# Genetic diversity in the oral pathogen *Porphyromonas gingivalis*: molecular mechanisms and biological consequences

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## Abstract

*Porphyromonas gingivalis* is a Gram-negative anaerobic bacterium that colonizes the human oral cavity. It is implicated in the development of periodontitis, a chronic periodontal disease affecting half of the adult population in the USA. To survive in the oral cavity, these bacteria must colonize dental plaque biofilms in competition with other bacterial species. Long-term survival requires *P. gingivalis* to evade host immune responses, while simultaneously adapting to the changing physiology of the host and to alterations in the plaque biofilm. In reflection of this highly variable niche, *P. gingivalis* is a genetically diverse species and in this review the authors summarize genetic diversity as it relates to pathogenicity in *P. gingivalis*. Recent studies revealing a variety of mechanisms by which adaptive changes in genetic content can occur are also reviewed. Understanding the genetic plasticity of *P. gingivalis* will provide a better framework for understanding the host–microbe interactions associated with periodontal disease.

## Keywords

adaptive virulence; genetic diversity; genetic variability; horizontal DNA transfer; pan-genome; *Porphyromonas gingivalis*

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*Porphyromonas gingivalis* is a Gram-negative anaerobe that colonizes dental plaque biofilms in the human oral cavity, and is one of the few major pathogens responsible for the development of chronic periodontitis. Periodontitis is one of the most common bacterial infections of humans and 47% of the US adult population is affected by some form of this disease [1] . The pathological process in periodontitis destroys the structures supporting the tooth and is the leading cause of tooth loss in adults. The development of this disease is a multifactorial process involving interactions between the host and the microorganisms that colonize the oral cavity [2,3]. Persistent bacterial colonization by certain pathogenic microbes, coupled with a self-damaging host inflammatory response, results in destruction of soft tissues and loss of the bone surrounding the tooth. This disease process is chronic in nature and the inflammatory–infectious state can continue for years [4]. The natural outcome of this process is exfoliation of the tooth, which resolves the infection by removing the tooth-associated bacterial biofilm. Clinical intervention is primarily mechanical and involves scaling of the subgingival tooth surface to remove the plaque biofilm. In the event of

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significant loss of tooth-supporting tissues, surgical procedures are sometimes carried out to restore the periodontal architecture and stimulate regeneration of supporting tissues. There is a significant body of evidence linking the presence of active periodontitis to increased morbidity of certain systemic conditions including diabetes, cardiovascular disease, osteoporosis, preterm birth, respiratory diseases and rheumatoid arthritis [5]. While cause and effect relationships remain unclear, treatment of periodontal disease is beneficial in that it reduces chronic inflammation and the risks associated with the oral cavity as a focal point of infection [6].

*P. gingivalis* colonizes multispecies plaque biofilms at and below the gingival margin, and microbiome studies have also identified the deep crypts of the tongue as an additional habitat for these obligate anaerobes [7]. Residence under the gumline places *P. gingivalis* and other dental plaque microorganisms in contact with the host epithelium, and neutrophils and other innate immune effectors minimize the total number of bacteria present in the gingival sulcus (Figure 1A). This host immune response, supplemented by excellent oral hygiene, is sufficient to maintain healthy periodontal tissues. However, failures in immune defense or plaque control result in a higher bacterial burden and the subsequent development of gingival inflammation. Although present at low proportions in the oral microbial community, *P. gingivalis* is considered a 'keystone' pathogen, able to disrupt host–microbe homeostasis by molecular manipulation of select host protective mechanisms [8]. A multitude of studies have shown that *P. gingivalis* modulates innate host defense functions by subversion of IL-8 secretion, complement activity and TLR4 activation. This impairs the ability of the host to defend against the oral microbial community at large, resulting in an altered oral flora composition and subsequent inflammatory responses that contribute to the pathogenesis of periodontitis [9].

The collateral damage resulting from the battle between host and flora is loss of clinical attachment between tooth and bone, due to apical migration of the epithelial and connective tissue attachment together with alveolar bone loss [10]. In most untreated periodontal patients, the clinical attachment loss and ongoing periodontal inflammation generates a periodontal pocket, manifested as a deepened space beneath the gumline that exceeds 3 mm in depth, as measured from the margin of the gingiva to the bottom of the gingival sulcus (Figure 1A). This periodontal pocket can be very deep, reaching the apex of the root in some severe cases and provides a gradient of environmental conditions [11], with areas closest to the surface subjected to variable concentrations of oxygen, pH and temperature owing to local influence from the oral cavity. Deeper regions of the periodontal pocket are protected from disturbance and are more likely to be anaerobic [12,13], rich in blood and serum protein, and have a stable, slightly alkaline pH. Deep periodontal pockets evidently provide ideal conditions for *P. gingivalis* propagation, as cross-sectional surveys of periodontal pocket composition show the highest concentrations of *P. gingivalis* to be located in the deepest regions of the pocket [14]. Thus, the end-game strategy for this keystone pathogen is to manipulate host–flora interactions to create the ideal niche for survival. In this sense, *P. gingivalis* is considered a host-adapted pathogen, as chronic periodontal disease produces a slow-progressing morbidity with low risk of mortality.

Although *P. gingivalis* can be frequently detected in periodontal pockets, not all strains of *P. gingivalis* are equally pathogenic. Genetic heterogeneity within the species has been clearly linked to differences in outcomes related to host–pathogen interactions. There appears to be genetic adaptation occurring in this species as they transition from colonizers of healthy plaque to a major species in deep periodontal pockets, as species isolated from healthy sites have distinct genetic traits relative to those found in disease sites, consistent with a process of 'inhost evolution' or adaptive virulence [15] . In this review, the authors discuss the

biological impact of genetic diversity on *P. gingivalis* pathogenicity and describe recent studies on gene transfer mechanisms underlying adaptive virulence.

