Interbacterial predation as a strategy for DNA acquisition in naturally competent bacteria

Veening, Jan-Willem; Blokesch, Melanie

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OPINION

Interbacterial predation as a strategy for DNA acquisition in naturally competent bacteria

Jan-Willem Veening and Melanie Blokesch

Abstract | Natural competence enables bacteria to take up exogenous DNA. The evolutionary function of natural competence remains controversial, as imported DNA can act as a source of substrates or can be integrated into the genome. Exogenous homologous DNA can also be used for genome repair. In this Opinion article, we propose that predation of non-related neighbouring bacteria coupled with competence regulation might function as an active strategy for DNA acquisition. Competence-dependent kin-discriminated killing has been observed in the unrelated bacteria *Vibrio cholerae* and *Streptococcus pneumoniae*. Importantly, both the regulatory networks and the mode of action of neighbour predation differ between these organisms, with *V. cholerae* using a type VI secretion system and *S. pneumoniae* secreting bacteriocins. We argue that the forced release of DNA from killed bacteria and the transfer of non-clonal genetic material have important roles in bacterial evolution.

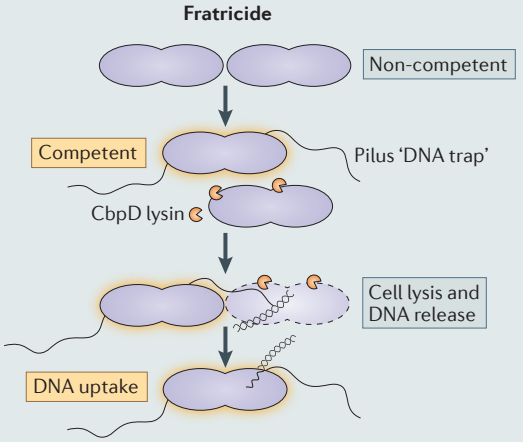
The emergence of new pathogens that have mosaic-like genomes[1], together with the increased spread of antibiotic resistance among bacteria, requires increased efforts into research on horizontal gene transfer (HGT). The three primary modes of HGT, phage transduction, conjugation and natural competence for transformation, enable the exchange of genetic material between bacteria[2]. Natural competence refers to a physiological state of a bacterium in which it is able to take up DNA from the environment[3]. Once this exogenous DNA reaches the cytoplasm, recombination with the genome of a competent bacterium might occur if there are sufficient homologous regions between the exogenous and host DNA. Natural competence for transformation is a widespread phenomenon among bacteria and several original publications and reviews have covered its distribution and regulation (see REFS 4–9).

Many bacteria enter the state of competence under specific conditions[6], but the evolutionary function of competence remains controversial[7]. Several suggestions for a function have been made, such as a source of nutrients (that is, nucleotide components) or phosphate, acquiring DNA for genome repair or for the acquisition of novel genetic material for evolution. These functions might not be mutually exclusive but could be species-specific or context-dependent. Moreover, the quality of the exogenous DNA substantially contributes to its fate after absorption by competent bacteria. Free DNA in the environment is often heavily fragmented[10] and is therefore unsuitable for the transfer of intact genes or larger operons, which, in theory, argues against a primary role of natural competence in genome repair or HGT.

Although the transfer of DNA from one bacterium to another through phage transduction and conjugation heavily depends on a donor cell, a common assumption is that natural competence is solely driven by the acceptor bacterium without the action of a DNA-releasing donor bacterium. In this Opinion article, we argue that neighbour predation coupled with competence induction might contribute to the transfer of genetic material from a donor cell to a competent acceptor bacterium. This strategy ultimately drives genome repair (or maintenance) and evolution. Previous studies on competent *Streptococcus pneumoniae* that provided evidence for bacterial fratricide support a hypothesis of DNA repair[11,12]. Fratricide is a form of killing in which closely related bacteria or even genetically identical bacteria are targeted by a competence-activated killing factor (BOX 1). Interestingly, recent studies on two unrelated naturally competent opportunistic pathogens, the Gram-negative bacterium *Vibrio cholerae*[13] and the Gram-positive bacterium *S. pneumoniae*[14,15], demonstrated that competence could also be linked to kin-discriminated interbacterial killing. Consequently, such neighbour predation leads to the transfer of non-clonal genetic material, which favours the hypothesis of evolution over genome maintenance. In this Opinion article, we also discuss the inducing signals, regulatory networks and predatory mechanisms, including the type VI secretion system (T6SS) and bacteriocins, of these bacterial species. Despite the regulatory and mechanistic differences between both systems, the biological outcomes are similar, resulting in a common biological theme. For example, environmental triggers, such as quorum sensing molecules, have an important role in the activation of competence in both species and DNA that is released by prey is subsequently absorbed through conserved DNA-uptake machineries. Therefore, we hypothesize that the coupling of competence and kin-discriminated killing functions as an active strategy for DNA acquisition. Others have suggested that the uptake of exogenous DNA is unlikely to result in the acquisition of beneficial traits in naturally competent bacteria, as the DNA is acquired from dead, and therefore less fit, cells. We speculate that the combined action of neighbour predation of non-related cells and DNA uptake is used by competent bacteria to target living and niche-adapted cells. The mechanisms that underlie kin-discriminated predation linked with natural competence and their role in bacterial evolution will also be discussed.

