Ecological Populations of Bacteria Act as Socially Cohesive Units of Antibiotic Production and Resistance

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the reported TS_{DIR} and TS_{CT} structures located along the BLA coordinate on opposite sides of the CI appear to be a manifestation of the geometric-phase theorem.

The key to understanding the origin of the thermal noise in rod photoreceptors is the existence of two electronically different TSs, with the lower displaying the same charge-transfer character as the Rh excited state. This is a consequence of the properties of the chromophore electronic wavefunction in the region of the CI (*27*, *28*, *30*). Therefore, the Barlow correlation represents a manifestation of the existence of a CI in Rh and complements the evidence provided by spectroscopic studies (*3*–*5*, *9*). Without this CI, the thermal isomerization would be controlled by the TS_{DIR} barrier and, therefore, high visual sensitivity would be achieved at the red edge of the visible spectrum rather than the blue. Further evidence supporting this theory could be provided by the observation of an "anti-Barlow" correlation (i.e., a decrease of $-\log k$ as a function of $1/\lambda_{max}$) for mutants or pigments containing PSB11 but absorbing radiations shorter than 470 nm.

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Supplementary Materials

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Ecological Populations of Bacteria Act as Socially Cohesive Units of Antibiotic Production and Resistance

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In animals and plants, social structure can reduce conflict within populations and bias aggression toward competing populations; however, for bacteria in the wild it remains unknown whether such population-level organization exists. Here, we show that environmental bacteria are organized into socially cohesive units in which antagonism occurs between rather than within ecologically defined populations. By screening approximately 35,000 possible mutual interactions among Vibrionaceae isolates from the ocean, we show that genotypic clusters known to have cohesive habitat association also act as units in terms of antibiotic production and resistance. Genetic analyses show that within populations, broad-range antibiotics are produced by few genotypes, whereas all others are resistant, suggesting cooperation between conspecifics. Natural antibiotics may thus mediate competition between populations rather than solely increase the success of individuals.

The ratio of intra- versus interspecific competition is a key element regulating populations and determining their success within diverse communities. It is especially important in structured animal and plant populations, in which closely related individuals live in patches and encounter each other often (*1*). In these cases, modulation of intraspecific antagonism or cooperation can mitigate the detrimental effects of niche overlap. However, for bacteria in the wild it has been postulated that populations merely represent loose assemblages of individuals driven by ecological opportunity (*2*, *3*). The reasons given include high dispersal rates and rapid horizontal gene transfer (HGT), which can both rapidly erode population structure by mixing unrelated individuals and introducing novel, potentially advantageous genes to their genomes. This may initiate a dynamic process of rapid but locally and/or temporarily limited expansion of individuals (clones). A classical example of such interactions is interference competition via colicin-type bacteriocins (*4*, *5*), which are almost always encoded by plasmids and are able to kill closely related competitors in a highly specific manner. In these cases, population dynamics are primarily driven by the cyclic invasion of antibiotic production and resistance genes. Similarly, a recent high-throughput screen of mutual interactions among soil isolates indicated changing types of interactions occur over relatively short evolutionary distances. This was interpreted as short-lived dynamics of gene gain and loss, in which antibiotic production selects resistance, which subsequently promotes loss of production and reversion to sensitivity (*6*). In contrast to this gene-centric view of bacterial population dynamics, recent fine-scale environmental mapping of bacterial diversity has suggested that population structure may exist beyond individual clones. Such ecologically defined populations consist of phylogenetic clusters of closely related but nonclonal individuals, which share common ecological associations (*7*, *8*). However, it remains unknown whether individuals within such populations interact sufficiently strongly to allow for

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the development of cohesive population-level social organization akin to structured animal and plant populations.

