates. *Environ. Microbiol.* 13:265-75
95. Preheim SP, Timberlake S, Polz MF. 2011. Merging taxonomy with ecological population prediction in a case study of Vibrionaceae. *Appl. Environ. Microbiol.* 77:7195-206
96. Purcell EM. 1977. Life at low Reynolds number. *Am. J. Phys.* 45:3-11
97. Quirke AM, Reen FJ, Claesson MJ, Boyd EF. 2006. Genomic island identification in *Vibrio vulnificus* reveals significant genome plasticity in this human pathogen. *Bioinformatics* 22:905-10
98. Rajanna C, Wang J, Zhang D, Xu Z, Ali A, et al. 2003. The *vibrio* pathogenicity island of epidemic *Vibrio cholerae* forms precise extrachromosomal circular excision products. *J. Bacteriol.* 185:6893-901
99. Reen FJ, Almagro-Moreno S, Ussery D, Boyd EF. 2006. The genomic code: inferring Vibrionaceae niche specialization. *Nat. Rev. Microbiol.* 4:697-704
100. Reynaud Y, Saulnier D, Mazel D, Goarant C, Le Roux F. 2008. Correlation between detection of a plasmid and high-level virulence of *Vibrio nigripulchritudo*, a pathogen of the shrimp *Litopenaeus stylirostris*. *Appl. Environ. Microbiol.* 74:3038-47
101. Salmond GP, Fineran PC. 2015. A century of the phage: past, present and future. *Nat. Rev. Microbiol.* 13:777-86
102. Salomon D, Klimko JA, Trudgian DC, Kinch LN, Grishin NV, et al. 2015. Type VI Secretion System Toxins Horizontally Shared between Marine Bacteria. *PLoS Pathog.* 11:e1005128
103. Seed KD, Faruque SM, Mekalanos JJ, Calderwood SB, Qadri F, Camilli A. 2012. Phase variable O antigen biosynthetic genes control expression of the major protective antigen and bacteriophage receptor in *Vibrio cholerae* O1. *PLoS Pathog.* 8:e1002917
104. Seed KD, Lazinski DW, Calderwood SB, Camilli A. 2013. A bacteriophage encodes its own CRISPR/Cas adaptive response to evade host innate immunity. *Nature* 494:489-91
105. Seed KD, Yen M, Shapiro BJ, Hilaire IJ, Charles RC, et al. 2014. Evolutionary consequences of intra-patient phage predation on microbial populations. *eLife* 3:e03497
106. Seitz P, Blokesch M. 2013. DNA-uptake machinery of naturally competent *Vibrio cholerae*. *Proc. Natl. Acad. Sci. USA* 110:17987-92
107. Seitz P, Blokesch M. 2014. DNA transport across the outer and inner membranes of naturally transformable *Vibrio cholerae* is spatially but not temporally coupled. *mBio* 5:e01409-14
108. Seitz P, Pezeshgi Modarres H, Borgeaud S, Bulushev RD, Steinbock LJ, et al. 2014. ComEA Is Essential for the Transfer of External DNA into the Periplasm in Naturally Transformable *Vibrio cholerae* Cells. *PLoS Genet.* 10:e1004066
109. Seymour JR, Amin SA, Raina JB, Stocker R. 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. *Nat. Microbiol.* 2:17065