Box 1 | **Fratricide in *Streptococcus pneumoniae***

Competence has been linked to the killing of kin, or so-called fratricide, in which a competent subpopulation of cells produces killing factors that lyse their non-competent siblings[12,108] (see the figure). In *Streptococcus pneumoniae*, activation of competence triggers the expression of *cbpD*, which encodes a cell wall-degrading enzyme or autolysin. Competent cells also express ComM, which provides immunity against CbpD[109,110]. Competence-controlled cell wall-degrading muralytic enzymes, such as CbpD, are present in almost all streptococci. Strains that do not encode CbpD, such as *Streptococcus gordonii*, contain an unrelated autolysin, LytF[111]. It was recently proposed that pneumococcal competence initiates stochastically in single cells and then spreads through cell–cell contact[49]. Activation of competence in a subset of cells in a clonal population might result in the lysis of non-competent cells and thus enable the uptake of intact highly homologous DNA, which can then be used for DNA repair[112,113]. Alternatively, activation of competence in the entire pneumococcal population coupled to the secretion of CbpD might be sufficient to target surrounding, but not yet competent, *S. pneumoniae*, thereby liberating their genomic DNA. The released DNA can then be captured through the DNA-uptake complex present on competent pneumococci that contains a micrometre-sized type IV pilus-like structure[114]. Notably, similar to kin-discriminated killing by bacteriocins, CbpD can also target non-clonal cells, and this predatory mechanism can increase the efficiency of horizontal gene transfer in *S. pneumoniae*, *Streptococcus mitis* and *Streptococcus oralis*[92].



Competence induction and regulation

V. cholerae, the causative agent of cholera, is an important model bacterium for the study of virulence regulation, biofilm formation and quorum sensing. *V. cholerae* inhabits aquatic environments in which it frequently associates with the exoskeletons of zooplankton[16]. Such exoskeletons are mainly composed of chitin, which is a polymer that consists of long chains of *N*-acetylglucosamine. Growth to high cell densities on these chitinous surfaces induces natural competence in *V. cholerae*[17]. The induction of competence in *V. cholerae* relies primarily on three signals (reviewed in REF. 8): chitin sensing (signalled through the main regulator of transformation in *V. cholerae*, TfoX), the secondary messenger cyclic adenosine monophosphate (cAMP; which is bound by the cAMP receptor protein (CRP) and accumulates in the absence of preferred carbon sources)[18–20] and a high abundance of secreted autoinducer molecules, which enable *V. cholerae* to monitor population density through quorum sensing (FIG. 1).

The sensing of chitin degradation products (*N*-acetylglucosamine oligomers) by the membrane-bound proteins ChiS[21,22] and TfoS[23,24] triggers the chitin signalling cascade (FIG. 1a). ChiS is an orphan two-component system sensor kinase, for which the precise mode of action is unknown[21,22,24]. TfoS is a membrane-bound transcriptional regulator that has a large periplasmic domain and a cytoplasmic AraC-type DNA-binding domain[23,24]. Following the binding of chitin oligomers to the periplasmic domain, TfoS induces the expression of the small RNA *tfoR*[23,24]. TfoR binds to *tfoX* mRNA, which is under the control of the cAMP–CRP complex[20]. Following TfoR binding, the Shine–Dalgarno sequence of the *tfoX* transcript becomes accessible to ribosomes, resulting in the synthesis of the TfoX protein[25]. TfoX is a homologue of the Sxy protein of *Haemophilus influenzae* and is the major regulator of natural competence for transformation in both organisms[17,26]. The synthesis of TfoX in *V. cholerae*, in conjunction with the formation of the cAMP–CRP complex[18], leads to increased transcript levels of chitin catabolism genes[17] and genes that encode components of the DNA-uptake complex[27] (labelled as competence genes I in FIG. 1a). Most of these genes, as well as competence genes II (described below), also receive input from the nucleoside scavenging protein CytR[28]. Neither the molecular mechanisms of this regulation nor the biological link between CytR and chitin catabolism, or alternatively DNA uptake, are fully understood.

On reaching a high cell density, in which autoinducers are abundant in the extracellular environment, the main regulator of quorum sensing in *V. cholerae*, HapR, is synthesized[29,30]. HapR and TfoX are both required for the expression of *qstR*, which encodes a quorum sensing and TfoX-dependent regulator[31]. QstR subsequently initiates the expression of a second subset of competence genes (labelled as competence genes II in FIG. 1a) that are essential for DNA uptake[19,27,31–33]. Concurrently, HapR also represses the expression of a gene that encodes the extracellular nuclease Dns, thereby preventing the degradation of exogenous DNA, which can be acquired by competent *V. cholerae*[8,19,34,35] (FIG. 1a).

V. cholerae produces two main autoinducer molecules that integrate into the quorum sensing system, autoinducer 2 (AI-2) and cholera autoinducer 1 (CAI-1). AI-2 is a spontaneously rearranged derivative (with or without boron for *Vibrio* spp. and enteric bacteria, respectively) of the precursor molecule 4,5-dihydroxy-2,3-pentanedione (DPD)[30,36]. Owing to the interconverting nature of these derivatives, AI-2 molecules are thought to form a universal inter-species cell–cell communication system[37]. Indeed, the DPD synthase LuxS is found in more than 500 species of bacteria, including both Gram-positive and Gram-negative bacteria[30].