We asked whether ecologically defined populations show social cohesion beyond association with similar sets of resources. We reasoned that an obvious and important test case would be interference competition mediated by antibiotic production. This required mapping the network of potential antagonistic interactions between bacteria onto their fine-scale genotypic structure in the environment. Thus far, such an exercise has been impeded by the lack of data on genotypically and ecologically delineated natural microbial populations. In recent years, however, we have taken bacteria of the family Vibrionaceae as a model for the ecology and evolution of bacterial populations (*8*–*10*). These populations were originally identified by using an unsupervised machine learning approach that combines genetic similarity and micro-habitat specialization so as to cluster clades with cohesive ecology (*8*) in a way that is agnostic to any preconceived notion of species. The populations thus identified consist of groups of individuals coexisting in micro-habitats and closely related in protein housekeeping gene sequences (genotypic clusters). Many of these clusters cannot be differentiated by the most commonly used marker for phylogenetic classification of microbes, the ribosomal RNA gene, suggesting recent evolutionary age (*8*). Although these genotypic clusters are distinguished by their propensity to occur as free-living or associated with different types of suspended organic particles and zooplankton (*8*, *11*), they co-occur in the guts and on the surfaces of larger marine animals (*11*). Because of their genotypic cohesion and differential environmental distributions, these clusters are hypothesized to represent natural populations and provide a platform to inquire whether factors beyond similarity in environmental association enforce population structure.

We first determined the potential for interference competition among individuals from different populations by measuring antagonistic interactions in an all-against-all framework using 185 *Vibrio* isolates that have been characterized to high genetic resolution by the sequencing of several protein-coding, housekeeping genes (table S2). We used the classic Burkholder plate assay (*12*, *13*), which allows testing for local growth inhibition between bacteria co-plated on nutrient agar (*14*). Testing inhibition in this way provides somewhat realistic conditions for ocean bacteria because interference competition is most likely to occur among individuals coexisting on particle surfaces or in the guts of animals, where local population density can be high (*13*). After screening ~35,000 possible interaction pairs, we obtained a large network containing 830 antagonistic interactions between naturally co-occurring *Vibrio* strains (individual genotypes). Replication of the antagonism assay for a selected number of strains showed this data to be very robust, with more than 97% replicability of interactions (*14*). The data show that nearly half (44%) of the strains were able to inhibit at least one other strain, whereas 86% were inhibited by at least one strain. A few (<5%) "super-killer" strains were able to inhibit more than 25% of all other strains in the collection (Fig. 1A). Using a membrane diffusion method (*14*), we estimate that 96% of the antagonistic interactions are mediated by small molecules and not by proteins such as bacteriocins.

Mapping the network of antagonistic interactions onto the fine-scale genotypic structure of the tested strains shows that the potential for interference competition is much lower within natural populations than between them. This is expressed by the conditional probability of observing an antagonistic interaction as a function of genetic distance, $P(A|d)$ (Fig. 1B), where distances were computed on the basis of a concatenated alignment of six housekeeping genes. $P(A|d)$ has a sigmoidal shape, with a 75 to 80% reduction in the probability of observing antagonism over relatively short genetic distances. Despite the strong influence that super-killer strains have on these data, this trend holds when considering only narrow-range antagonists with ≤5 inhibited strains (fig. S1), and the bias to long-distance killing is statistically significant ($P = 0.001$) even when controlling for phylogenetic structure and killing activity per strain (*14*). The sharp increase in $P(A|d)$ at a threshold distance indicates that a natural genetic boundary exists for interference competition. This boundary coincides almost exactly with the average value of d between strains in different populations, d_{pop}—that is, the average genetic boundary between ecologically cohesive genotypic clusters determined in previous studies (Fig. 1C) (*8*, *10*). This means that antagonism occurs mostly between rather than within natural *Vibrio* populations.

The observed low antagonism within populations is not a result of resistance between near-clonal strains as would be expected from dynamics of clonal expansion followed by gradual gene loss (*6*). Although the *Vibrio* populations consist of isolates with high sequence similarity in the set of shared genes, there is considerable gene content diversity between strains. In 41 sequenced genomes representing 10 *Vibrio* populations (table S4), we find that although populations are clustered by gene content, the average percentage of shared genes between genomes at distances $< d_{pop}$ is only 72% (fig. S2). Moreover, these genomes are highly recombinogenic and show no evidence of a clonal origin (*15*). This implies that the pattern of low intrapopulation antagonism is not likely to be explained by simple vertical inheritance and gene loss; rather, this pattern is generated and maintained in a regime of fast allelic turnover and potential for losing and acquiring new genes.