## *P. gingivalis* genetic diversity & virulence

*P. gingivalis* pathogenesis is well studied and these bacteria possess multiple virulence factors including gingipains (proteinases), major and minor fimbriae, hemagglutinins, hemolysin, iron uptake transporters, capsule and toxic outer membrane blebs [16,17]. These bacteria are also accomplished biofilm-formers, coaggregate with many other species found in dental plaque and contribute to the production of a protective biofilm exopolysaccharide matrix [3,18,19]. *P. gingivalis* directly binds to and invades gingival epithelial cells, and is able to spread from cell to cell into periodontal tissues (Figure 1C) [20,21]. Attachment of *P. gingivalis* to host cell surfaces is primarily mediated by the major fimbriae, which are long filamentous appendages on the surface of the bacterial cell (Figure 2). Interaction of the fimbriae with integrin host cell receptors mediates internalization of the bacteria and alters host cell signaling pathways (Figure 1D) [22–25]. Production of both gingipains and major fimbriae are mechanistically important for deregulation of immune function [2 6 –3 0], and expression of a capsule aids in evasion of immune effectors [31]. Many strains produce prolific membrane blebs, containing endotoxin from the outer membrane, which can stimulate innate immune responses (Figure 2). *P. gingivalis* is also capable of disseminating beyond the oral cavity via bacteremia and may contribute to distant sites of infection and aggravation of systemic diseases [5, 32–35] . *In vitro* and clinical studies have shown *P. gingivalis* to invade and survive in nonoral human cells, such as coronary arterial endothelial and placental cells, where they could potentially contribute to localized inflammatory responses [36–38].

*P. gingivalis* can be detected in the gingival sulcus of healthy individuals and the periodontal pocket of periodontitis patients. As the conditions found in a healthy sulcus and a diseased pocket are quite different, these sites represent distinct environmental niches in the oral cavity. Furthermore, *P. gingivalis* may reside in a dental plaque biofilm or take up residence in host tissue. This ability to permanently colonize distinct niches implies that these bacteria have sophisticated mechanisms to adapt to the local environment. *In vitro* and animal model studies confirm the clinical observations: comparison of distinct *P. gingivalis* strains demonstrates extensive differences in behavior and pathogenic potential [28,30,31,39–52].

Underlying these behavioral differences, genetic variations exist for many of the major virulence factors of *P. gingivalis* (Table 1), and numerous studies have attempted to associate specific virulence alleles with initiation of disease. Most commonly, these studies have tracked a single genetic marker, the major fimbrial gene *fimA*. The *fimA* gene encodes the protein subunit that assembles to form the *P. gingivalis* surface fimbriae. The *fimA* gene has six alleles (types I, Ib, II, III, IV and V), as determined by DNA sequencing, and these alleles can easily be distinguished by PCR [53] . The genetic variability of *fimA* probably has a significant influence on host–pathogen interactions, as the fimbriae mediate several key aspects of virulence, including manipulation of host cell responses to bacteria. In studies of *P. gingivalis* isolates from healthy and diseased individuals, *fimA* type Ib, II and IV are more commonly associated with periodontal disease, whereas types I and III are found primarily in isolates from healthy individuals [45,46,52,54]. The human studies have been confirmed by animal studies, which found that strains containing *fimA* type II and IV are more cytotoxic and invasive, while strains with *fimA* type I and III are less inflammatory and more likely to form localized selflimiting infections [55]. The *fimA* type II allele is believed to contribute to improved virulence owing, in part, to its increased binding affinity for epithelial cell receptors compared with other *fimA* types [52,56].

## Population structure

Numerous studies have measured genome-wide genetic variability in the *P. gingivalis* population using techniques such as random amplified polymorphic DNA analysis [57], restriction fragment length polymorphism [58], insertion sequence (IS) hybridization [59], cross-species microarray hybridization [41,60] and ribosomal intergenic spacer region analysis [61] . Several research groups have utilized multilocus sequence typing to measure the allele distribution of multiple housekeeping genes and, subsequently, utilize this measure of genetic variability to determine the population structure of these bacteria, defined as clonal or nonclonal [45,49,62,63]. In clonal populations, cells are descended from, and are genetically identical to, a single common ancestor; genetic diversity might arise by point mutation; however, there is little horizontal transfer of DNA. In nonclonal populations, cells are descended from multiple ancestors; therefore, some form of DNA exchange occurs between cells to generate diverse offspring. An Index of Association score is a quantitative measure of population structure: on one end of the spectrum, fully clonal populations score one; at the other end of the range, nonclonal populations are zero. According to the Multilocus Sequence Typing website for *P. gingivalis* [201], the *P. gingivalis* population structure falls in the nonclonal range with a score of 0.37 [64].

In nonclonal bacteria, the population displays a spectrum of phenotypes from commensal to pathogenic [65], and many different allele combinations can cause disease. Nonclonal population structures are common to human pathogens involved in chronic infections, resulting from horizontal DNA transfer between strains *in vivo*. This sexual behavior is thought to contribute to adaptation and persistence in the human host during changing niche conditions [65–67]. In well-characterized nonclonal pathogens such as *Helicobacter pylori* and *Neisseria* spp *.,* natural competence is the mechanism that facilitates DNA exchange between strains that are coresidents in the host environment [15, 68 –70].

Regarding *P. gingivalis*, studies have attempted to more broadly link nonclonal population structure to pathogenic heterogeneity by analyzing the combinations of virulence alleles present in collections of *P. gingivalis* clinical isolates [31, 45] . In a study correlating *fimA* allele type to multilocus sequence profile, *fimA* type II was found to be the most common (34%) in a population of 82 strains of worldwide origin [45]. The *fimA* type II allele was found in seven distinct genetic backgrounds [61], suggesting that this allele confers a selective advantage during the disease process that ensures its distribution throughout the *P. gingivalis* population. Two other studies have attempted to correlate virulence in *P. gingivalis* strains to the presence of proteases, capsule production alleles and *fimA* alleles (Table 1). Inaba *et al.* found that strains containing the *fimA* type II allele were most virulent in mice if they possessed capsule types 4 or 5 (from seven types), and were more invasive if they had high levels of protease activity [44]. Yoshino *et al.* assessed the genotypes of 62 clinical isolates, determining the allele combinations for *fimA* (type I, Ib–V), gingpain *kgp* (I or II) and gingipain *rgpA* (A, B or C). Out of 36 possible allele combinations, 15 were found in the isolate collection. Of these 15 combinations, two allele profiles were found in 50% of the isolates. The two frequent allele combinations both contained the *fimA* type II allele [31] . Taken together, these results indicate that many different *P. gingivalis* strains have the potential to contribute to the pathogenesis of periodontitis; however, particular allele combinations are favored over others. This is consistent with the nonclonal population structure of *P. gingivalis*, in that many strains may cause disease, but some allele combinations are more virulent.