CAI-1((*S*)-3-hydroxytridecan-4-one) of *V. cholerae* is used for species-specific or genera-specific communication[38]. CAI-1 autoinducer synthase (CqsA) is present in all *Vibrio* species (reviewed in REF. 30); however, CAI-1 varies with respect to acyl chain length and other modifications in different species[30]. Consequently, different affinities exist for the binding of CAI-1 and its derivatives by the cognate sensory proteins in different species[39]. Therefore, some crosstalk (signals that can also be detected by other members of the same genus) may occur between closely related *Vibrio* spp.[39,40], which is reminiscent of the 'dialects' of competence-inducing oligopeptides in Gram-positive bacteria. Efficient chitin-induced competence in *V. cholerae* is dependent on the presence of CAI-1 when grown on chitinous surfaces, as the bacterium does not respond efficiently to AI-2 alone (at least when grown in

monoculture)[19,35]; therefore, it was suggested that CAI-1 acts as a bona fide competence pheromone[35] (FIG. 1a).

S. pneumoniae (also known as the pneumococcus) is an opportunistic human pathogen that colonizes the human nasopharynx. The nasopharynx constitutes a polymicrobial environment and humans are frequently colonized by more than one pneumococcal strain[41–43]. From this niche, pneumococci can spread and cause non-invasive disease (for example, inner ear infection and sinusitis) and invasive disease (for example, pneumonia, bacteraemia and meningitis)[44]. To initiate invasion, pneumococci need to persistently colonize the nasopharynx and successfully compete with other bacteria that occupy the same niche.

In *S. pneumoniae*, competence is controlled by a quorum sensing system that is based on the secretion and sensing of extracellular competence-stimulating peptides (CSPs; the gene product of *comC*) (FIG. 1b). After a threshold level of CSP in the extracellular environment has been reached, the membrane-bound histidine kinase ComD autophosphorylates and activates the response regulator ComE[45]. Phosphorylated ComE then activates the expression of several genes, including the alternative sigma factor SigX. SigX then activates the expression of genes that are required for DNA uptake and integration into the bacterial chromosome[46]. Competence in *S. pneumoniae* can be triggered by several environmental conditions, such as high cell density, changes in pH and several antibiotics[47,48]. All of these conditions result in changing the concentration of CSPs that is required to activate gene expression (effective concentration)[48]. Interestingly, competence of a small subset of cells can also be transmitted through cell–cell contact[49]. Together, the combination of cell–cell contact and diffusion and sensing of CSP through classical quorum sensing ensures that most pneumococci in the population only become competent during stress or when there is a high local cell density[48]. Among *S. pneumoniae* strains, there are only two major types of CSP autoinducer (the ComC proteins; FIG. 1b), which indicates that most pneumococci can respond to each other's competence signals[50]. Other species in the genus *Streptococcus*, such as *Streptococcus pyogenes*, *Streptococcus mutans*, *Streptococcus equinus* (formerly known as *Streptococcus bovis*) and *Streptococcus suis*, produce various different CSPs (denoted ComS in these organisms or *sigX*-inducing peptide (XIP) after processing)[7], which therefore enables species-specific communication among streptococci[51,52].

Evolutionary implications

Once the competence cascade is activated, both *V. cholerae* and *S. pneumoniae* produce a DNA-uptake complex, which enables them to actively take up genetic material from their extracellular environments[5,7,53]. The evolutionary benefits of natural competence and, in particular, DNA uptake, have been extensively discussed in the past[6,7,54–56]. In most of these discussions, three main benefits were considered: 'DNA for food', 'DNA for genome repair or maintenance' (recently supported by the notion that transformation might function as a curing system against selfish mobile genetic elements[57], which, conversely, can themselves interfere with natural transformability of the host bacterium[58]) and 'DNA for evolution' (which defines natural transformation as a mode of HGT). Consistent with the involvement of starvation-mediated signals in the onset of natural competence in some naturally transformable bacteria, it has long been argued that newly acquired DNA can provide nutrition to the cell, such as nucleotides that can be recycled for DNA synthesis (as *de novo* synthesis of nucleotides is very costly) or further shuttled into central metabolism[59–61] and therefore immediately benefit competent bacteria[62]. Moreover, a major argument that has been put forward against the 'DNA for genome repair' and 'DNA for evolution' hypotheses, and therefore in favour or the 'DNA for food' idea, has been that transformation occurs through the uptake of genetic material that is derived from dead cells that have "died due to natural selection" and contain on average more mutations than surviving bacteria[63]. Nonetheless, comparative genomics has provided strong evidence for highly frequent horizontal gene transfer in *V. cholerae*[64] and between related *Streptococcus* spp.[65–67]. For *Streptococcus* spp., the evolutionary advantages of HGT are direct. For example, rapid spread of antibiotic resistance was observed when people took antibiotics, as shown by whole-genome sequencing of more than 3,000 pneumococcal carriage strains isolated from individuals in a 2.4 km^2 refugee camp[67]. Furthermore, the introduction of vaccines that target the pneumococcal capsule resulted in a rapid increase in immune escape 'capsule switch' strains that have altered capsule types, owing to an increased incidence of horizontal transfer of capsule operons[68,69]. The incredible genetic plasticity observed in opportunistic human pathogens underlines the importance of HGT, and thus competence, in bacterial evolution.