To further explore whether antibiotic production might have coevolved with populations or was horizontally acquired, we increased the isolate sampling around the most prolific super-killer

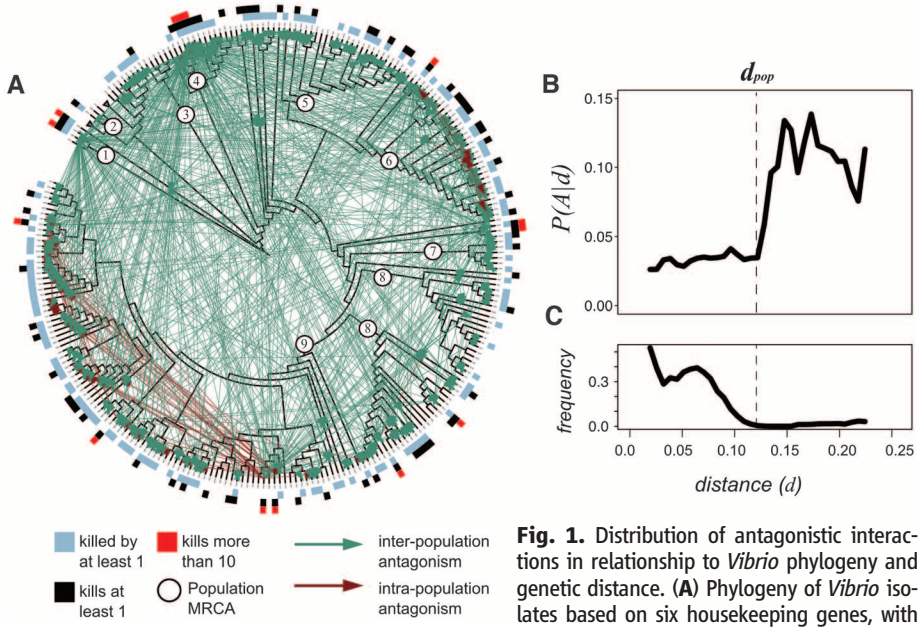

Fig. 1. Distribution of antagonistic interactions in relationship to *Vibrio* phylogeny and genetic distance. (**A**) Phylogeny of *Vibrio* isolates based on six housekeeping genes, with outer, colored rings highlighting antagonists and sensitive strains. Green arrows connect antagonists to sensitive strains. Circles identify the most recent common ancestor (MRCA) of previously identified ecologically cohesive populations: 1, *V. ordalii*; 2, *V. fischeri*; 3, *V. breoganii*; 4, *V. alginolyticus*; 5, *V.* sp. F12; 6, *V. crassostreae*; 7, *V. cyclotrophicus*; 8, V. tasmaniensis; 9, *V. splendidus*. (**B**) The conditional probability of antagonism as a function of genetic distance, $P(A|d)$, shows that antagonistic interactions occur mostly between strains whose genetic distance exceeds a critical threshold. This threshold coincides with the average distance between previously defined populations (dashed line). (**C**) Frequency distribution of within-population genetic distances, showing that the transition point for $P(A|d)$ matched the average boundary of populations.

in our collection, strain 12B09, belonging to a population of *V. ordalii*. We added a tight cluster of 29 highly related coisolates ($d < 0.01$), which we used to study the population genomics of the super-killer phenotype. Using random transposon mutagenesis, we identified the genetic basis of antibiosis in 12B09 and studied its evolution using whole-genome sequences of both producers and nonproducers. This genomic approach was complemented with chemical screening and identification of active compounds.