If certain allele combinations are favored in disease, why do other allele combinations persist in the *P. gingivalis* population? An important consideration in any population study is the presence of bias in the sample selection. The population structure and genetic diversity

studies described above were done with clinical isolates from periodontal patients. The oral environment in these patients is distinct from individuals with healthy gingiva or with gingivitis. From an ecological perspective, these states of health or disease represent unique environmental niches, and certain alleles or allele combinations may favor survival in one niche over another. In the aforementioned studies, bacterial strains were selected mainly from periodontal patients, thus the diseased environmental niche is best represented. However, *P. gingivalis* is able to colonize both healthy and diseased gingival sulci. In the study comparing *fimA* alleles in diseased patients versus healthy individuals, healthy oral niches were more likely to be colonized by strains with *fimA* alleles I and III. *P. gingivalis* strain 33277 is less virulent in abscess models than isolates from diseased patients, has *fimA* allele I, produces no capsule and excretes low levels of gingipains (Table 1). Strain 33277 could therefore be considered a prototype for strains that successfully colonize the healthy oral cavity, and illustrative of strains that are under-represented in population genetics studies.

## Whole-genome comparisons

Studies of population structure provide a wide-angle view of genetic diversity within a bacterial species, and alignment of whole genome sequences provides a complementary and a highly magnified assessment of variability. Whole-genome sequences are currently available from three strains of *P. gingivalis*: W83, 33277 and TDC60 [71–73]. These three strains are representative of the *P. gingivalis* nonclonal population structure in that they have diverse phenotypic behaviors. The W83 and 33277 isolates are well-studied laboratory strains. W83 has been demonstrated to be more cytotoxic and likely to disseminate in the host, while 33277 is better at biofilm formation and localized host cell invasion. The TDC60 strain is a strain recently isolated from a periodontal pocket and exhibits relatively greater pathogenicity in mice than strains W83 and 33277 [71] . The genome of strain 33277 contains 2090 protein-coding sequences (CDSs), while strain W83 contains 2023 CDSs and strain TDC60 has 2220 CDSs.

Computational alignment of these three genomes reveals that approximately 75% of the CDSs are conserved at greater than 90% identity. Strain 33277 has 461 CDSs, W83 has 415 CDSs and TDC60 has 382 CDSs that are designated as nonidentical or strain specific. Many of these strain-specific CDSs have functional homologs in the opposing strain sequence, implying that these CDSs represent allelic forms of the same gene. Others are unique to individual strains and many are hypothetical in function, thus providing an unknown benefit to the host strain. As shown in Figure 3, genetic variability between strains can be extensive and encompass entire genes or operons; these regions are considered strain-specific and compose approximately 20–25% of a given strains genome. Genetic variability can also be detected within open reading frames as point mutations or other genetic changes that are limited in size (Figure 3). Although the latter genetic changes are not dramatic, the resulting amino acid changes could result in significant functional alterations. Genetic differences can also be detected in intergenic regions and, therefore, have the potential to influence promoter or transcriptional terminator activity (Figure 3).

Beyond bioinformatic alignment of the three sequenced genomes, other multiple strain comparisons have been made using comparative genome hybridization microarrays. Brunner *et al.* compared seven *P. gingivalis* strains to a W83 microarray and identified genes that were: fully conserved in all strains; 'aberrant' or not identical between strains (allelic); or absent in the test strain. Similar to the genomic alignments, this study identified a common core genome comprised of approximately 80% of the genes in the W83 genome [74]. Interestingly, many of the genes that are divergent between strains are found in clusters, creating localized 'hotspots' of genetic diversity within the genome sequence [60,74]. One

cluster of divergent genes is the capsule operon, which is composed of 14 open reading frames [72,75]. Other divergent regions contain open reading frames predicted to encode IS (Figure 3), transposases, CRISPR-associated gene sequences, restriction endonucleases or hypothetical proteins. The presence of transposases and CRISPR-associated genes implies that at least some of the *P. gingivalis* genome is 'mobile' and capable of rearrangement or horizontal transfer from one strain to another.

## Mobile DNA & genetic diversity

### Insertion sequences

ISs are the smallest forms of mobile DNA. They consist of one open reading frame encoding a transposase enzyme, surrounded by short repeated DNA sequences. If the transposase gene is expressed, the enzyme can bind to the DNA repeats, cut the DNA to remove the IS and reinsert the IS in a new location. At least seven distinct IS elements have been identified in strains of *P. gingivalis* and have been given a standardized nomenclature of IS*Pg* (1–7) [76–80]. Most strains have multiple copies of more than one IS*Pg* element and the IS element profile and copy number has been used to differentiate a collection of laboratory and clinical isolates (Table 1) [59]. The presence of IS elements in a bacterial genome can have multiple effects, including the inactivation of open reading frames, up- or down-regulation of gene expression via polarity effects, and can also result in wide scale genomic rearrangements. There is some evidence that IS elements in *P. gingivalis* are active. IS*Pg* elements have been shown to influence levels of gingipain expression by insertion upstream in the promoter region [77, 81], and strain W83 lacks minor fimbriae owing to an IS*Pg1* insertion in the *mfa* gene [73,82]. These transposition events have a significant impact on genetic diversity and pathogenicity, as both gingipains and minor fimbriae are important for interactions with the host.

Whole genome alignments of the sequenced strains reveal a significant level of genomic rearrangements [71]. It is possible that transposonmediated recombination between repeated IS*Pg* sequences dispersed on the genome might allow the rearrangement of the genomic structure (Figures 3 & 4C). Such genomic rearrangements can impact gene expression levels, as genes located in proximity to the chromosomal origin of replication may have a higher transcription level. Interestingly, a recent transcriptional mapping experiment demonstrated that during growth in standard laboratory conditions, antisense mRNA is expressed for IS*Pg* elements. This would have the predicted effect of silencing expression of IS*Pg* transposase genes, and preventing transposition activity [83]. I t will be interesting to discover if this transposition silencing i s released du r i ng cell stress, as low levels of IS*Pg* activity could provide a mechanism of genetic adaptation to changing and stressful environmental conditions via random alteration of gene expression.