Neighbour predation

Following biofilm growth on chitinous surfaces, pandemic *V. cholerae* strains induce the expression, biosynthesis and assembly of a T6SS[13], which is a molecular killing device that shows structural similarity to inverted contractile phage tails[70]. Precisely, the T6SS is a macromolecular complex that consists of an outer and inner membrane-spanning core complex to which a tubular cytoplasmic structure is attached. This inner tube is wrapped by outer sheath proteins, which, on contraction, propel the tube out of the bacterium. Attached to the tip of this molecular spear are T6SS effector proteins (for example, toxins), which, owing to the contraction force, are transported into neighbouring cells. Several types of T6SS effector protein have been identified[71] and it is the activity of these specific effectors, and not the mechanical puncturing, that causes the lysis and death of target cells[70]. Previous studies have shown that non-pandemic strains of *V. cholerae* propel their constitutively active T6SSs in random directions[72], which was also confirmed for pandemic strains, in which the T6SS is chitin-induced[13]. Theoretical calculations suggested that smaller distances between *V. cholerae* (as a predator) and its prey result in a higher likelihood of a successful T6SS strike, whereas larger distances between adjacent cells are less likely to have a successful strike[70], which highlights the benefits of inducing the T6SS at high cell densities in surface-attached biofilms. Importantly, T6SS-mediated attacks do not kill kin, owing to the production of immunity proteins, which neutralize the translocated effector proteins[73–77]. Indeed, recent work showed that kin cells can even reuse a subset of the translocated T6SS components for T6SS reassembly[78].

When the immunity proteins that protect against the major effector proteins of the T6SS of *V. cholerae* were identified[74], it became clear that they are typically encoded adjacent to the respective effector-encoding genes[79] (FIG. 2). A bioinformatics approach was used to further investigate and characterize the T6SS gene clusters of diverse *V. cholerae* isolates (37 isolates collected during the past 77 years). It was found that the encoded effector–immunity gene pairs often differ between isolates and that their

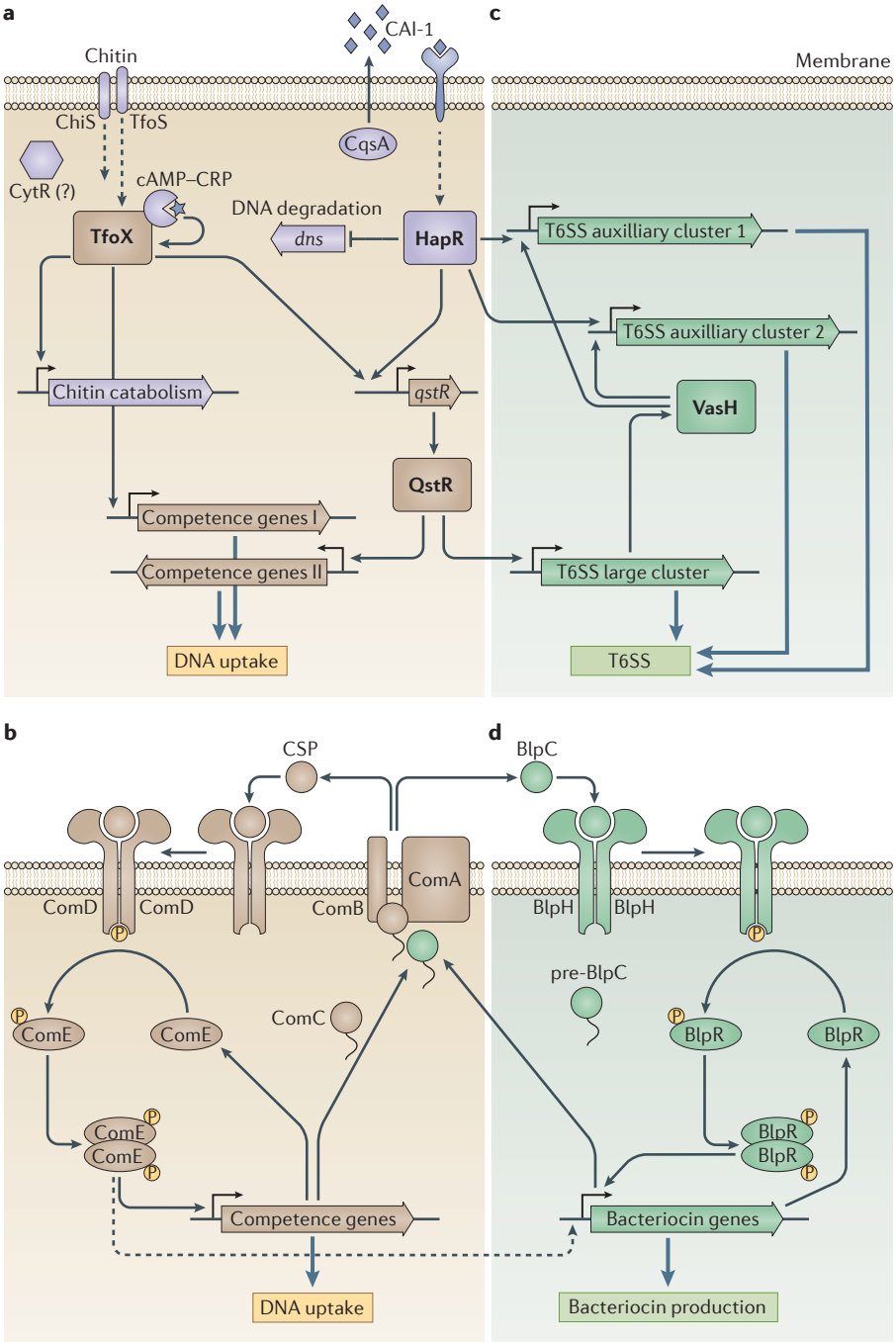
observed lower GC content than the rest of the genome is indicative of recent horizontal acquisition[79] (FIG. 2). Consequently, strains of *V. cholerae* that have different effector–immunity gene pairs are unable to protect against the action of the T6SS effector proteins of the other strains[79] and therefore undergo T6SS-mediated interbacterial killing. The horizontal movement of effector–immunity gene clusters was recently also predicted *in silico* for other *Vibrio* spp., such as *Vibrio alginolyticus* and *Vibrio parahaemolyticus*[80].

