Article: **Bacterial plasmid-associated and chromosomal proteins have fundamentally different properties in protein interaction networks**

Authors and affiliations: Tim Downing[1]*, Alexander Rahm[2].

[1,]*Author for Correspondence: School of Biotechnology, Dublin City University, Dublin, Ireland. Corresponding author. Email: Tim.Downing@dcu.ie. Orcid: 0000-0002-8385-6730.
[2] GAATI Lab, University of French Polynesia, Tahiti, French Polynesia. Email: Alexander.Rahm@upf.pf. Orcid: 0000-0002-5534-2716.

Abstract

Plasmids facilitate horizontal gene transfer, which enables the diversification of pathogens into new anatomical and environmental niches, implying that plasmid-encoded genes can cooperate well with chromosomal genes. We hypothesise that such mobile genes are functionally different to chromosomal ones due to this ability to encode non-essential functions like antimicrobial resistance and traverse distinct host cells. The effect of plasmid-driven gene gain on protein-protein interaction network topology is an important question in this area. Moreover, the extent to which these chromosomally- and plasmid-encoded proteins interact with proteins from their own groups compared to the levels with the other group remains unclear. Here, we examined the incidence and protein-protein interactions of all known plasmid-encoded genes across representative specimens from most bacteria using all available plasmids. We found that such plasmid-encoded genes constitute ~0.7% of the total number of genes per bacterial sample, and that plasmid genes are preferentially associated with different species but had limited taxonomical power beyond this. Surprisingly, plasmid-encoded proteins had both more protein-protein interactions compared to chromosomal proteins, countering the hypothesis that genes with higher mobility rates should have fewer protein-level interactions. Nonetheless, topological analysis and investigation of the protein-protein interaction networks' connectivity and change in the number of independent components demonstrated that the plasmid-encoded proteins had limited overall impact in >96% of samples. This paper assembled extensive data on plasmid-encoded proteins, their interactions and associations with diverse bacterial specimens that is available for the community to investigate in more detail.

Keywords: AMR, plasmid, genome, protein-protein interaction, network.

Significance statement

It is well-established that plasmids drive new traits in their bacterial hosts, but the extent to which host-plasmid co-evolution is evident at the level of protein-protein interactions remains unclear. To address this, we compiled and analysed all available valid bacterial plasmids and associated proteins to explore the compositional differences between chromosomal and plasmid-encoded proteins and their interaction levels. We found that plasmid-encoded genes were highly correlated across the bacterial samples such that they had a high association with taxonomic context. Contrasting with the complexity hypothesis, plasmid-encoded proteins had far more interactions on average than chromosomal ones, though they had minimal effects on protein-protein interaction network structure. This demonstrated that host-plasmid co-evolution is evident and detectable at the level of protein interactions.

Introduction

Plasmids are short extrachromosomal DNA elements that are typically circular and with variable copy numbers. Conjugative plasmids possess machinery enabling horizontal gene transfer (HGT) between bacterial cells, a key mechanism of bacterial evolution. Plasmid DNA may also transfer infrequently between cells by transformation, vesicles and phages (Canosi et al 1982, Erdmann et al 2017, Zhang et al 2018, Wein & Dagan 2020). This allows plasmid-encoded genes to move between bacterial cells in the same niche (Wein et al 2021), where these genes may allow new phenotypes (Norman et al 2009, Downing 2015, Hall et al 2017, Decano et al 2020). Plasmid-encoded genes encode a wide range of functions, including antimicrobial resistance (AMR), virulence, metabolism, and symbiosis (Ahmer et al 1999, Stasiak et al 2014, San Millan 2018), facilitating spread into new environmental niches (Niehus et al 2015). Importantly, they depend on the environmental context to be beneficial, such that they can be lost when no longer required (Niehus et al 2015).

Mobile genes form part of the accessory genome, distinct from chromosomal genes that typically compose the core genome (Brockhurst et al 2019), though some chromosomal genes may not be universal within a collection, and so are part of the accessory genome. Some genes can be encoded on both plasmids and chromosomes, and there are numerous instances in which beneficial genes have been mobilised to spread from plasmids to chromosomes, such as *bla*CTX-M-15 (Agyekum et al 2016, Huang et al 2017, Irrgang et al 2017, Decano et al 2019a, Decano et al 2019b, Yoon et al 2020, Ludden et al 2020, Bevan et al 2021, Shawa et al 2021). In this study, we examined the broad patterns across bacteria using the categorisation of plasmid-encoded versus chromosomal.