### Conjugative transposons

The genome sequences for *P. gingivalis* strains have regions that are similar to conjugative transposons (cTns) found in their close taxonomic relatives, the *Bacteroides*. *Bacteroides* species comprise the majority of the flora in the mammalian GI tract and are well-characterized genetically [84]. These bacteria have been shown to transfer DNA to other bacterial species in the intestine, and the primary mode of genetic exchange between *Bacteroides* spp. is mediated by cTns. The *Bacteroides* cTns are a diverse group o f 40–60 kb elements that encode multiple genes required for formation of a conjugal pore, with the genetic designation *tra*. The cTns do not have an origin of replication and are usually integrated in the chromosome of the host cell. In preparation for conjugation, these elements excise themselves from the chromosome, forming a circular intermediate (Figure 4b). This circular form is the substrate for conjugation and the conjugal pore transfers a single strand

to the recipient cell. Once conjugation is complete, the single strands in both the donor and recipient are replicated, and the circular molecules integrate back into the chromosomal DNA. In addition to their own transfer, the cTns also mobilize *Bacteroides* plasmids and other transposons by providing the promiscuous conjugal pore encoded by the *traAQ* locus. This extrachromosomal transfer of plasmids and transposons by the *traAQ* locus has been implicated in the high rates of antibiotic resistance in *Bacteroides* species [85].

There are four putative cTns in strain 33277; these elements have been given the designation cTn*Pg*. Three of the 33277 cTns encode a partial *tra* conjugation locus (cTn*Pg*1-b, cTn *Pg*1-c and cTn*Pg*2) as compared with functional *tra* gene regions encoded in the genome sequences of *Bacteroides spp*. One 33277 element, cTn*Pg*1-a has a complete *traAQ* region, and has been shown to be a functional conjugative transposon, with behaviors similar to those of *Bacteroides* cTns [72,86]. As both oral and intestinal bacteria have similar cTns, it seems that these elements could play an important role in horizontal gene transfer between related strains or species of bacteria. cTn*Pg*1 carries a gene that codes for a Na$^+$-driven multidrug efflux pump; however, it was not determined if transfer of this element conferred antibiotic resistance. Naito *et al*. demonstrated that cTn*Pg*1 could be transferred to the closely related genera *Bacteroides* and *Prevotella* (Figure 4D); therefore, this family of cTns could contribute to genetic diversity by mediating gene sharing within the *Bacteroidetes* phylum [86].

**Plasmid mobilization**

In the *Bacteroides*, the cTns can mobilize plasmids and other transposons by providing the promiscuous conjugal pore encoded by the *traAQ* locus. To date, no natural plasmids have been identified in *P. gingivalis*; however, plasmids introduced from close relatives, such as the *Bacteroides*, are functional and are frequently used for genetic manipulation of *P. gingivalis*[87]. Two *Bacteroides*- derived plasmids were used to test the mobilization functions of cTn-like elements in eight different strains of *P. gingivalis*[88]. Strain W83 and two others could not transfer plasmids to a recipient. By contrast, strain 33277 and four others could conjugate plasmids at frequencies between $10^{-5}$ and $10^{-7}$[88]. Therefore, the cTn*Pg*1 found in 33277 is functionally similar to *Bacteroides* cTns in that, in addition to its ability to transfer itself, it can also mediate the transfer of other genetic elements. The other four strains capable of plasmid mobilization contain DNA sequences highly similar to cTn *Pg*1 [Tribble GD, Unpublished Data]. Strain W83 also contains a *traAQ* genomic locus that has a similar gene arrangement, but less than 36% homology at the DNA level, to cTn*Pg*1 of 33277.

The 33277 cTn*Pg*1-a *traAQ* locus contains a *traP* gene and in *Bacteroides*, the *traP* gene is required for plasmid conjugation; the W83 *traAQ* locus does not contain a *traP* homolog, which may explain the lack of plasmid transfer for W83 [88,89]. The locus of *P. gingivalis* W83 does not have genes for integration or excision, implying that this locus is not a conjugative transposon and is permanently anchored in the chromosome [88]. Based on these collective data, it appears that some strains of *P. gingivalis* have the ability to facilitate transfer of cTns and other accessory mobile elements, while others do not.

**Chromosomal DNA transfer**

While the presence of mobile DNA elements, such as ISs and cTns, can contribute to genetic diversity, these elements alone do not account for the extensive genetic variability and nonclonal population structure demonstrated by the *P. gingivalis* pan-genome. In other nonclonal bacterial species, mechanisms to exchange chromosomal DNA are present, most commonly via natural competence or by integrated plasmids, similar to the high frequency recombination systems found in *Escherichia coli*[90]. Recent investigations into *P.*

*gingivalis* chromosomal transfer have identified both natural competence and conjugation mechanisms for DNA exchange between *P. gingivalis* strains [91]. Using antibiotic resistance markers engineered into the *P. gingvalis* genome, it was found that strains W83 and 33277 could exchange chromosomal DNA with each other. The resulting offspring were shown to be chimeric, as they contained DNA from both donor and recipient strains and the chimeras were demonstrated to have altered phenotypic properties as measured by biofilm assays [88]. It was also demonstrated, by whole genome footprinting with IS*PG*1, that widespread genome rearrangements did not occur during interstrain DNA transfer; however, exchange of regions large enough to swap entire genes did occur. This study illustrates that essential features of nonclonal population structure exist in *P. gingivalis* at the mechanistic level: transfer of chromosomal DNA between strains swaps alleles and generates diverse phenotypic behaviors.