110. Shapiro BJ, Friedman J, Cordero OX, Preheim SP, Timberlake SC, et al. 2012. Population genomics of early events in the ecological differentiation of bacteria. *Science* 336:48-51
111. Shapiro BJ, Levade I, Kovacikova G, Taylor RK, Almagro-Moreno S. 2016. Origins of pandemic *Vibrio cholerae* from environmental gene pools. *Nat. Microbiol.* 2:16240
112. Stocker R, Seymour JR. 2012. Ecology and physics of bacterial chemotaxis in the ocean. *Microbiol. Mol. Biol. Rev.* 76:792-812
113. Suckow G, Seitz P, Blokesch M. 2011. Quorum sensing contributes to natural transformation of *Vibrio cholerae* in a species-specific manner. *J. Bacteriol.* 193:4914-24
114. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, et al. 2015. Ocean plankton. Structure and function of the global ocean microbiome. *Science* 348:1261359
115. Suttle CA. 2005. Viruses in the sea. *Nature* 437:356-61
116. Szabo G, Preheim SP, Kauffman KM, David LA, Shapiro J, et al. 2013. Reproducibility of Vibrionaceae population structure in coastal bacterioplankton. *ISME J.* 7:509-19
117. Takemura AF, Chien DM, Polz MF. 2014. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. *Front. Microbiol.* 5:38
118. Takemura AF, Corzett CH, Hussain F, Arevalo P, Datta M, et al. 2017. Natural resource landscapes of a marine bacterium reveal distinct fitness-determining genes across the genome. *Environ. Microbiol.* 19:2422-33
119. Taylor RK, Miller VL, Furlong DB, Mekalanos JJ. 1987. Use of *phoA* gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. *Proc. Natl. Acad. Sci. USA* 84:2833-7
120. Thomas J, Watve SS, Ratcliff WC, Hammer BK. 2017. Horizontal Gene Transfer of Functional Type VI Killing Genes by Natural Transformation. *MBio* 8
121. Thompson FL, Iida T, Swings J. 2004. Biodiversity of vibrios. *Microbiol Mol Biol Rev* 68:403-31
122. Trucksis M, Michalski J, Deng YK, Kaper JB. 1998. The *Vibrio cholerae* genome contains two unique circular chromosomes. *Proc. Natl. Acad. Sci. USA* 95:14464-9
123. Udden SMN, Zahid MSH, Biswas K, Ahmad QS, Cravioto A, et al. 2008. Acquisition of classical CTX prophage from *Vibrio cholerae* O141 by El Tor strains aided by lytic phages and chitin-induced competence. *Proc. Natl. Acad. Sci. USA* 105:11951-6
124. Unterweger D, Miyata ST, Bachmann V, Brooks TM, Mullins T, et al. 2014. The *Vibrio cholerae* type VI secretion system employs diverse effector modules for intraspecific competition. *Nat. Commun.* 5:3549
125. Van der Henst C, Clerc S, Stutzmann S, Stoudmann C, Scrignari T, et al. 2017. Molecular insights into *Vibrio cholerae*'s intra-amoebal host-pathogen interactions. *bioRxiv (preprint)* https://doi.org/10.1101/235598
126. Van der Henst C, Scrignari T, Maclachlan C, Blokesch M. 2016. An intracellular replication niche for *Vibrio cholerae* in the amoeba *Acanthamoeba castellanii*. *ISME J.* 10:897-910
127. Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, et al. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304:66-74
128. Vezzulli L, Grande C, Reid PC, Helaouet P, Edwards M, et al. 2016. Climate influence on *Vibrio* and associated human diseases during the past half-century in the coastal North Atlantic. *Proc. Natl. Acad. Sci. USA* 113:E5062-71
129. Waldor MK, Mekalanos JJ. 1996. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 272:1910-4
130. Waldor MK, Tschäpe H, Mekalanos JJ. 1996. A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. *J. Bacteriol.* 178:4157-65
131. Weill FX, Domman D, Njamkepo E, Tarr C, Rauzier J, et al. 2017. Genomic history of the seventh pandemic of cholera in Africa. *Science* 358:785-9