In contrast to the T6SS of *V. cholerae*, *S. pneumoniae* uses bacteriocins for neighbour predation. Bacteriocins are small heat-stable antimicrobial peptides that are secreted by both Gram-negative and Gram-positive bacteria. Similar to the T6SS effector proteins of *V. cholerae*, bacteria that produce bacteriocins have specific immunity mechanisms in place to prevent cell lysis[81–85]. Bacteriocins are diverse and are classified into two classes: the class I peptides, which are typically post-translationally modified and can contain, for example, polycyclic thioether amino acids such as lanthionine, making them very stable; and the class II peptides, which are unmodified[83].

S. pneumoniae strains can express more than seven different bacteriocins, including both class I and class II peptides[86,87]. Although the activities of bacteriocins are mainly targeted towards other *Streptococcus* spp., they can also target others such as *Listeria* spp., *Lactococcus* spp. and *Micrococcus* spp.[87–89]. Although it is thought that most bacteriocins target cells through a receptor-mediated mechanism, the identity of the receptor is unknown for most bacteriocins[90]. It is also unclear how far bacteriocins can diffuse in the human nasopharynx. Experiments in which susceptible cells were cultured in the presence of bacteriocin producers on agar plates showed relatively large zones of clearance, which indicates that cell killing that is observed *in vitro* is not limited to bacteria that are in close proximity[87]. The precise role that bacteriocins have in human colonization remains unknown, but *in vitro* overlay assays combined with genetic analyses on the class II 'Blp' bacteriocins demonstrated no correlation between bacterial inhibition and co-colonization in the human nasopharynx[91]. Notably, when the production of bacteriocin is triggered in *S. pneumoniae*, genes that are required for immunity are also activated, which protects kin from being killed by these bacteriocins. Although fratricide specifies 'killing of brothers' (that is, kin) and was first discovered in *S. pneumoniae* monocultures, in principle, the fratricide effectors that are induced by competent cells can also target non-competent non-kin cells[92]. It should be noted that fratricins are typically cell wall-degrading enzymes, whereas bacteriocins typically cause pore formation in the cytoplasmic membrane. Thus, both competence-induced fratricins and bacteriocins can augment horizontal gene transfer through the acquisition of non-clonal DNA (BOX 1; FIG. 3).

Linking competence with predation

Although natural competence and neighbour predation have been investigated separately for many years, recent studies have highlighted a regulatory and phenotypic link between these biological processes (FIG. 4). Specifically, it was demonstrated that the T6SS of *V. cholerae* is part of the chitin-induced competence regulon (that is, the T6SS is co-induced by TfoX, HapR and QstR; FIG. 1a,c)[13]. Therefore, the coupling of interbacterial predation and



DNA uptake enables the bacterium to absorb recently released genetic material from neighbouring prey attacked by the T6SS, as was directly visualized through time-lapse microscopy of competent *V. cholerae*[13]. This observation contradicts the general assumption that transforming DNA is short in length owing to extensive degradation by abundant extracellular nucleases, especially after extended persistence of such DNA in the environment[10]. Instead, killing an adjacent cell using the T6SS might enable *V. cholerae* to initiate 'controlled' killing of the prey, as T6SS effectors often attack the cell wall of other bacteria[71]. Degrading the cell wall ultimately results in the lysis of the prey cell, followed by the release of its intact genetic material, which can subsequently function as transforming material and be taken up by the DNA-uptake complex of the predator[13]. Interestingly, and in contrast to other T6SS-containing bacteria that are non-transformable, such as *Agrobacterium tumefaciens*[93], a bioinformatics analysis of the effector proteins of 37 sequenced strains of *V. cholerae* showed that nucleases are uncommon T6SS effector proteins in *V. cholerae* isolates[79]. We hypothesize that this finding underlines the importance of maintaining the released DNA of the prey intact. Indeed, despite hundreds of *V. cholerae* genome sequences being present in various genome databases, T6SS effector proteins that have nuclease-containing domains (toxin_43 domain; PF15604)[93] have only been identified in two environmental isolates of *V. cholerae*[93,94] (strain CT 5369–93, an isolate from sewage in Brazil, and strain HE48, an environmental isolate from Haiti[95]) and therefore seem to be rare.

In addition to *V. cholerae*, a molecular link between the competence regulatory network and kin-discriminated interbacterial competition through the production of bacteriocins has been found in *S. pneumoniae*[14,15] (FIG. 4), *S. mutans* and *Streptococcus gordonii*[96–99]. Indeed, for *S. pneumoniae*, the class II Blp bacteriocins are under competence control[14,15] (FIG. 1b,d). Competence and the Blp bacteriocins are controlled by a dual quorum sensing system: first, CSP is secreted and sensed (see above; FIG. 1b), which is followed by the secretion and sensing of a second quorum sensing peptide, BlpC. Once a threshold concentration of mature BlpC has been reached and sensed by the BlpH histidine kinase receptor, and the response regulator BlpR has been phosphorylated by BlpH, a positive feedback loop is activated, which results in more BlpC being produced. This ultimately results in the enhanced expression of bacteriocins (FIG. 1d). This signalling cascade ensures that pneumococci only become competent and produce bacteriocins during stress or when there is a high local cell density. Therefore, such kin-discriminated neighbour predation through Blp bacteriocins might have an important role in active DNA acquisition and the exchange of intact DNA, similar to *V. cholerae*.