Plasmids impose a metabolic fitness cost on their host cells (San Millan & MacLean 2017, Baltrus 2013, Harrison & Brockhurst 2012) and yet persist widely thanks to infectious spread, stability mechanisms, and chromosomal compensatory mutations (Stalder et al 2017). Plasmids are genetically very diverse, and this is paralleled by extensive variation in AMR gene profiles across bacteria (Ho et al 2020, Coelho et al 2021). The co-evolution of plasmid-encoded genes with chromosomal ones are consequently of keen interest. This matters because a suitable host-recipient genetic background is needed such that the transferred gene can be acquired and replicated (Forsberg et al 2014, Soucy et al 2015, Andam & Gogarten 2011). In addition, donor & recipient cells may be genetically distinct and yet have high HGT rates (Smillie et al 2011, Brito et al 2016), suggesting additional factors affecting gene and plasmid retention to quantify.

HGT depends on the physiological compatibility so that a plasmid-bourne gene can be expressed and useful in the transconjugant recipient cells by interacting with host proteins in a beneficial manner (Porse et al 2018). The complexity hypothesis (Jain et al 1999, Novick & Doolittle 2020) asserts that protein-protein interaction (PPI) network connectivity and HGT rates are negatively correlated. In line with this, previous work on small sets of protein orthologs has found that less essential genes with higher HGT rates have fewer PPIs (Nakamura et al 2004, Aris-Brosou 2005, Puigbo et al 2010). HGT rates are higher for rarer genes like those in the accessory genome on plasmids, and lower for core genes (Dewar et al 2021, Touchon et al 2020). Moreover, gene context and physical gene distance are related to network proximity (Babu et al 2006) such that accessory genes and newly transferred genes are likely to be peripheral in PPI networks, including plasmid-encoded ones, whereas the chromosomal proteins should be more central.

Plasmid properties vary extensively based on the host cell (De Gelder et al 2007, Dunn et al 2021, Alonso-Del Valle et al 2021, Kottara et al 2018, Sheppard et al 2020, Gama et al 2020, Alderliesten et al 2020), suggesting that the interaction between host chromosomal proteins with plasmid-encoded ones is important. The evidence collated so far implies that chromosomes adapt to plasmids more than the reverse, typically by single nucleotide polymorphisms (SNPs) (Stalder et al 2017, Harrison et al 2015, San Millan et al 2014, Hall et al 2020), but sometimes by larger gene deletions (Modi et al 1991, Porse et al 2016, Lee & Marx 2012). This co-evolution of plasmid-encoded and chromosomal proteins

means more systematic approaches to examining the extent to which these proteins interact across bacterial species could provide insights into plasmid-host compatibility.

In this study, we assessed the hypothesis that chromosomal and plasmid-encoded genes have different interaction levels based on the host species, PPI context and PPI connectivity. Using a core dataset of 4,363 bacterial samples, we examined PPI network connectivity using PPI data for all chromosomal proteins and for plasmid-encoded ones. Importantly, HGT is not strongly affected by gene sequence composition and features (Porse et al 2018), supporting our simple classification of genes as present or absent here. We examined the changes in PPI network structure based on changes in indirect connections when plasmid-encoded proteins enter a host's chromosomal PPI network. We identified distinctive features associated with plasmid-encoded proteins and their PPIs, assessed their covariation, and thus provide a pilot study of plasmid-related PPIs s across all bacteria.

Methods

Protein-protein interaction data extraction

We retrieved PPI information for all available valid bacterial genomes (n=4,445) from STRING database v12 (Szklarczyk et al 2021) with R v4.0.1 (R Core Team 2021) and RStudio v2022.2.3.492 (RStudio Team 2022) via Bioconductor v3.11 (Ihaka & Gentleman 1996) and packages Rentrez v1.2.2 (Winter 2017), STRINGdb v2.0.1 (Szklarczyk et al 2018), and StringR v1.4.0 (Wickham 2019) (Figure 1). Of the initial 4,445 potential samples listed, 22 were not accessible and four had more >300,000 PPIs, suggesting potential accuracy issues and so they were excluded from analysis. The valid data (n=4,419 samples) was processed and collated using Dplyr v1.0.8 (Wickham et al 2002), Forcats v0.5.1 (Wickham 2021), Grid v4.4.1, ReadR v2.1.2 (Wickham et al 2022), Readxl v1.4.0 (Wickham & Bryan 2022), Tibble v3.1.6 (Müller & Wickham 2021), TidyR v1.2.0 (Wickham & Girlich 2022), Tidyverse v1.3.0 (Wickham et al 2019), VennDiagram v1.7.3 (Chen 2022) and Xlsx v0.6.0. The total number of unique protein names in the entire dataset was 9,551,828, though the true number of gene clusters is likely smaller due to name inaccuracy and redundancy. Using a STRINGdb score threshold of >400, we used this data to compute the numbers of unique proteins and pairwise PPIs on chromosomes: this score threshold of >400 was used throughout this study. These 4,419 post-QC samples had 3,628.5±1,710.0 (mean±SD) proteins and a mean of 57,105.8±34,698.6 PPIs each. Not all data was accessible for 56 samples among these 4,419, leaving 4,363 samples. This and subsequent data below were visualised with R packages ggplot2 v3.3.5 (Wickham 1996) and ggrepel v0.9.1 (Slowikowski 2021). All reported p values were corrected for multiple testing using the Benjamini-Hochberg approach in R.