132. Werner KM, Perez LJ, Ghosh R, Semmelhack MF, Bassler BL. 2014. *Caenorhabditis elegans* recognizes a bacterial quorum-sensing signal molecule through the AWC^{ON} neuron. *J. Biol. Chem.* 289:26566-73
133. WHO. 2016. World Health Organization: Cholera, 2015. *Weekly Epidemiological Record* 91:433-40
134. Wozniak RA, Fouts DE, Spagnoletti M, Colombo MM, Ceccarelli D, et al. 2009. Comparative ICE genomics: insights into the evolution of the SXT/R391 family of ICEs. *PLoS Genet.* 5:e1000786
135. Wozniak RA, Waldor MK. 2010. Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nat. Rev. Microbiol.* 8:552-63
136. Yawata Y, Cordero OX, Menolascina F, Hehemann JH, Polz MF, Stocker R. 2014. Competition-dispersal tradeoff ecologically differentiates recently speciated marine bacterioplankton populations. *Proc. Natl. Acad. Sci. USA* 111:5622-7
137. Yip ES, Grublesky BT, Hussa EA, Visick KL. 2005. A novel, conserved cluster of genes promotes symbiotic colonization and sigma-dependent biofilm formation by *Vibrio fischeri*. *Mol. Microbiol.* 57:1485-98
138. Yooseph S, Nealson KH, Rusch DB, McCrow JP, Dupont CL, et al. 2010. Genomic and functional adaptation in surface ocean planktonic prokaryotes. *Nature* 468:60-6
139. Zahid MS, Udden SM, Faruque AS, Calderwood SB, Mekalanos JJ, Faruque SM. 2008. Effect of phage on the infectivity of *Vibrio cholerae* and emergence of genetic variants. *Infect. Immun.* 76:5266-73
140. Zettler ER, Mincer TJ, Amaral-Zettler LA. 2013. Life in the "plastisphere": microbial communities on plastic marine debris. *Environ. Sci. Technol.* 47:7137-46

TERMS AND DEFINITIONS LIST

- Horizontal gene transfer (HGT): the transfer of DNA between microbes that does not follow vertical transmission.

- Mobile genetic element (MGE): Gene or gene clusters that can move within an organism's genome or horizontally to other organisms.

- Genomic island: Genome region that is part of the dispensable genome and differs in GC-content compared to the rest of the genome.

- Pathogenicity island (PAI): Genomic island that encodes virulence factors. Mostly present in pathogenic bacteria and absent in non-pathogenic members of same species.

- Integrative and conjugative elements (ICE): MGEs that reside in bacterial chromosomes but maintain their ability to excise and transfer to a new host by conjugation.

- Superintegron (SI): Gene-capturing system for novel gene cassettes that leads to their storage on a large chromosomal island.

- Type VI secretion system (T6SS): A molecular killing device used by bacteria to translocate effector proteins into neighboring cells.

