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The impact of *Vibrio fischeri* strain variation on host colonization

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Strain-level epidemiology is a key approach to understanding the mechanisms underlying establishment of any host-microbe association. The squid-vibrio light organ symbiosis has proven to be an informative and tractable experimental model in which to discover these mechanisms because it involves only one bacterial species, *Vibrio fischeri*. In this horizontally transmitted symbiosis, the squid presents nutrients to the bacteria located in a bilobed light-emitting organ, while the symbionts provide bioluminescence to their host. To initiate this association, *V. fischeri* cells go through several distinct stages: from free-living in the bacterioplankton, to forming a multicellular aggregation near pores on the light organ's surface, to migrating through the pores and into crypts deep in the light organ, where the symbiont population grows and luminesces. Because individual cells must successfully navigate these distinct regions, phenotypic differences between strains will have a strong impact on the composition of the population finally colonizing the squid. Here we review recent advances in our understanding of behavioral characteristics that differentially drive a strain's success, including its effectiveness of aggregation, the rapidity with which it reaches the deep crypts, and its deployment of type VI secretion.

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Introduction

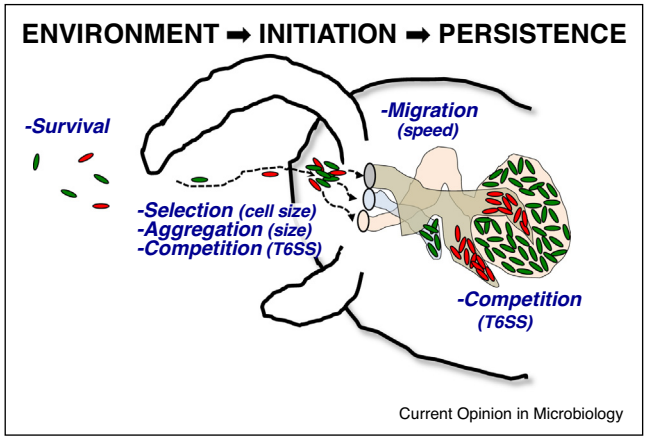
The squid-vibrio symbiosis has been a powerful model for identifying and deciphering the mechanisms by which strain-level differences ultimately impact the horizontal transmission of a symbiont. The newly hatched bobtail squid, *Euprymna scolopes*, has an aposymbiotic light organ, which becomes colonized by harvesting a few *Vibrio fischeri* cells present in the ambient seawater [1]. Different bacterial species, including *V. fischeri*, enter the mantle cavity as seawater is drawn across the gills and the ciliated surface of the nascent light organ. Ciliary activity delivers bacterium-sized particles to a location near the light organ's pores, where they form aggregates (Figure 1) [1,2]. *V. fischeri* cells pass into the pores on the nascent light organ's surface, and migrate through different micro-environments until a few cells reach and colonize the crypts (Figure 1) [3]. Strains isolated from wild-caught *E. scolopes* have shown distinct phenotypic traits [4,5], genomic composition [6] and competition behaviors [7••,8••]. Here, we present an overview of recent discoveries explaining the roles these differences play in determining colonization efficiency and effectiveness that drive symbiont population biology in the host.

Dominant and sharing strains

Swiftness of colonization

When squid are experimentally co-inoculated with different symbiont strains, two behaviors were observed: (i) a dominant strain ('D' strain; corresponding to the previously described 'A-type' strains [4]) would be found as the only one colonizing the squid, or (ii) a sharing strain ('S' strain) would share the light organ with another S strain [6]. Animals collected in the field are typically colonized by 6–8 strains [5]; surprisingly, in spite of this hierarchy of colonization dominance, some wild-caught squid harbor a mixture of both D and S strains. The co-occurrence of these two kinds of strains may be explained by a sequential encounter of different strains during the initial colonization of the juvenile host [7••]. In the laboratory, a D strain needed a shorter exposure time than an S strain to colonize >50% of exposed hosts [7••]. In addition, a D strain required less time than an S strain to migrate into the crypts, conferring a competitive priority effect (Figure 1). Such a priority effect has also been reported when two strains of *Borrelia burgdorferi* were co-inoculated into ticks [9], but has principally been described when different species compete for colonization of a host. For example, in the cnidarian-dinoflagellate symbiosis, a prior exposure to one species of alga gives it an advantages over the subsequent colonization by a more thermo-resistant species, even under elevated-temperature selection [10]. Similarly, among *Bacteroides* spp., a 'commensal colonization factor' is involved in priority effects of the bacteria during colonization of the mouse gut [11,12]. The presence and importance of competitive priority effects is understudied in many other models of simple or complex community symbioses, such as in the bee gut [13] and among and between the communities making up the human microbiome [14,15]. Thus, if the