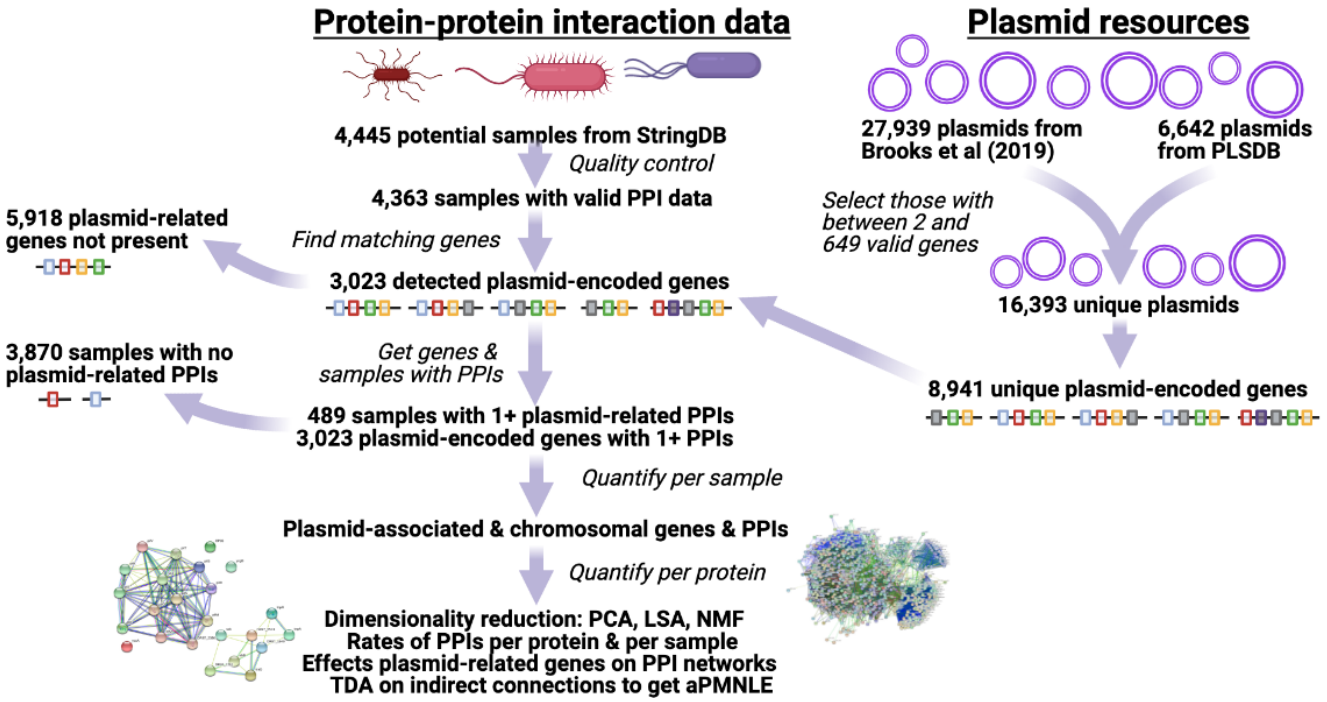


Figure 1. Summary of data sources and analytical steps. The plasmid resources (top right) and protein-protein interaction (PPI) data from StringDB were ultimately merged based on gene matches. Plasmid-encoded genes with PPIs were the focus, and gene prevalence and PPI level per sample were computed, as well as more general patterns across sample based on a range of complementary methods. Created with BioRender.com.