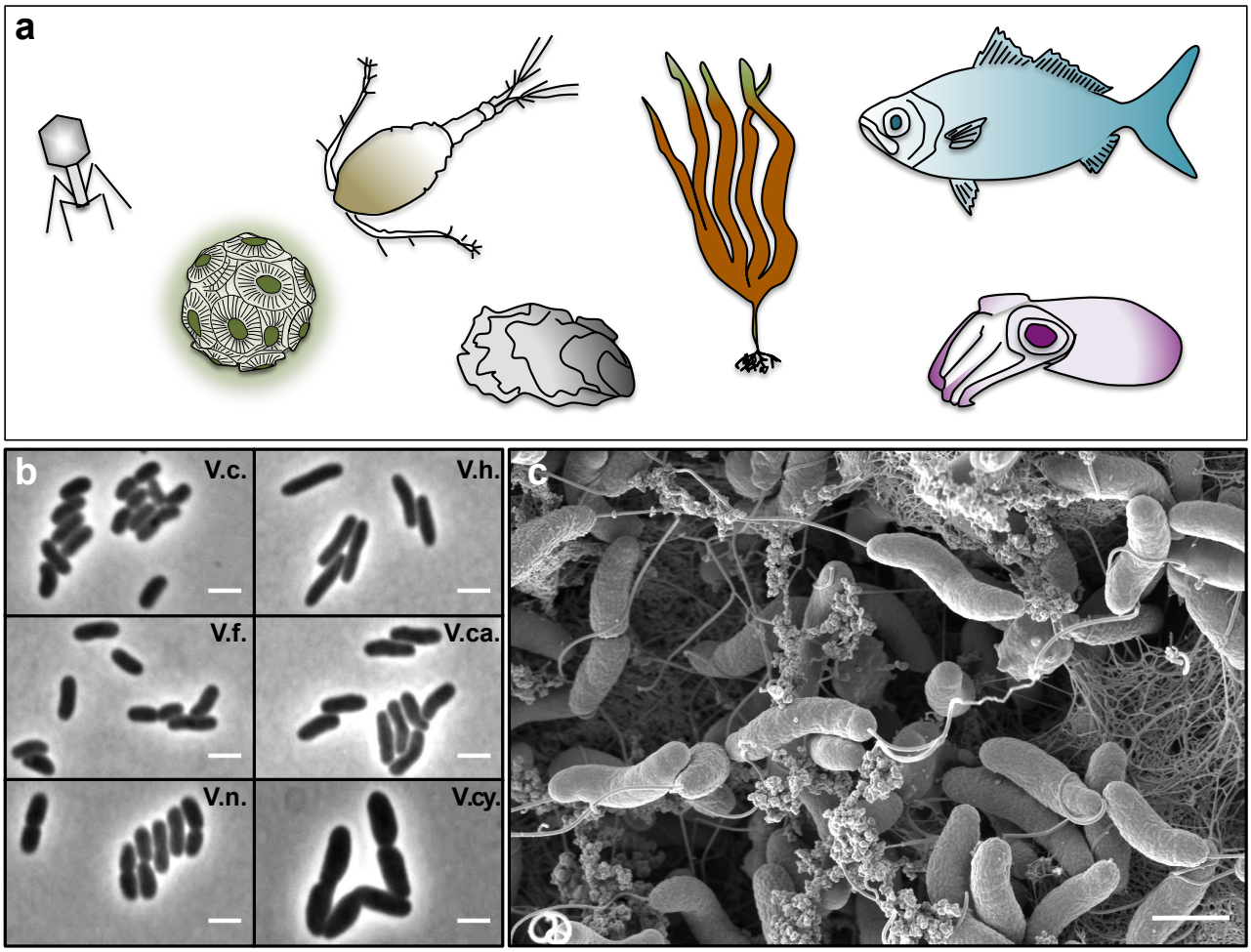
FIGURE LEGENDS

Figure 1: The *Vibrio* universe. (a) In the aquatic environment, vibrios engage in organismal interactions, which can include phages, phyto- and zooplankton, mollusks, algae, squid, and fish (from left to right). (b) Different *Vibrio* species imaged by light microscopy. V.c., *V. cholerae*; V.h., *Vibrio harveyi*; V.f., *Vibrio fischeri*; V.ca., *Vibrio campbellii*; V.n., *Vibrio natriegens*; V.cy., *Vibrio cyclitrophicus*; Scale bar = 2 μm. (c) Biofilm formation by *V. cholerae* on chitinous surfaces. Scanning electron micrograph. Scale bar = 1 μm.

Figure 2: *Vibrio* population structures provide a framework to explore the functional unit of virulence. Despite genetic diversity, vibrios are divided into phylogenetic clades (symbolized by different colors; middle) with habitat preferences (left). Habitats: free-living/planktonic (blue), associated with particles (yellow/green) or with larger organisms (red). For most human pathogens, virulence is caused by strains that emerged after HGT and clonal expansion. In the case of oysters, populations can be the functional unit of virulence.

Figure 3: Genome architecture and plasticity in vibrios. (a) Medium genome size of the indicated *Vibrio* species: *V. cholerae* [29], *V. anguillarum* [11], *V. vulnificus* [12], *V. parahaemolyticus* [17], *V. alginolyticus* [11], *V. alginolyticus* [11], *V. natriegens* [4], *V. coralliliticus* [4], *V. campbellii* [6]. Numbers in parenthesis indicate the number of closed genomes analyzed for each species. (b) Representation of the two chromosomes of *V. aestuarianus* strain 02_041. The outmost circle shows genes that are unique to this species (yellow bars; flexible genome) or common with other vibrios (n= 229; dark blue). The 2nd circle corresponds to the location of mobility-mediating genes (e.g., transposases and integrases). The inner circle shows the GC skews. The location of the integrase gene and therefore the beginning of the superintegron on the small chromosome is indicated by the red arrow (adapted from (47)).