By screening a library of 20,000 12B09 transposon mutants against a sensitive indicator strain, we identified 10 mutants with no antagonistic activity, which all had transposon insertions within a hybrid polyketide synthase (PKS)/nonribosomal peptide (NRP) gene cluster (Fig. 2A). A genetic knockout of the central NRP biosynthesis gene shows complete loss of activity, demonstrating a single specific antibiotic biosynthesis cluster is responsible for the antagonistic activity. This is consistent with results obtained from screening a chemical extract from cell-free 12B09 supernatant separated by means of high-performance liquid chromatography, showing that 100% of the activity could be accounted for by a single peak. Accordingly, this peak was absent from the knockout mutant 12B09-HW44, which had no antibiotic activity (figs. S4 and S5). Genes in the PKS-NRP cluster possess sequence similarity to cyclic lipopeptide antibiotic synthases, which typically cause membrane depolarization or pore formation, triggering cell lysis (*16*).

Using the cluster of 29 highly related strains from the expanded *V. ordalii* population, we obtained a high-resolution all-against-all antagonism network comprising 91 strains subdivided roughly equally across three populations of *V. ordalii*, *V. crassostreae*, and *V. tasmaniensis* (table S3). This network revealed that the super-killer phenotype is present in a small fraction (5 out of 29) of the highly related strains within *V. ordalii* (Fig. 2B) and was absent in any of the other populations. Moreover, all *V. ordalii* strains were resistant, confirming our previous result at a much higher genetic resolution level. A polymerase chain reaction (PCR) screen with multiple specific primers diagnostic for the PKS-NRP biosynthetic cluster confirmed that this specific pathway is found in all of the five super-killers identified in the plate assays and not in any of the other *V. ordalii* strains (*14*). This was consistent with data from the 185 strain network (Fig. 1A) showing that individuals resistant to each other did not antagonize the same set of strains (Pearson's $\varphi = 0.04$). Overall, this result shows that the super-killer phenotype in the population is caused by differential presence/absence of genes, not by transcriptional regulation or silencing mutations.

The study of sequenced genomes from two super-killers and three resistant conspecifics confirmed our prediction that recent horizontal gene transfer mediated the acquisition of production and resistance genes in *V. ordalii*. Different lines of evidence supported this. First, a whole-genome phylogeny of the sequenced strains (fig. S3) showed that super-killers share a recent common ancestor, suggesting that the gene cluster is not ancestral to the population but that it was acquired in a single recent event. Second, BLAST (*17*) searches against public databases of fully sequenced genomes identified the antibiotic cluster only in two previously sequenced *Vibrio* isolates from the Pacific Ocean with very low genomic similarity to *V. ordalii*: the shellfish pathogen *V. tubiashii* and the particle-attached SWAT-3 (*14*, *18*). Third, a whole-genome alignment of 12B09 and SWAT-3 revealed a colinear and conserved fragment of 16.3 kb containing only the antimicrobial cluster, which indicated recent acquisition. Moreover, in SWAT-3 the antimicrobial peptide cluster is flanked by a large arrangement of transposases and integrases (Fig. 1B), suggesting that the cluster comprises a mobile element, which was recently acquired in different *Vibrio* populations across distant regions of the ocean. The resistance factors did not appear to be coded within the same mobile cluster because none of the genes in the cluster were present in the resistant but nonproducing *V. ordalii* strains. This suggested that these genes for antimicrobial production are unlinked from their resistance factors and can only invade in populations in which some individuals carry preadaptations that enable them to survive the acquisition of antimicrobial-production genes.