The accuracy of gene names in annotation is a pervasive issue where previous work has shown ~46±9% of genes can be functionally assigned (Zhou et al 2021). To explore that limiting factor here,

we examined the frequency of four-digit (short) gene names to longer ones with five or more digits, after removing any instances of names ending in an underscore or dash or dot followed by any digit. This showed that these samples had a median of 411±410 short names and a median of 2,931±1,744 long names each (Figure S1).

Identification of plasmid-encoded genes and interactions across bacteria

To get all available plasmid-encoded genes, we collated 32,839 plasmids: 27,939 from Brooks et al (2019) and 6,642 from PLSDB (v2020_11_19, Galata et al 2019) and retrieved gene annotation for each plasmid using Genbankr v1.16.0 (Figure 1). We focused on 16,383 with between two and 640 annotated genes (<640 to avoid chromosome-related contigs). We verified that the plasmids were annotated as plasmids, including 12 with lengths >2 Mb for *Ralstonia solanacearum* and *Rhizobium gallicum*. A gene was defined as plasmid-encoded if it had been found on a plasmid originating in the same species, yielding two categories for genes: plasmid-encoded or chromosomal. These plasmids had a median of 12 unique annotated genes per plasmid and had 8,941 unique plasmid-encoded genes across 98,534 unique sample-gene associations (Table S1, see full table FigShare doi: https://doi.org/10.6084/m9.figshare.19525630). Analyses focused on the 288 samples with at least one plasmid gene, and the 5,538 plasmid-encoded genes detected in at least one sample here.

Next, we identified the numbers of PPIs per protein per sample. A PPI was defined as plasmid-related if either (or both) protein(s) were found on a plasmid, yielding three categories for proteins: chromosomal, plasmid-related with one plasmid-linked protein in the pair, or exclusively plasmid-related where both protein pairs were plasmid-encoded – in practise the latter category was rare and was not investigated in detail here. The numbers of PPIs in which both proteins were exclusively chromosomal was large (286,364,425) compared to the totals for plasmid-related PPIs (390,592) and PPIs exclusively on plasmid-encoded proteins (46,772). This meant the fraction of PPIs that were plasmid-related was 0.136%, and 0.016% of all PPIs were exclusively plasmid-related, and the remainder chromosomal (99.86%). In the samples with at least one plasmid-related PPI, we used the probabilities of having a plasmid-related or chromosomal protein like allele frequencies to compare to the observed frequencies of plasmid-restricted, plasmid-chromosome and chromosome-restricted PPIs per sample in a manner analogous to Wright's F-statistic (Wright 1951) as $F = 1 - obs/exp$ where *obs* was the fraction of plasmid-chromosome PPIs, and *exp* was twice the product of the frequencies of plasmid-related and chromosomal proteins. This compared the expected probabilities of plasmid-restricted, plasmid-chromosome and chromosome-restricted PPIs against the observed rates to test for structure (or absence thereof) between the plasmid-related and chromosomal proteins.

We examined the taxonomic classifications of the samples across families, orders, classes and phyla using R package taxize v0.9.98 (Chamberlain et al 2020) to examine their scaled pairwise Euclidean distances of the plasmid presence-absence data in a dendrogram from hierarchical clustering from R packages stats v3.6.2 and dendextend v1.15.2 (Galili 2015). This identified totals of 100 families, 46 orders, 20 classes and 10 phyla.

Screening for correlated bacterial samples and plasmid-encoded genes

We examined patterns of covariance across the set of the samples and 3,023 plasmid-encoded genes using three approaches. To resolve highly correlated sets of samples, the 1st assessed genes and sample-gene pairs using principal components analysis (PCA) implemented with R packages stats v3.6.2 and factoextra v1.0.7 (Kassambara & Mundt 2020). The 2nd was the distributional semantics method, latent semantic indexing (LSA): the probabilistic version has been used to examine other bacteria's drug resistance genomic data previously (Rusakovica et al 2014). Here, it applied a classical vector space model and singular value decomposition to determine the samples' plasmid-encoded genes: allocating genes to samples with which they are strongly associated. LSA allocated the samples across the maximum possible 360 dimensions here, which represented the plasmid-encoded genes.