Figure 1



Vibrio fischeri cells (green ovals) are found at different stages: free in the environmental bacterioplankton, colonizing the squid, and persisting in the crypts of the light organ (pink, brown and light blue). The dashed arrows indicate the trajectory of the bacterial strains during colonization of the juvenile squid. Different activities (dark blue) influence the bacteria, depending on which stage they are in. In this figure, only the left side of the light organ is shown.

squid encounters an S strain sufficiently sooner than a D strain, it may become colonized by both [7••]. The mechanism(s) underlying this timing advantage remains unknown, especially because it is not simply a case of swimming speed (D strains swim more slower in a soft-agar medium [5]), and the bacterial migration pathway passes through several tissue microenvironments [3] that are not yet possible to reproduce experimentally.

Population dynamics between the planktonic and symbiotic environments

The first parameter that influences the composition of the light organ population is the strain diversity in the environment [16]. Both D and S strains are typically found in adult squid [5] and, thus, will be in the ambient bacterioplankton to which the hatchling squid are exposed. However, if the D strains have such an advantage over the S strains during colonization [6], and the light organ is essentially the bacterium's only growth environment [16], one would predict that D strains will eventually sweep the bacterioplankton population. One mitigating factor would be if D strains are less fit in the environment, and decrease in relative abundance over time; this hypothesis was experimentally supported when cells of D (but not S) strains become non-culturable after 48 h in natural ocean water [4]. Thus, an ecological trade-off may occur in which S strains survive better in the bacterioplankton, but D strains are more effective colonizers. As a result, even in an environment containing more S than D strains, a sequential encounter with the juvenile squid would create conditions for stochastic colonization by both types of strains [7••]. Finally, it is ecologically significant that only a miniscule portion of the millions of symbionts that an adult releases each dawn into the bacterioplankton will have an opportunity to colonize a juvenile squid, emphasizing the importance of the symbiont's population biology both in the host and in seawater [16,17].

Aggregation behavior during colonization

Bacterial specificity

On either side of the nascent light organ of a newly hatched *E. scolopes*, there are two surface appendages that are covered by ciliated fields [3]; the activity of these fields moves seawater in the mantle cavity, winnowing bacteria-sized particles into an accumulation zone near the light organ's pores [1]. At bacterial concentrations typical of seawater, *V. fischeri* and certain other Gram-negative species attach to the short cilia found in this zone [18] forming aggregates of a few cells (Figure 1). If these cells are *V. fischeri*, they specifically induce host responses and chemotax toward the light organ's pores [19]. In contrast, all tested Gram-positive bacteria did not form such aggregates [2,20••]. If *V. fischeri* cells are added at a concentration above that found in seawater, the bacteria begin to attach to each other, forming aggregates of hundreds to thousands of cells [2]. Some Gram-negative species (e.g. *Vibrio campbellii* strain KNH1) form larger aggregates than symbiotic *V. fischeri* strains [20••]; however, when co-occurring with other species, *V. fischeri* cells interfere in an unknown way with aggregation by the other species [2]. Thus, while there is no direct correlation between the size of an aggregate and the colonization capability or efficiency of a bacterium, this step, together with flagellar motility and chemotaxis, is a necessary step in the selection of the correct bacterial species [21–23]. Although the symbionts appear to be passive participants in their accumulation at the pores [1], they must subsequently detach from the aggregates and proceed to and through the pores to reach the crypts [18,24••].