A recent study analysed more than 4,000 pneumococcal genomes and early findings show that the presence of the genes that encode both CSP and BlpC is highly conserved, with 99% of strains encoding both quorum sensing peptides[100]. However, BlpC was found to be more polymorphic than CSP, with 20 different mature forms of BlpC being identified[101]. Interestingly, preliminary experimental data showed extensive crosstalk and 'eavesdropping' (the detection of multiple signals by cells) between BlpC variants[100]. As bacteriocin and immunity-encoding genes are co-regulated, eavesdropping will result in the activation of bacteriocin production to defend against predators and might also induce immunity against the incoming bacteriocins.

◀ Figure 1 | **Linking the regulation of competence-induced DNA uptake and the production of killing factors in *Vibrio cholerae* and *Streptococcus pneumoniae*. a** | *Vibrio cholerae* induces natural competence following growth to high cell densities (HCD) on chitinous surfaces[17]. Chitin is sensed through membrane receptors (ChiS[21,22] and TfoS[23,24]), which indirectly (dotted arrows) leads to the production of the major transformation regulatory protein of *V. cholerae*, TfoX. The induction and effect of TfoX on downstream genes require the presence of the cAMP–cAMP receptor protein complex (cAMP–CRP complex)[18,20]. The production of TfoX leads to increased transcript levels of genes that are involved in chitin catabolism and a subset of competence genes (labelled as competence genes I)[13,17]. Cell density is sensed by quorum sensing through the production and sensing of the primary autoinducer cholera autoinducer 1 (CAI-1)[38]. Under conditions of HCD, the quorum sensing regulator HapR is produced. HapR represses the nuclease gene *dns*[34] and, in conjunction with TfoX, induces the production of the transcription factor QstR[31]. QstR subsequently induces further competence genes (labelled as competence genes II)[13,31], which, together with a subset of competence genes I, encode the DNA-uptake complex[27]. CytR, through an unknown mechanism, is also involved in the TfoX-dependent induction of chitin catabolism and competence genes[28]. For further details see REF. 8. **b** | In *S. pneumoniae*, the competence-stimulating peptide (CSP) is produced at a basal level by readthrough from an upstream tRNA locus[115]. ComC is processed and exported to mature CSP by the ComA–ComB complex. CSP is sensed by the histidine kinase ComD, which then phosphorylates the response regulator ComE[45]. ComE, in turn, activates transcription of the gene that encodes the alternative sigma factor SigX, which then activates the genes that are required for DNA uptake and integration. When the local concentration of CSP increases (for example, by more cells producing and secreting CSP or by antibiotics that increase *comCDE* copy numbers)[102], a positive feedback loop is activated and all of the cells in the population will produce CSP. **c** | The production of the type VI secretion system (T6SS) is co-regulated with the DNA-uptake complex in pandemic isolates of *V. cholerae*[13]. The quorum sensing and TfoX-dependent transcriptional regulator QstR (see panel **a**) induces the expression of the major gene cluster that encodes the T6SS of *V. cholerae*[13]. After induction of the large cluster, the auxiliary cluster 1 and auxiliary cluster 2 are further induced through the RpoN-activating protein VasH[116,117]. **d** | Phosphorylated ComE (see panel **b**) induces SigX, the competence genes that encode the DNA-uptake complex and the *blp* gene cluster, which results in the production of BlpC. As most pneumococcal strains lack an intact cognate BlpC transporter[14], BlpC is transported across the membrane by the same transporter as CSP, the ComA–ComB complex. Once a threshold level of BlpC is reached, another positive feedback loop is activated and all of the cells produce BlpC to high levels and subsequently produce bacteriocins and their cognate immunity proteins.

Consequences of DNA acquisition

Several arguments have been made against the upholding of natural transformability in bacterial species for the purpose of acquiring genetic material for DNA repair or evolution. As outlined above, one of the main arguments against these hypotheses is that free DNA is mostly derived from dead cells that were less fit than cells that survived natural selection and are therefore unable to overcome specific selective pressures[63]. However, coupling natural competence to fratricide or kin-discriminated neighbour predation circumvents this argument. Indeed, fratricide of *S. pneumoniae* (BOX 1) can even occur in clonal populations in which cell-to-cell variability in the timing until competence is switched on in the population is the main factor, which eventually determines the fate of the cell. Together with the observation that antibiotics and replication stress can induce competence in *S. pneumoniae*[47,102], the DNA repair hypothesis still holds, as under such conditions of genotoxic stress the acquisition of external DNA will ameliorate the stress.