In a series of experiments to better define the molecular events occurring during DNA exchange, it was determined that using a 'dead donor' assay was much more efficient at DNA transfer than mixing two living strains. In a dead donor assay, one strain is heat-killed before mixing with a live strain. In this scenario, the only transfer that can occur requires the live recipient strain to take up free DNA released by the heat-killed donor. The high transformation frequencies in dead donor assays implied that a natural transformation system is present in *P. gingivalis* strains. This was confirmed by mutating a predicted competence-associated gene *comF* in strain W83. The resulting *comF* mutant was unable to be transformed in a dead donor assay. These studies also documented the presence of extracellular DNA in *P. gingivalis* biofilms, which could provide a source of DNA for transformation and reassortment of alleles between strains (Figure 4A) [91]. Interestingly, two live *comF* mutants were still able to exchange chromosomal DNA at very low frequencies. A further series of experiments revealed that this residual DNA transfer was due to conjugation of chromosomal DNA mediated by the *traAQ* region (Figure 4b & C) [91]. In summary, overlapping mechanisms for chromosomal DNA exchange exist with *P. gingivalis*: natural competence occurs at the highest frequency and conjugation of chromosomal DNA is also possible, although this occurs at a lower frequency. As predicted by the nonclonal population structure of *P. gingivalis*, horizontal transfer of chromosomal DNA is possible, and as *P. gingivalis* has redundant mechanisms to ensure this process, it seems likely to be a critical component of its lifestyle.

## Conclusion

Horizontal DNA transfer is well known for its spread of virulence traits such as antibiotic resistance genes and pathogenicity islands; however, its significance as a driving force in species diversity and niche adaptation is only recently becoming more obvious. The pan-genome concept describes a bacterial species as a pool of alleles that may be acquired, deleted or reassorted between coresident strains in response to pressures from the ecological niche [92]. For many bacteria, humans are their primary or only habitat, and adaptation and evolution is in response to selective pressures from the host environment. As each human host represents a unique habitat, bacterial genetic factors that contribute to colonization and persistence may need to be tailored to the specific host in order to maximize fitness of the microorganism. For potential pathogens, genetic adaptation to the host might contribute to the initiation of an infection after colonization, persistence and propagation in a hostile environment, evasion of the immune response and different disease outcomes in different hosts. In *P. gingivalis*, multiple forms of genetic transfer and recombination are occurring within the species by conjugation and transformation, and interspecies transfer of cTns and mobilizable elements can also occur. Therefore, mechanisms for maintaining genomic plasticity are well provided for and in the complex communities associated with oral biofilms, sources of exogenous DNA are plentiful [93,94].

In periodontal patients, elevated numbers of *P. gingivalis* are found in the periodontal pocket, and may compose up to 7% of the bacteria found in plaque samples. Individuals can be transiently coinfected by two, three or more strains simultaneously [95]. Theoretically, exchange of DNA between multiple strains occupying the same dental plaque biofilm could produce a pool of chimeric offspring with diverse phenotypes. Over a period of time, these offspring would compete with each other, with the most fit for the prevailing host conditions becoming the dominant strain within the plaque community. Over the lifetime of the host, one could predict multiple co-colonization events could occur, introducing new alleles to the local *P. gingivalis* gene pool. Assuming *P. gingivalis* is able to 'dial-up' the correct allele combinations, DNA exchange would allow continued fine-tuning of bacterial fitness and contribute to persistence in the ever-changing oral niche of the host. Beyond persistence, DNA exchange between strains could also be contributing to the clinical development of chronic periodontitis, by providing this organism with the correct ways in which to hack the host cellular signaling network.

In the complex lifestyle of *P. gingivalis*, initial colonization of plaque biofilms and survival in the nutrient deprived conditions of a healthy gingival sulcus will favor certain strains; whereas long-term survival and immune evasion in an inflamed periodontal pocket may favor different allelic combinations. Hence, selective pressure from the diverse oral environment maintains allelic diversity in *P. gingivalis*. The current authors predict that once a strain successfully colonizes the gingival sulcus, co-colonization with additional strains, even transiently, will allow genetic reassortment and the subsequent selection of new genetic variants with improved fitness for the changing environment [66]. At present, an interesting perspective is that strains that colonize the dental plaque of a healthy gingival sulcus are considered 'less pathogenic' than strains isolated from deep periodontal pockets. However, the true pathogenicity of *P. gingivalis* lies in its role as a keystone pathogen: defined by its ability to subvert early immune responses and send the host–pathogen interaction down a darker path. Therefore, one could redefine stages of pathogenicity for this bacterium and 'healthy host' colonizers involved in early subversion events are likely in 'stealth' mode, working to modify the host environment and continuing to persist in a shallow sulcus until the upper hand is gained [96]. Strains found in deeper periodontal pockets in the presence of a chronic immune response have likely shifted to a different mode, one that favors survival and transmission to a new sulcus or even a new host.

## Future perspective

The study of *P. gingivalis* as a keystone pathogen in the polymicrobial development of periodontitis illustrates the difficult tasks faced by modern microbiologists and immunologists. With the advent of microbiome research, scientists and clinicians are challenged with dissecting the molecular interactions between the host and a highly complex flora. Changes in the composition of the human microbiome are being linked to a variety of chronic health conditions, including obesity [97], ulcerative colitis and irritable bowel syndrome [98,99], pulmonary exacerbations in cystic fibrosis [100] and even immunoregulatory dysfunction in Type 1 diabetes [101]. As with studies of periodontitis, determining if changes in the microbiome are a cause or effect of disease, identifying bacterial species that may act as a 'keystone' to disease progression and characterizing underlying molecular pathology will be key to linking microbiome data to other chronic health conditions.

Beyond the role of microbiome composition, understanding *in vivo* bacterial adaptations to the host is key to understanding fundamental questions about the role of microbial fitness, adaptation and evolution in chronic infections. Nonclonal bacterial species are a moving target, allowing them to persist within the host and potentially transform into a more

pathogenic type. Our standard weapons against acute bacterial infection are ineffective at targeting chronic microbial disorders, but hopefully understanding the genetic plasticity of nonclonal bacteria such as *P. gingivalis* will provide a better framework for intervening in the host–microbe interactions associated with chronic disease.