202 The 3rd approach was non-negative matrix factorisation (NMF) (Lee & Seung 1999) to examine the
203 associations of individual plasmid-encoded genes with individual bacterial samples using R packages
204 nmf v0.23.0 (Gaujoux & Seoighe 2010) and Biobase v2.52.0 (Huber 2015). This was applied to 331
205 samples with at least three plasmid-encoded genes and 1,733 plasmid-encoded genes found in at three
206 samples as presence-absence data. The optimal rank was determined by examining changes in the
207 cophenetic correlation coefficient across ranks 4-26 for 10 runs per rank to ensure clustering was stable
208 and to select the maximum rank that retained a high cophenetic coefficient. This reduced the
209 complexity of the data per sample to six groups (ranks) (cophenetic correlation coefficient maximised
210 at 0.971). We also examined the covariation of samples with the PPI levels per gene for 481 samples
211 and 2,363 plasmid-encoded genes where the input data was the number of PPIs per protein per sample
212 using NMF as above where each protein had at least 30 PPIs across the sample and each sample had at
213 least ten PPIs in total. The smaller dataset was due to a number of samples and genes with zero
214 plasmid-related PPIs. The optimal rank was determined as above, and an estimate of seven groups
215 (ranks) was obtained (cophenetic correlation coefficient maximised at 0.9856).
216
217 **Topological data analysis of the bacterial protein-protein interaction networks**
218
219 We developed computationally efficient metrics to investigate the topology of a PPI network, with in
220 our focus the PPI network's indirect connectivity. Constructing the Vietoris-Rips complex (Vietoris
221 1927) on the PPI network allows counting "non-trivial loops": loops made of chains of PPIs (the PPIs
222 are the edges of our Vietoris-Rips complex), such that some of the proteins involved were not directly
223 connected to each other by a PPI. A pair of such proteins was then indirectly connected by two chains
224 of PPIs along the loop (each obtained by starting from one protein and following the loop in one of the
225 two directions until reaching the protein which was not directly connected to it by a PPI). For this
226 reason, we called a non-trivial loop an "indirect connection".
227
228 The number of indirect connections and the number of PPIs are positively correlated, and previously
229 we observed that the ratio of these two (number of indirect connections divided by number of PPIs)
230 varies only moderately when we varied the StringDB combined score threshold, which modified the
231 PPI network topology by reducing the number of PPIs (Decano et al 2020). If the indirect connectivity
232 of the PPI network was defined as this ratio for one score threshold, it would lose information about
233 the strength of the PPIs. Therefore, we introduced here a measurement which takes the PPI network
234 topologies at all thresholds into account; we called it the Persistent Maximum of Non-trivial Loops per
235 Edge (PMNLE). This is the maximal ratio (number of indirect connections divided by number of PPIs)
236 which was reached or exceeded across an interval of at least 100 score thresholds. Measuring the
237 PMNLE by calculating the indirect connections at all score thresholds was computationally inefficient,
238 so to reduce the ecological impact and computation time 20-fold, we used an approximate PMNLE
239 (aPMNLE), which was the PMNLE measured only at score thresholds from 400 to 900 with a step of
240 20. For 16 samples, the scaled deviation between the PMNLE and aPMNLE was small at just 0.02%
241 with a standard deviation of 0.25% (Table S2).
242
243 The PPI networks of the 491 samples with plasmid-encoded proteins were analysed with all proteins,
244 and then with chromosomal ones only. This used a specialised open-source tool (Rahm 2019) that
245 constructed the two-dimensional part of the Vietoris-Rips complex by registering the trios of proteins
246 (namely, triangles consisting of three proteins connected to one another by three PPIs) and then ran
247 sparse matrix computations with the LinBox library (with LinBox v1.1.6) to obtain the 1st Betti
248 number, which is the number of indirect connections, and the 0th Betti number, which is the number of
249 connected components (subnetworks of the PPI network in which each pair of proteins is joined by a
250 chain of PPIs - hence there is no chain of PPIs joining two distinct connected components).
251 Additionally, we use the numbers of PPIs, trios, connected components and indirect connections
252 computed at the combined score threshold 400 for comparison: where unstated, the combined score
253 threshold used was 400. In many bacteria's PPI networks, the purely chromosomal part determined the
254 indirect connectivity, i.e., if the aPMNLE for the full PPI network was similar to the aPMNLE of the
255 chromosomal PPI network, then the influence of the plasmids on the aPMNLE was negligible. We

256 examined the difference of the former minus the latter of these two aPMNLE values, which we scaled
257 by the aPMNLE of the full PPI network to measure the percentage change when plasmid-linked
258 proteins were removed. The aPMNLE was associated with the numbers of PPIs per sample (r=0.42,
259 p=8e-182).