Figure 4: Chitin-induced neighbor predation fosters HGT. Chitin, derived from the exoskeletons of zooplankton, is the most abundant polysaccharide in marine environments and a preferred carbon source of most vibrios. The bacteria chemotact towards these surfaces following a gradient of chitin degradation products [$(GlcNAc)_2$]. After attachment and microcolony formation, vibrios form biofilms in which the bacteria are in close proximity to each other. Under such conditions, *V. cholerae* produces its type VI secretion system (T6SS) killing device (middle), which allows predation of non-kin neighboring cells. As the natural competence-induced DNA-uptake complex is concomitantly produced (right), the prey released genetic material is absorbed by *V. cholerae*, which can lead to its transformation. Images above the scheme show the different stages: Scanning electron micrographs of motile (left image) or biofilm-forming bacteria (second image from left). Green fluorescently labeled T6SS inside chitin-induced *V. cholerae* cells (imaged by light microscopy; second image from right). Fluorescent micrograph of T6SS-attacked prey cells (green) that round up and lyse. Lysed cells release their genetic material, which serves as transformation material for the attacking *V. cholerae* (red; right image).



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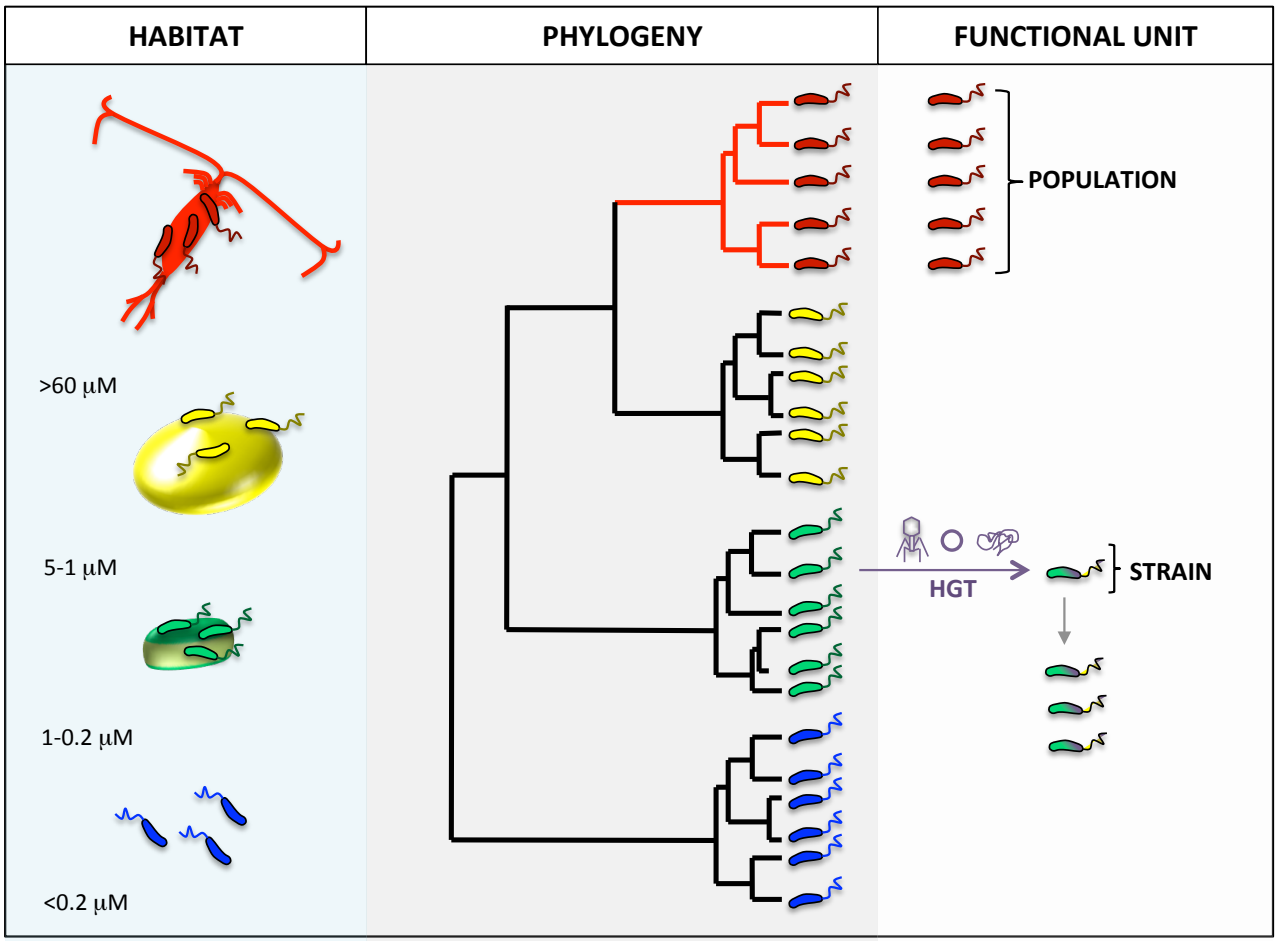