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## Website

201. *Porphyromonas gingivalis* MLST database. http://pubmlst.org/pgingivalis

## Executive summary

**Porphyromonas gingivalis**

- *Porphyromonas gingivalis* is a Gram-negative, strictly anaerobic bacterium that colonizes the human oral cavity and is associated with the development of chronic periodontal disease.

- *P. gingivalis* is found in anaerobic sites in the mouth such as the deep crypts of the tongue and in subgingival dental plaque.

- Transmission occurs between household contacts, with elderly residents often transmitting to younger members.

- *P. gingivalis* can be found in a healthy oral cavity in low numbers; in periodontitis the subgingival burden is much higher.

- In the subgingival plaque biofilm, *P. gingivalis* comes into direct contact with host gingival tissues and circulating immune cells.

**Host–pathogen interactions**

- *P. gingivalis* is defined as a keystone pathogen. Even in small numbers in the flora, the bacterium interacts with the host defense system to suppress cytokine expression, complement activity and Toll-like receptor activation.

- Localized immune subversion ultimately leads to a pathologic relationship between host and oral flora, resulting in a chronic inflammatory response. Collateral damage to the periodontium results in the clinical signs of periodontal disease.

- *P. gingivalis* possesses multiple virulence factors. For example, secreted protease enzymes called gingipains interfere with complement activity. Major fimbriae on the bacterial surface mediate adherence and internalization into host cells.

**Genetic variability**

- The *P. gingivalis* population is composed of many different strains.

- Pathogenic potential is variable between strains and most virulence factors have multiple alleles.

- Particular combinations of virulence alleles are more common in active disease sites, while other allele combinations are present in healthy sites.

- The nonclonal population structure predicts that new allele combinations are created by horizontal DNA transfer.

**Gene-transfer mechanisms**

- *P. gingivalis* possesses multiple mechanisms for DNA transfer and recombination.

- *P. gingivalis* insertion sequences can modify gene expression and mediate genomic rearrangements.

- Conjugative transposons can transfer between strains and mobilize other elements and chromosomal DNA to recipients.

- *P. gingivalis* secretes extracellular DNA into biofilms and displays natural competence; different strains occupying the same biofilm can therefore share DNA by transformation.

- Gene transfer and recombination likely produce a diverse population of strains, which is then subject to fitness selection by the challenging conditions of the oral cavity.
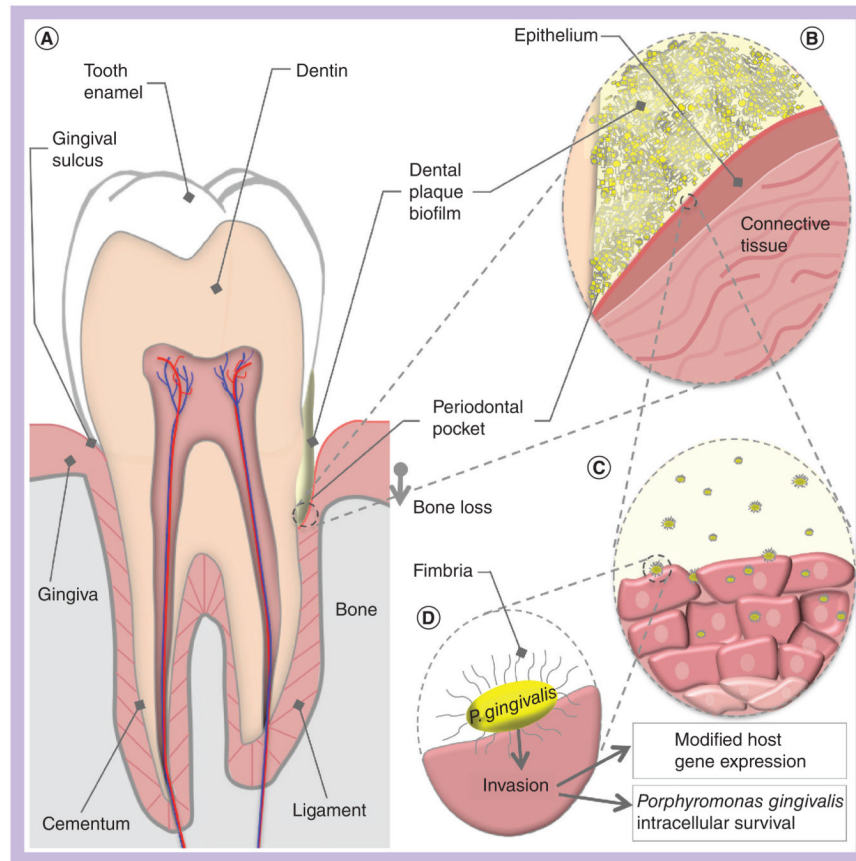
**Figure 1. *Porphyromonas gingivalis* host environment and interactions**
**(A)** A cross-sectional schematic diagram of a human tooth. The periodontium is composed
of the gingiva (pink) and the alveolar bone (grey). The left side represents periodontal
health, while the right represents the changes that occur in periodontitis. Dental plaque is
represented in yellow, in contact with the gingiva and tooth surfaces. Development of a
chronic host inflammatory response ultimately results in a loss of connection between the
gingiva and the root surface, creating a periodontal pocket. Bone is also resorbed as the
bacteria advance down the surface of the tooth. **(B)** A magnified view of the periodontal
pocket. The biofilm (yellow) is composed of many species of bacteria, of which
*Porphyromonas gingivalis* is a small portion. *P. gingivalis* in the biofilm comes into direct
contact with gingival epithelium. Secretion of *P. gingivalis* gingipains aids in the destruction
of host antibodies and complement, and contributes to localized tissue destruction. **(C)** A
number of *P. gingivalis* cells may leave the biofilm community and invade host cells.
Internalized bacteria move from cell to cell into the deeper layers of the connective tissue.
**(D)** Major fimbriae on the bacterial cell surface act as adhesins for host integrin receptors,
and mediate internalization of the bacteria into the host cell cytoplasm. Postinvasion host
cell gene expression is significantly modified; for example, reductions in cytokine
production interferes with neutrophil recruitment to the periodontal pocket. The ability of *P.
gingivalis* to modify local host responses to the biofilm flora results in the skewed immune
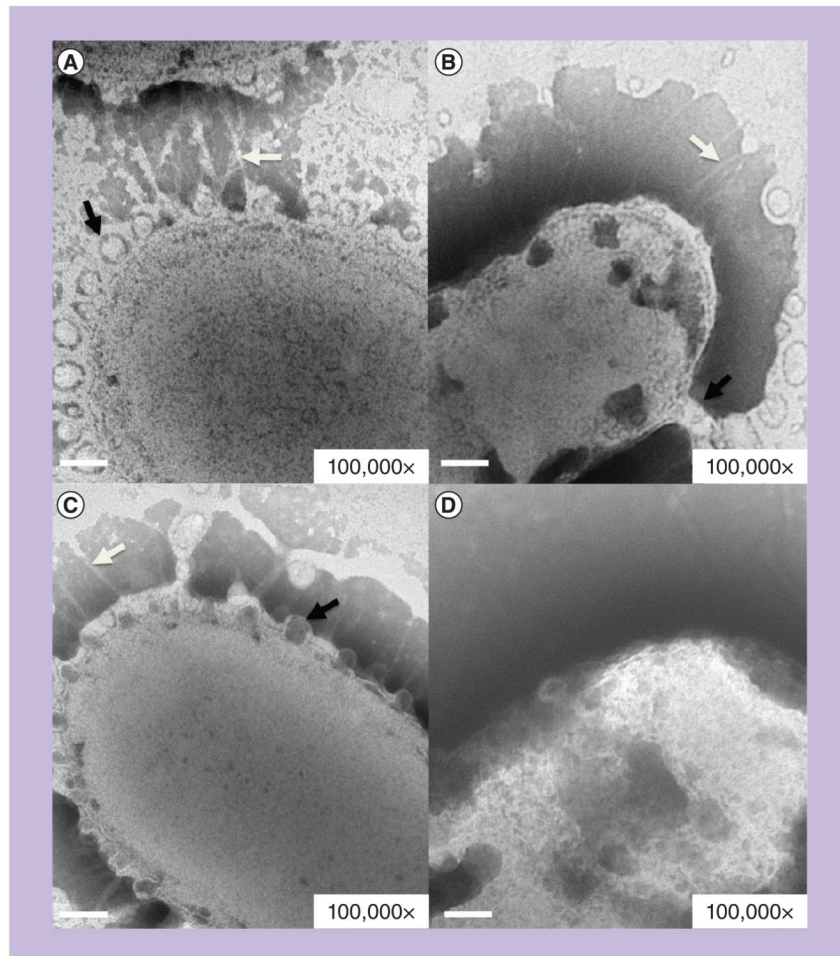response that drives periodontitis progression.