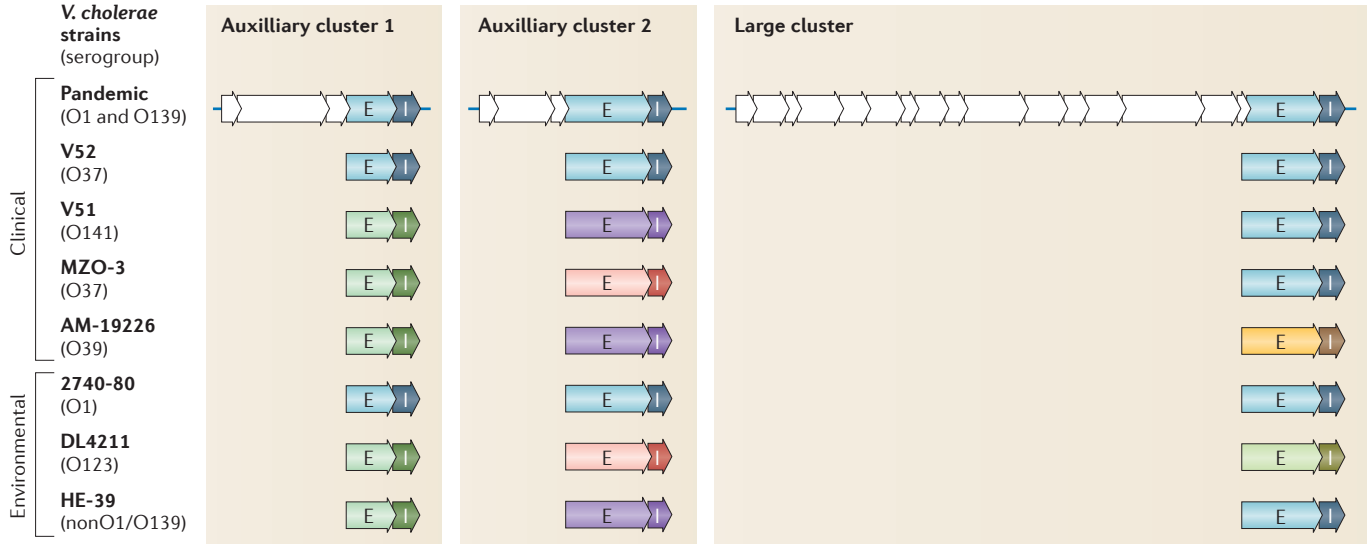
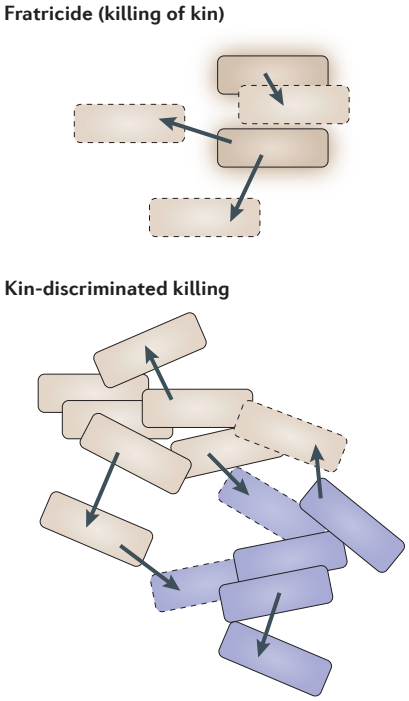


Figure 2 | **Diversity of effector–immunity gene pairs in different *Vibrio cholerae* strains.** The three type VI secretion system (T6SS)-encoding gene clusters of *Vibrio cholerae* are shown at the top of the figure. Each cluster encodes an effector (E) and immunity (I) gene, which often vary between isolates of *V. cholerae*. Representative strains are shown on the left together with their serogroup (O antigen numbers) and their source of isolation (clinical or environmental). The corresponding effector–immunity gene pairs are colour-coded depending on the cluster-specific family to which they belong, according to a recent classification[79]. This classification grouped the effector–immunity gene modules of 37 strains of *V. cholerae* into 15 families[79], which was recently extended to 19 groups on the basis of comparative genomics of more than 500 strains of *V. cholerae* and closely related non-cholera *Vibrio* spp.[104].

Moreover, T6SS-mediated or bacteriocin-mediated killing of prey is directed towards living bacteria. Indeed, experimental data provided evidence that the induction of the T6SS and the expression of bacteriocins enhance DNA exchange in *V. cholerae*[13] and *S. pneumoniae*[15], respectively, which suggests an important role for kin-discriminated killing in HGT. Importantly, recently released prey DNA has not yet undergone heavy fragmentation by surrounding nucleases. Therefore, in this model, this non-fragmented DNA could bring several genes and larger genomic islands to the predator cell. Therefore, we hypothesize that the link between neighbour predation and DNA uptake might represent a common biological theme for naturally competent bacteria and we expect that future studies will address this intriguing hypothesis.