Results

We retrieved PPI data from StringDB to compare the properties of plasmid-encoded versus chromosomal proteins, with a view to the potential compatibility and signals of co-evolution for plasmid-chromosome combinations. This focused on the numbers of chromosome-linked PPIs, plasmid-associated PPIs, taxonomical classification based on plasmid gene profiles, relative rate of PPIs, indirect connections, and the effect on PPI network structure.

A positive correlation between the numbers of proteins and the numbers of interactions

We examined the association between the numbers of proteins and PPIs across representatives spanning 4,363 valid bacterial samples based on their unique annotated proteins on StringDB. As expected, the numbers of proteins and PPIs was highly correlated (adjusted r=0.914, p=5.2e-15) (Figure 2, Table S3 – see full data at FigShare https://doi.org/10.6084/m9.figshare.19525708). This suggested that larger chromosomes have more proteins and thus PPIs. It also suggested variation in protein and PPI numbers across bacteria did not skew the association between the numbers of proteins and PPIs, enabling evaluation of features of plasmid-encoded proteins in different samples combinations in more detail.

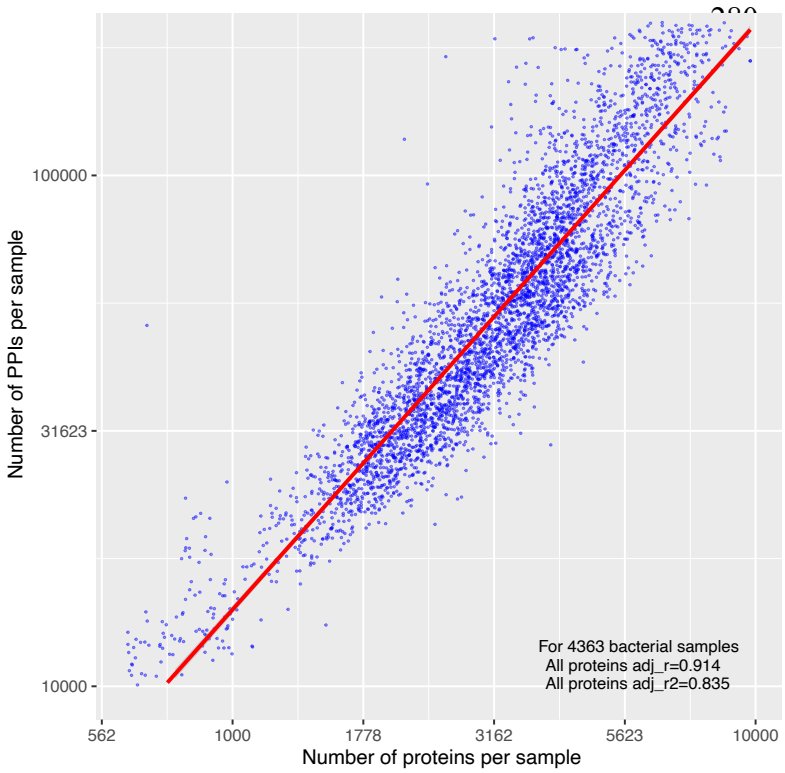


Figure 2. The number of proteins per bacterial genome (x-axis) was highly predictive of the number of PPIs (y-axis) (adjusted r^2=0.835). The data was for 4,363 bacterial samples, each of which is shown by a blue dot, with a linear model line of best fit shown in red.

Distinct patterns of plasmid-encoded gene retention across bacterial samples

We compared these 4,363 samples' PPI data with the set of 8,941 unique plasmid-linked genes where a gene was defined as plasmid-encoded if it was detected on a plasmid from the same species. 66.2% (5,918) of these genes were not detected, yielding 3,023 detected genes. 491 samples (11.2%) had plasmid-encoded genes, leaving 88.8% (3,872) samples with no such genes detected. In this set of 491, the median number of samples in which a plasmid-encoded gene was found was three (interquartile range four, Figure 3A), and the median number of plasmid-encoded genes per sample was five, with an interquartile range of 12 (Figure 3B). The fraction of plasmid-encoded genes per sample was 0.65±2.5% (27±112 out of 3,927±1,367, mean±SD) in these 491 (Figure 3). Six *E. coli* had a median of 1,035 plasmid-encoded genes per sample and were the only samples with over 400 plasmid-encoded

genes. These results illustrated that most plasmid-encoded genes were rare, with few common ones, and that widely shared plasmid-encoded genes were unusual (Figure S2, see full figure FigShare doi: https://doi.org/10.6084/m9.figshare.19525453).