**Figure 2. Transmission electron micrograph of *Porphyromonas gingivalis* strains 33277, 53977, 49417 and W83**

*Porphyromonas gingivalis* cells from 2 days' growth on blood agar stained with 2% phosphotungstic acid, shown at 100,000× magnification. Membrane blebs are indicated with black arrows, major fimbriae with white arrows. **(A)** Strain 33277 (*fimA* type I). **(B)** Strain 53977 (*fimA* type II). **(C)** Strain 49417 (*fimA* type III). **(D)** Strain W83 (*fimA* type IV) does not display any fimbriae nor any obvious evidence of membrane blebbing. Scale bar: 100 nm.
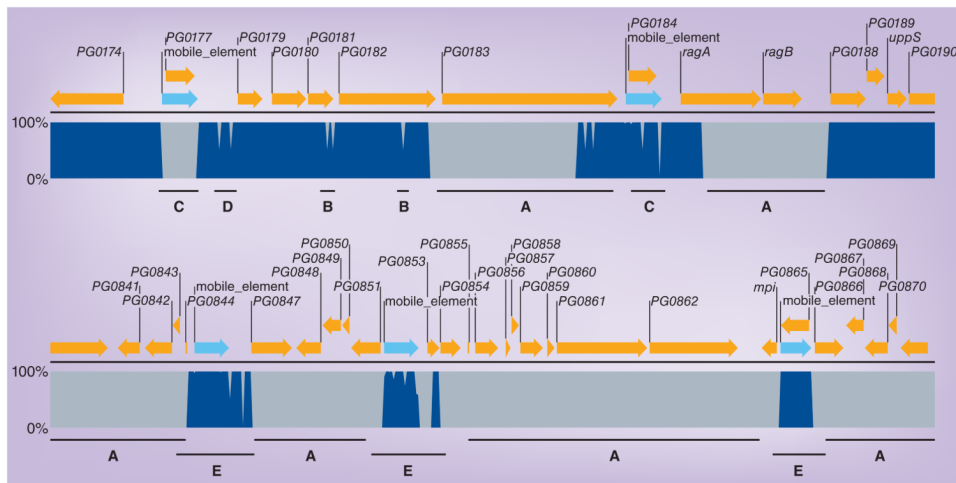
Sample preparation: Jennifer Kerr.