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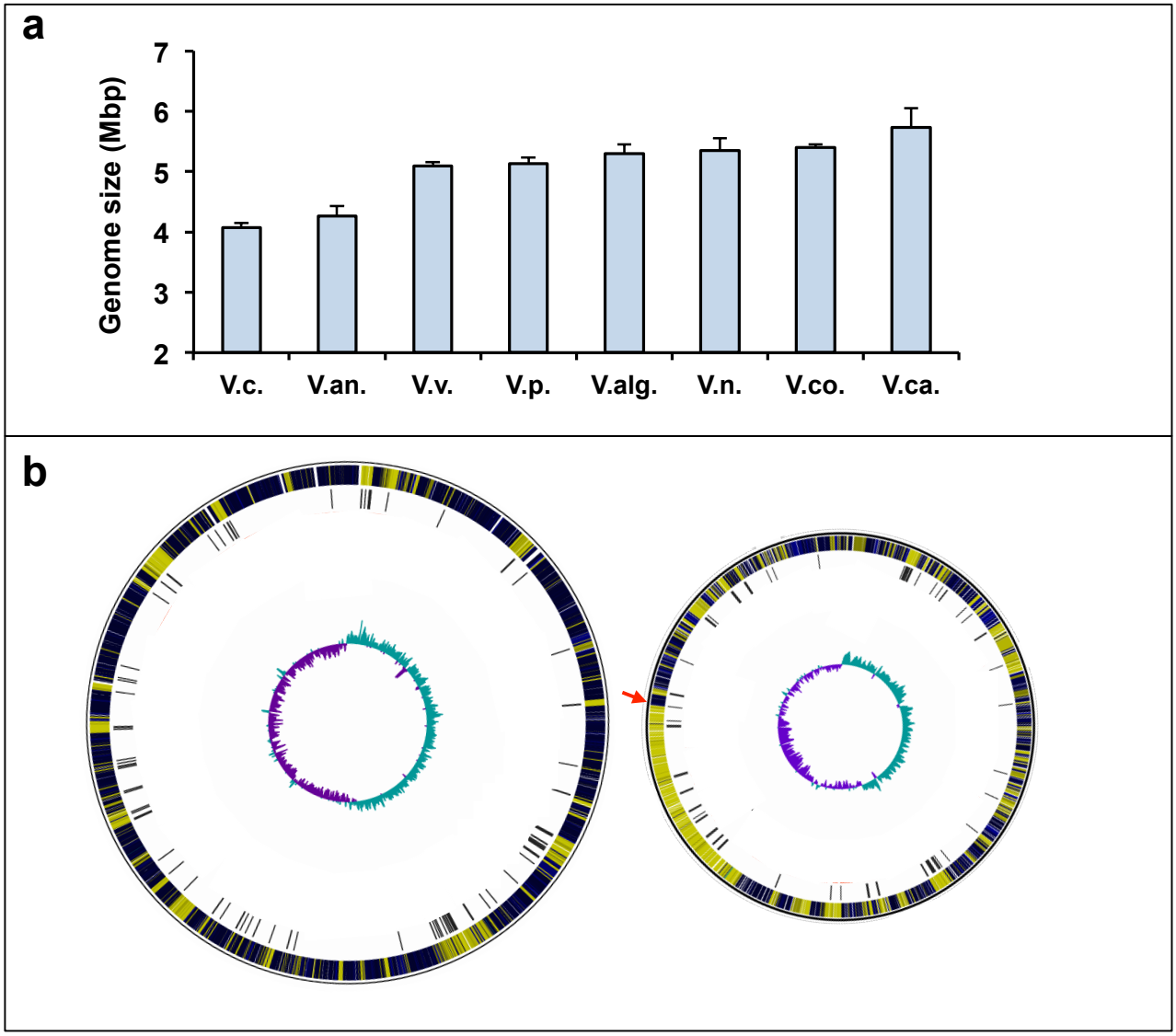