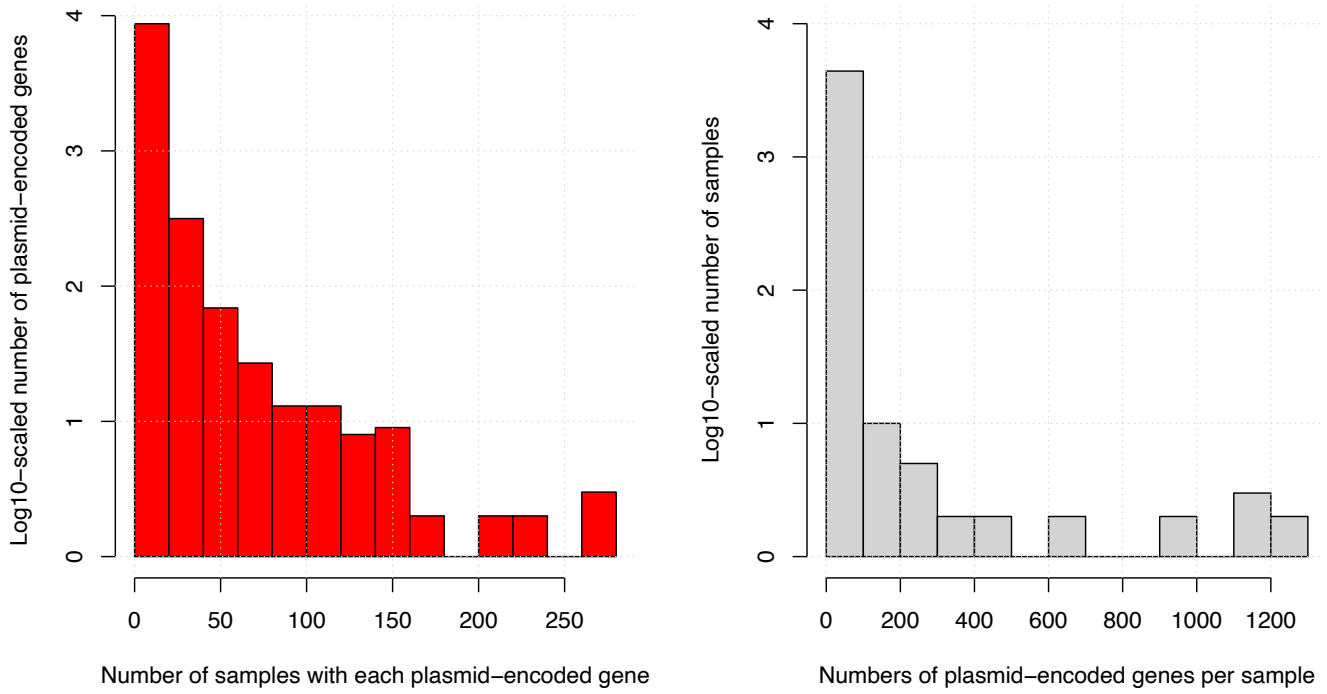


Figure 3. Left: The number of samples in which each plasmid-encoded gene (red) was found was generally small with a median of five. Right: The number of plasmid-encoded genes per sample (grey) had a median of three, including six *E. coli* samples with a median of 1,035 plasmid-encoded genes per sample that represent all samples with >400 genes. These figures excluded samples with no plasmid-encoded genes.

Plasmid-encoded genes are structured and reflect taxonomic groups

The samples were clustered by similarity based on their binary 3,023 unique plasmid-encoded gene data (presence-absence). Taxonomic classifications across the 100 families, 46 orders, 20 classes and ten phyla in these 491 samples showed that the *Enterobacteriaceae* were the most diverse family (n=24 samples, Z=1.49), and that the *Enterobacterales* (n=57, Z=0.77) was the most variable order, with limited changes at the class and order levels. Overall, the plasmid-encoded gene profiles per taxonomic group did not yield an informative taxonomic resolution (Figure S3, see full figure FigShare doi: https://doi.org/10.6084/m9.figshare.19525435).