Aggregation behavior has been observed for a number of different strains of symbiotic *V. fischeri*, revealing a range of sizes and speeds of aggregation [20••]. While there is no direct correlation between the number of cells in an aggregate and the ability to compete for colonization, strains producing an aggregate above a certain size seem to have an advantage. In fact, while the more rapid detachment and subsequent migration of cells from the aggregates into the crypts [7••] appears to explain at least some of the dominant behavior, the expression of other as yet unknown adaptability traits specific to D strains may also play a role.

Regulation of aggregate formation

In *V. fischeri* aggregation is dependent on the expression of the symbiosis polysaccharide (*syp*) locus, which encodes capsule-synthesis genes, and is under a complex regulation that includes a number of factors that function upstream of the proximal regulatory protein, SypG

Table 1

Regulation of the *V. fischeri syp* locus

Regulatory factor		Type[a]	Effector/activator(s)	Effect on *syp* transcription	Other effects[b]	Homologs present in:	Reference
SypG	VFA1026	RR	SypF; BinK	+	nd[c]	other *Vibrio spp*	[25]
SypE	VFA1024	RR	SypF	–	post-transcr.	*V. fischeri*	[21]
SypA	VFA1020	STAS	SypE	–	post-transcr.	other *Vibrio spp*	[25]
SypF	VFA1025	HSK	RscS; HahK	+	nd	other *Vibrio spp*	[25]
RscS	VFA0237	HSK	nd	+	nd	certain *V. fischeri*	[30••]
HahK	VFA0072	SK	HnoX	+	nd	other Proteobacteria	[36]
BinK	VFA0360	SK	nd	–	nd	other *Vibrio spp*	[31]
HnoX	VFA0071	S	nitric oxide	–	HahK (-)	other Proteobacteria	[24••]
–	–		Ca^{2+}	+	Bcs (+)	–	[36]

[a] HSK (hybrid sensor kinase); RR (response regulator); S (sensor); SK (sensor kinase); STAS (anti-sigma factor antagonist and sulfate transporter domain).
[b] Downstream targets other than the *syp* locus; Bcs = bacterial cellulose synthesis.
[c] nd (none determined).

(Table 1) [26,27]. Studies of this regulatory pathway have been confined to the *V. fischeri* symbiont strain ES114 and its mutant derivatives [21]. The first regulator of symbiont aggregation (and biofilm formation in culture) to be identified was RscS [28,29]. In various *V. fischeri* strains, *rscS*, is either present, absent or frameshifted [30••]. Specifically, RscS is not required for host colonization by several symbiont strains, since they colonize the squid even though they don't encode RscS, or their *rscS* gene contains an inactivating frame-shift; nevertheless, they remain dependent on the downstream *syp* locus. For instance, the D strain MB13B2 forms large aggregates even with a frame-shifted *rscS*, but loses that phenotype if the structural gene *sypQ* is mutated [20••]. This finding indicated that RscS isn't the only factor controlling Syp-dependent biofilm formation [21]. In fact, a second key regulator is HahK, which, like RscS, activates the SypF sensor kinase upstream of SypG. In addition to these positive regulators, the negative regulator BinK, which reduces SypG function and antagonizes RscS action [31], appears to be present and functional in all *V. fischeri* strains examined [30••]. Even at this stage in our understanding of this tightly controlled pathway, the complexity of the system indicates *V. fischeri* aggregate/biofilm production is sensitive to both recognized, and as yet unknown, signals from the abiotic and biotic environment. Niche colonization by many symbionts involves-specific aggregation behavior as described, for example, in the reproducible spatial distribution of distinct gut microbes in the zebrafish [32]. Aggregation-driven colonization resistance by the normal microbiota has also been reported to protect against vaginal infection by inhibiting *Trichomonas vaginalis* adhesion to host cell [33]. Interestingly, *Fusobacterium nucleatum* protects itself from clearance by adhering to a specific previously attached species as part of its integration into the oral microbial community [34].