**Figure 3. Examples of genomic differences between strains W83, 33277 and TDC60**
The genome sequence of strain W83 was screened against strains 33277 and TDC60 to identify regions of conserved (dark blue) and divergent (grey) sequence. The upper alignment represents a region primarily encoding lipoproteins. The lower alignment demonstrates highly diverged regions flanked by conserved mobile elements. The genomic comparison was performed using CLC Genomics Workbench V4, using the internal BLAST function (Mask low-complexity; Expect 1; Match 1, Mismatch -2; Word size 30). The open reading frame annotations are from strain W83: yellow arrows represent coding sequence and light blue arrows represent mobile elements. 'A' denotes genes that are highly divergent between all three strains and are thus 'strain-specific'. In the upper panel, *ragAB* encodes an outer membrane lipoprotein and *PG0183* encodes a putative lipoprotein. 'B' denotes changes in DNA sequence in one of the three strains, TDC60, which result in limited changes in amino acid content. *PG0181* and *PG0182* encode putative lipoproteins; the genetic differences in strain TDC60 result in changes to 15 and 32 amino acids, respectively. 'C' denotes a mobile element that is found only in strain W83, IS*Pg*4 to the left; and a mobile element found in all three strains, IS*Pg*1 to the right. 'D' denotes genetic variation in the promoter region for *PG0179*. The promoter region is conserved between W83 and 33277, and divergent in TDC60. The two points of genetic variance begin 147 and 350 bp upstream of *PG0179*, and represent 28 and 25% genetic divergence over 127 and 385 bp, respectively. 'E' denotes conserved mobile elements flanking strain-specific genes. Two copies of the conserved IS*Pg*1, left and middle, and one copy of IS*Pg*2, right, flank regions containing W83-specifc genes encoding primarily hypothetical proteins. *PG0861* is predicted to be a helicase and *PG0862* a restriction endonuclease, suggesting that this region may encode functions associated with DNA transfer.
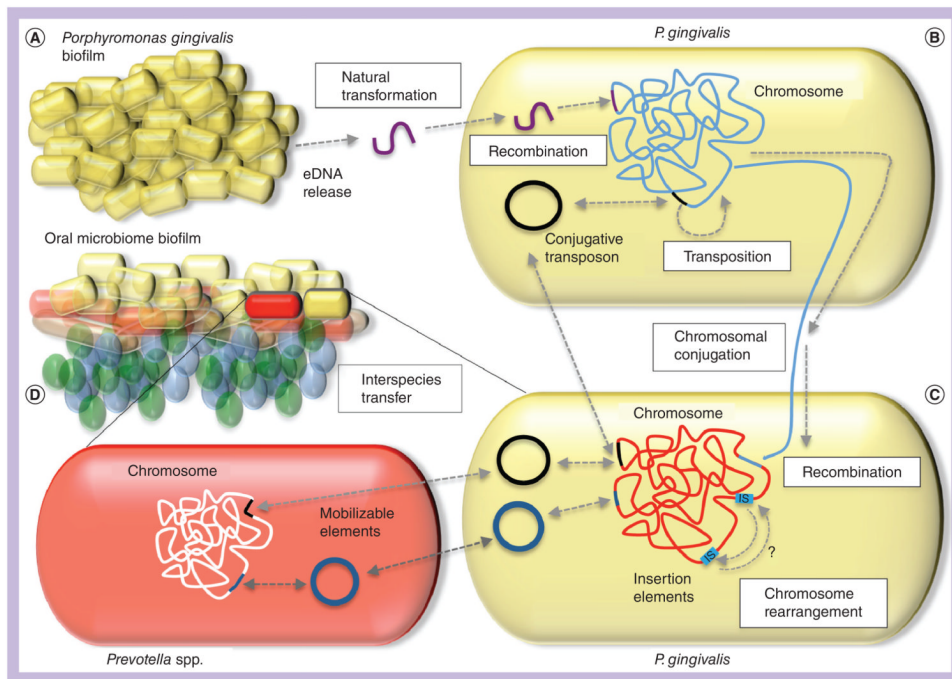
**Figure 4. Mechanisms of DNA transfer in *Porphyromonas gingivalis***
*Porphyromonas gingivalis* resides in dental plaque biofilms in the presence of diverse bacterial species and multiple strains of *P. gingivalis* may coexist within the same biofilm community. **(A)** *P. gingivalis* biofilms have been shown to release eDNA (purple) into the biofilm matrix. This DNA can be taken up by other strains of *P. gingivalis* by natural transformation. This process requires the *comF* gene in the recipient strain; comF proteins provide the molecular motor to bring DNA across the bacterial membrane. DNA that is homologous to the host cell genome may be integrated into the chromosome by homologous recombination. **(B)** Some strains of *P. gingivalis* contain conjugative transposons that are normally integrated into the chromosome (black line), but may excise and form a circular intermediate prior to conjugative transfer to a new host cell (black circle). Conjugative transposon cTn*Pg*1 has been shown to transfer between *P. gingivalis* strains, is able to transfer into other closely related species such as *Prevotella* **(D)**, and can also mobilize plasmids and other genetic elements between species (dark blue circles). **(C)** Conjugation can also mediate the transfer of chromosomal DNA between strains of *P. gingivalis*. This process is dependent on conjugative transposons or other *traAQ*-containing elements present in the donor strain and homologous recombination in the recipient. Allelic replacement of recipient DNA (red) with donor DNA (blue line) is mediated by homologous recombination. Finally, insertion elements (light blue boxes) are present in *P. gingivalis* and are capable of transposing into genes or their promoter regions, thus influencing expression. They are also likely responsible for the large scale genomic rearrangements that can be detected by comparison of multiple genome sequences. **(D)** cTnPg1 can be transferred to the closely related genus *Prevotella*. eDNA: Extracellular DNA.

**Table 1**

*Porphyromonas gingivalis* genetic variations.

| Genetic variation | W83 | 33277 | 49417 | 53977 | TDC60 |
|---|---|---|---|---|---|
| *fimA* type (I, Ib and II–V) | IV | I | III | II | II |
| *mfa* (+/−) | − | + | Unknown | Unknown | + |
| *ragAB* type (1–4) | 1 | 4 | 1 | 3 | $4^{\dagger}$ |
| Capsule (K1–6 and K−) | K1 | K− | K4 | K3 | Unknown |
| *kgp* gingipain type (I and II) | I | II | Unknown | Unknown | $II^{\dagger}$ |
| *rgp* gingipain type (A, B and C) | A | A | Unknown | Unknown | A |
| cTn*Pg*1a (+/−) | − | + | − | + | + |
| IS*Pg*4 copy number | 10 | 0 | 6 | 10 | $3^{\ddagger}$ |

Table demonstrates known genetic variations between common laboratory strains of *Porphyromonas gingivalis*. *fimA* is the major protein subunit of the major fimbriae, Mfa is the presence or absence of minor fimbriae and RagAB is a surface lipoprotein associated with virulence. The capsule is characterized as one of six types or absent (K−), Kgp and Rgp are subtypes of the gingipain proteases, cTnPg1 is a conjugative transposon and ISPg4 is an example of an insertion sequence that varies in copy number between strains.

$^{\dagger}$For strain TDC60, allele types were derived based on genetic comparison to genome sequences for W83 and 33277.

$^{\ddagger}$ISPg4 exists as three truncated copies in TDC60.