Contrary to the widespread idea that bacterial populations are driven by gene-centric and selfish dynamics, we have shown that ecological

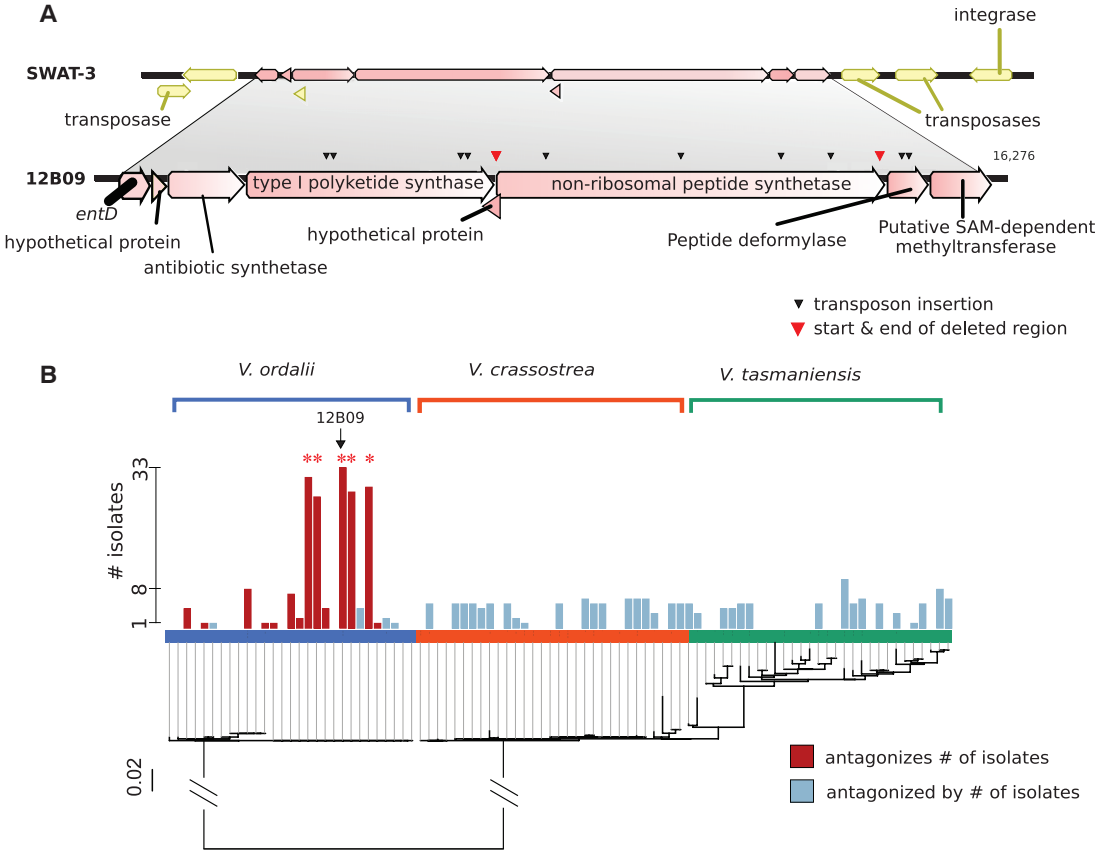

Fig. 2. Antimicrobial peptide cluster and its distribution in the *V. ordalii* population. (**A**) Alignment of NRP cluster from 12B09 showing transposon insertions and knocked-out gene, and homologous cluster from environmental isolate SWAT-3. The figure shows that in SWAT-3, a nearly identical cluster is flanked by transposases and integrases, which is consistent with the idea that the cluster comprises a mobile element. (**B**) Antibiotic activity of *V. ordalii* isolates against *V. ordalii*, *V. crassostreae*, and *V. tasmaniensis* populations. The phylogeny is based on the hsp60 genetic marker, and the bars next to each isolate indicate the number of isolates antagonized (red) and the number of *V. ordalii* isolates that antagonized the isolate (light blue). The red stars mark the five isolates in which the NRP gene cluster was detected by PCR.

populations defined by common microhabitat association also represent socially cohesive units. Although it remains unknown how widespread the observed phenomenon is, low frequency of antagonism within short genetic distances has also recently been observed among *Streptomyces* isolates (*6*). Our results indicate that similar to the case of marine vibrios, this pattern could reflect the ecological and genetic boundaries of structured populations and not a transient gene-centric dynamic (*6*). The fact that each antibiotic is produced by only a small fraction of the population whereas the rest is resistant supports the hypothesis that antibiotics can constitute public goods within populations, benefiting nonproducing but resistant conspecifics. Importantly, such social structure mediates competition between ecological populations rather than benefiting only the carrier of the antibiotic production gene. These results suggest that the ecological population structure of bacteria in the wild is much stronger than previously assumed.