In addition to these internal regulatory proteins, external nutrient and salt conditions play a modulating role in biofilm formation [35]. In particular, two-specific extracellular factors have been shown to control the extent of biofilm formation: calcium is an activator of *syp* gene expression [36], while nitrite oxide (NO) is an inhibitor [24••]. Interestingly, the presence of BinK is sufficient to prevent calcium's ability to induce biofilm in wild-type cells [36]. In addition, calcium increased the expression of a newly discovered bacterial cellulose-based biofilm through the *bcs* locus, which was also dependent on the regulator SypF. Nitric oxide synthase is secreted by the host and is present in the mucus, where it produces NO and affects bacterial production of NO-detoxifying activity in the aggregates [37,38]. In addition, host-derived NO serves as a signal to inhibit the formation of Syp polysaccharide, allowing the bacteria to dissociate and migrate from the aggregate and into the crypts of the light organ [24••,27]. The degree to which symbiotic *V. fischeri* cells aggregate and/or dissociate varies from strain to strain, a difference that is likely to contribute significantly to their relative success at squid colonization.

Type VI secretion system (T6SS) activity

The T6SS is a cell-contact mechanism that can be deployed by one bacterium to kill another [39]; such antagonistic behavior provides a fitness advantage during strain–strain competition for nutrients or ecological niches, especially during symbiosis [40]. T6SS also has a role both in shaping the normal host microbiota and in pathogenesis in complex communities [41]: for example, *Vibrio cholerae* uses a T6SS to attack the pre-existing gut microbiota to facilitate its colonization [42]. The impact of T6SSs in shaping bacterial communities raises the question of its involvement in the colonization of the squid by its symbiont. Two T6SSs have been identified in *V. fischeri* [8••]. Homologs of one system, T6SS1, are present in all strains studied but their specific function has not been described yet [8••]. The other one, referred to as T6SS2, is required for killing of conspecific strains when tested in a culture-based co-incubation assay [8••].

Homologs of genes encoding this system have been demonstrated in half of the 32 *V. fischeri* strains examined and, intriguingly, only some of the strains isolated from a given light organ would harbor it [8••]. Significantly, the carriage of this T6SS2 and the dominance phenotype are not correlated; in fact, this system is encoded by certain sharing or dominant strains, as well as some strains of *V. fischeri* that are incapable of colonizing the light organ. At present, six groups of compatible strains have been identified, where incompatible strains must be spatially separated from each other or else one will eliminate the other [8••]. These antagonistic interactions can effect colonization of the squid: in the rare event where two incompatible *V. fischeri* strains reached the same light organ crypt (Figure 1), the T6SS was involved in killing the strain that didn't encode it [8••]. A question of future interest is whether the T6SS2 is involved in strain selection as early as the aggregation step of the squid colonization. Except for when they are attached to particles [43], planktonic *V. fischeri* cells will rarely encounter each other in the environment; however, because the host concentrates them there [1,2], an aggregate provides a time and location at which they would likely come into contact.

Conclusion

Strain variation is a key factor in understanding the epidemiology of host colonization. Here, we described three behaviors — dominance, aggregation, and antagonism — that are employed to different degrees by strains of *V. fischeri* that are successful light organ symbionts. Other behaviors, such as bioluminescence and chemotaxis, while not described here, also play critical roles in the squid-vibrio association. Each of these mechanisms can be shown to contribute to a strain's fitness in laboratory assays, but because the symbionts are horizontally transmitted, and encounter different sets of conditions and selective pressures in both their symbiotic and their planktonic niches, evolutionary success is not dependent on only one mechanism. In addition, while this review focuses on strain-dependent behaviors during the initiation of symbiosis, there are distinct and equally stringent behaviors required for bacterial persistence in the association [44,45]. Understanding the dynamics of these interacting mechanisms, and the trade-offs different strains make, is fundamental to understanding the population biology of symbiosis.

Conflict of interest

The authors declare no conflict of interests.

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