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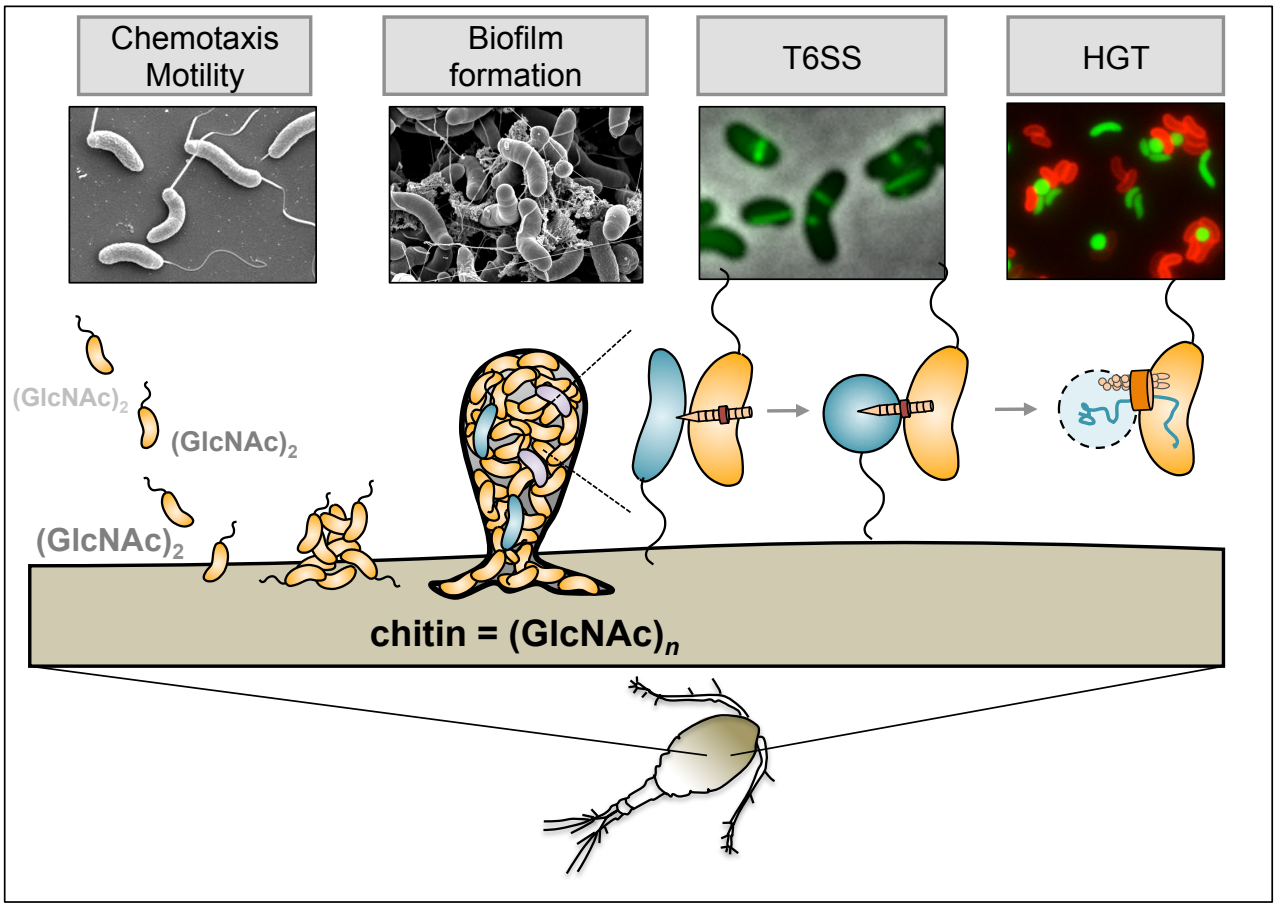

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