We argue that an important feature of kin-discriminated killing and competence is that, for both *Vibrio* spp. and *Streptococcus* spp., the horizontal transfer of novel T6SS effectors and immunity[79,80,103], and bacteriocin–immunity gene pairs[101], respectively, could result in an immediate fitness advantage to the transformed predator. For a certain period of time after DNA transfer, the transformant might 'memorize' its protection from kin (owing to its immunity status), while the onset of expression of the novel horizontally-acquired T6SS effector–immunity genes (or bacteriocin–immunity genes) could benefit the transformant in two ways. First, to attack and outcompete its kin[103] and, second, and maybe more importantly, to acquire immunity against potential attacks that are exerted by the kin of the prey. Notably, a recent comparative genomics study showed that the exchange of effector–immunity gene modules in *V. cholerae* might not always be precise, as the old gene pair is not always fully replaced by the novel gene pair. Instead, some isolates of *V. cholerae* isolates have arrays of immunity genes downstream of the cognate immunity gene that matches the adjacent effector gene[104]. This observation could be the result of a mechanism known as homology-facilitated illegitimate recombination, which has been described for several naturally competent bacteria[105–107]. Homology-facilitated illegitimate recombination is rare compared with homologous recombination, but it can lead to the integration of novel genetic information through RecA-dependent homologous recombination in the homologous region combined with illegitimate recombination (that is, joining of DNA that has no homology) in the heterologous DNA segment. This finding fully supports our argument that kin-discriminated neighbour predation coupled



◀ Figure 3 | **Fratricide versus kin-discriminated killing.** Bacteria are able to compete with neighbouring cells through the use of secretion systems or the release of toxins, such as bacteriocins. The lysis of the targeted kin cell (top) or non-kin cell (bottom) will lead to the release of clonal or non-clonal DNA, respectively, thereby forcing the prey to act as a donor cell. It should be noted that fratricins can also target non-competent non-kin cells (see BOX 1); however, the phenomenon was first described in monoculture.

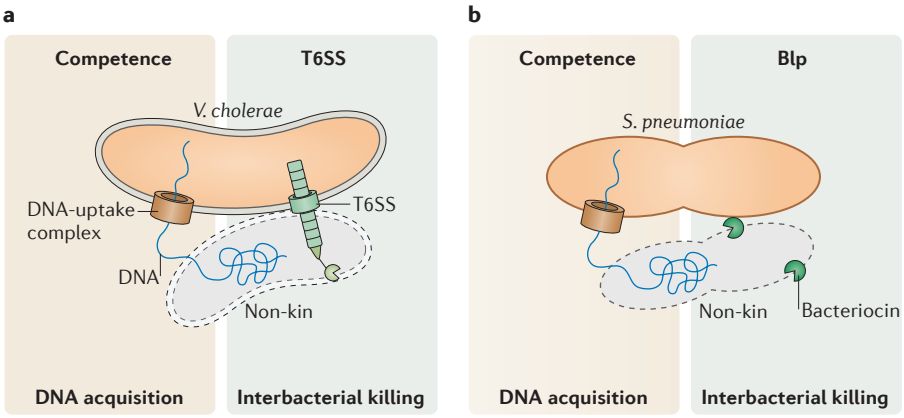


Figure 4 | **T6SS-mediated and bacteriocin-mediated neighbour predation fosters horizontal gene transfer in *Vibrio cholerae* and *Streptococcus pneumoniae*, respectively. a** | Following the induction of competence, pandemic isolates of *Vibrio cholerae* co-induce their type VI secretion system (T6SS)[13]. This results in the intoxication of adjacent non-clonal cells, which ultimately leads to their lysis and the release of their genetic material. The released DNA is then absorbed by the competence-induced DNA-uptake complex of the predatory *V. cholerae* cell[13]. In this scenario, the competent bacterium can acquire new genetic information, as was experimentally demonstrated[13]. **b** | The induction of competence in *Streptococcus pneumoniae* leads to the concomitant induction of bacteriocin production[14,15], which ultimately results in the kin-discriminated killing of neighbours. Comparable to the situation in *V. cholerae* (panel **a**), the genetic material that is released from lysed cells can then function as transforming material and enhance horizontal gene transfer (HGT)[15].

with DNA acquisition can provide an immediate fitness advantage to the naturally competent bacterium and might therefore be a key factor in interbacterial competition. Future studies will be required to test potential fitness advantages or disadvantages, taking the hypotheses presented in this article into consideration. Importantly, such studies will have to move from experimental monoculture conditions towards studying these processes in bacterial community structures, as kin-discriminated killing is only observed under such conditions. Indeed, monocultures might explain why studies of natural transformation have overlooked non-kin predation coupled with DNA acquisition in the past.

Conclusions

The recently discovered regulatory connections between the development of competence and the active killing of neighbouring bacteria in *V. cholerae* and *Streptococcus* spp. provide strong evidence for a functional link between both processes and the emergence of a common biological theme. Whereas *V. cholerae* uses a contact-dependent killing device (that is, the T6SS) for the acquisition of DNA, it is tempting to speculate that *S. pneumoniae* ensures that enough prey bacteria are in close proximity for the diffusion of their released DNA to be usable as transforming material by coupling the production of the killing bacteriocins to a quorum sensing system. Whether the released DNA is then used as food, to repair DNA or to foster HGT might be species-specific and context-dependent. In any case, evidence from genomic studies shows the enormous genetic plasticity of competent bacteria; thus, it is safe to say that competence contributes to the acquisition of new traits and to genome evolution.

Jan-Willem Veening was previously at the Molecular Genetics Group, Groningen Biomolecular Sciences and Biotechnology Institute, Centre for Synthetic Biology, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands. Present address: Department of Fundamental Microbiology, Faculty of Biology and Medicine, University of Lausanne, Biophore Building, CH-1015 Lausanne, Switzerland.

Melanie Blokesch is at the Laboratory of Molecular Microbiology, Global Health Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL; Swiss Federal Institute of Technology Lausanne), Station 19, EPFL-SV-UPBLO, CH-1015 Lausanne, Switzerland.

Jan-Willem.Veening@unil.ch; melanie.blokesch@epfl.ch

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