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Supplementary Materials

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Transforming Fusions of *FGFR* and *TACC* Genes in Human Glioblastoma

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The brain tumor glioblastoma multiforme (GBM) is among the most lethal forms of human cancer. Here, we report that a small subset of GBMs (3.1%; 3 of 97 tumors examined) harbors oncogenic chromosomal translocations that fuse in-frame the tyrosine kinase coding domains of fibroblast growth factor receptor (FGFR) genes (*FGFR1* or *FGFR3*) to the transforming acidic coiled-coil (TACC) coding domains of *TACC1* or *TACC3*, respectively. The FGFR-TACC fusion protein displays oncogenic activity when introduced into astrocytes or stereotactically transduced in the mouse brain. The fusion protein, which localizes to mitotic spindle poles, has constitutive kinase activity and induces mitotic and chromosomal segregation defects and triggers aneuploidy. Inhibition of FGFR kinase corrects the aneuploidy, and oral administration of an FGFR inhibitor prolongs survival of mice harboring intracranial FGFR3-TACC3–initiated glioma. FGFR-TACC fusions could potentially identify a subset of GBM patients who would benefit from targeted FGFR kinase inhibition.

Chromosomal translocations leading to production of oncogenic fusion proteins are critical events in the pathogenesis of human cancer (*1*–*3*). To examine whether such alterations are present in the tumor glioblastoma multiforme (GBM), we used massively parallel, paired-end sequencing of expressed transcripts (RNA-seq) to detect gene fusions in short-term cultures of glioma stemlike cells (GSCs) freshly isolated from nine patients with primary GBMs. Using TX-Fuse, a methodology that detects split reads and split inserts (see supplementary materials and methods section and fig. S1A), we discovered six rearrangements (all of which were intrachromosomal) that gave rise to in-frame fusion transcripts (table S1). We validated five in-frame fusion predictions by direct sequencing of polymerase chain reaction (PCR) products spanning the fusion breakpoint (Fig. 1 and fig. S1, B to E).

In Fig. 1, A and B, we show the prediction and cDNA sequence validation, respectively, for the fusion with the highest read support involving *fibroblast growth factor receptor 3* (*FGFR3*) fused in-frame with *transforming acidic coiled-coil 3* (*TACC3*) in GSC-1123 and GBM-1123 primary tumor. The cDNA contained an open reading frame coding for a protein of 1048 amino acids resulting from the in-frame fusion of the FGFR3 N terminus (residues 1 to 758) with the TACC3 C terminus (residues 549 to 838) (Fig. 1C and fig. S2A). FGFR3 is a member of the *FGFR* receptor tyrosine kinase (TK) family (*4*), whereas TACC3 belongs to the evolutionarily conserved *TACC* gene family, which also includes TACC1 and TACC2. The distinctive feature of TACC proteins is a coiled-coil domain at the C terminus, known as the TACC domain, which mediates localization to the mitotic spindle (*5*, *6*). TACC proteins are hypothesized to be oncogenic in several human tumors, including GBMs (*7*, *8*).

In the predicted fusion protein, the intracellular TK domain of FGFR3 is fused upstream of the TACC domain of TACC3 (Fig. 1C). Exon-specific gene expression analysis from the RNA-seq coverage in GSC-1123 and quantitative reverse transcription PCR showed that the expression of the fused *FGFR3-TACC3* exons is higher in GSC-1123 than in other GSCs or the normal brain (80- to 130-fold) (fig. S2, B and C). The FGFR3-TACC3 fusion protein was abundantly expressed in GSC-1123 and GBM-1123, and

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