Evidence for this low overall covariation came from PCA of this data where six *E. coli* and three *Ralstonia solanacearum* samples explained most variation across PC1 (34.5%) and PC2 (5.6%) (Figure S4A). These six *E. coli* had far more plasmid-encoded genes compared to the sample with the next highest number (*Ralstonia solanacearum* PSI07 with 347), which in turn exceeded the other samples substantially. Excluding these nine divergent *E. coli* and *R. solanacearum* samples, the remaining 482 samples' PC1 had 7.0% of variation and PC2 had 5.0% (Figure S4C). The plasmid-encoded proteins had a similar rate of covariation across all 491 samples where 16.8% of variation was allocated to PC1, and 14.9% to PC2 (Figure S4B), with higher heterogeneity in the 482 samples (6.8% to PC1, 4.9% to PC2, Figure S4D, see full figure at FigShare doi: https://doi.org/10.6084/m9.figshare.19525465, and data at FigShare doi: https://doi.org/10.6084/m9.figshare.19525471). These patterns were largely recapitulated by LSA of

349 all samples, which showed limited variation across the 1st (10.0% of variation) and 2nd (4.1% of
350 variation) dimensions (Figure S5).
351
352 **Plasmid-encoded proteins are rare and collectively interact with diverse chromosomal proteins**
353
354 We examined the samples' 3,023 unique plasmid-encoded proteins to identify PPIs where one or both
355 proteins were plasmid-related (termed plasmid-related PPIs here, see data at FigShare doi:
356 https://doi.org/10.6084/m9.figshare.19672527) to distinguish them from chromosomal PPIs where no
357 plasmid-encoded proteins were found, and we also tracked the rarer category of plasmid-related PPIs
358 where both protein pairs were plasmid-encoded (see data per sample at shorturl.at/mtFNZ). Overall, as
359 a percentage of all PPIs, plasmid-related PPIs constituted a median of 0.35±4.76% (mean±SD) PPIs
360 per sample, including 0.02% of all PPIs exclusively involving plasmid-encoded proteins. 3,874
361 samples had zero detected plasmid-related PPIs, 489 samples had at least one plasmid-related PPI (two
362 samples had one plasmid-encoded protein with no PPIs). These 489 samples had a median of 169 PPIs
363 where at least one protein was plasmid-related and a median of 53,947±27,590 chromosomal PPIs per
364 sample, which was comparable to the rate for all samples (48,834±46,615, Table S3). Their total
365 number of plasmid-related PPIs had no clear association with the numbers of proteins per sample
366 (r=0.05), but did with the numbers of chromosomal PPIs per sample (r=0.27, Figure S6). Of these 489
367 samples, 283 had at least one PPI involving only plasmid-encoded proteins, with a median of five such
368 PPIs.
369
370 We examined the plasmid-gene associations across 331 samples that had at least three of the 1,733
371 plasmid-encoded proteins detected in at least three samples using NMF (Figure S7). This allocated
372 these samples and proteins to six distinct groups based on their pattern of plasmid-encoded protein
373 sharing (Figure S8, see full figure at FigShare doi: https://doi.org/10.6084/m9.figshare.19525492), and
374 the proteins to these same six groups as mixture coefficients based on their prevalence in the samples
375 (Figure S9, see full figure at FigShare doi: https://doi.org/10.6084/m9.figshare.1952559). There were
376 just five samples in rank 3 that were five *E. coli* (all bar *E. coli* 536), which was far below the mean
377 number of samples per rank (77.3, Figure S10). These were associated with 1,065 plasmid-encoded
378 genes, far above the average number of proteins per rank (526.2, Figure S11).
379
380 Similarly, we investigated 2,363 plasmid-encoded proteins' associations based on PPI rates across the
381 same 481 samples where each protein had >30 PPIs and each sample had >10 PPIs. This allocated
382 these samples and proteins to seven distinct groups based on their pattern of PPI numbers (Figure S12,
383 see full figure at FigShare doi: https://doi.org/10.6084/m9.figshare.19611276), and these same proteins
384 to the seven groups as mixture coefficients based on their PPI numbers for each sample (Figure S13,
385 see full figure at FigShare doi: https://doi.org/10.6084/m9.figshare.19611282). Like above, all six *E.*
386 *coli* (including *E. coli* 536) were the sole members of a rank (five), and corresponded to 238 proteins'
387 PPI rates as mixture coefficients (Figure S14), which again was more than the average number of PPI
388 rates associated with each rank (748, Figure S15).
389
390 **Plasmid-encoded proteins have two-fold higher rates of protein-protein interactions**
391
392 The mean number of PPIs per protein was 2.2-fold higher for plasmid-related proteins compared to
393 chromosomal ones using data for all samples, though with large variations (n=13,348 plasmid-related
394 proteins in 491 samples: 39.0±35.0 vs n=15,450,151 chromosomal proteins in 4,363 samples:
395 17.6±5.7, mean±SD, t-test p=4.8e-16, Figure 4). This was not due to artefacts in the 489 samples with
396 plasmid-related PPIs: their rate of chromosomal PPIs at 14.8±3.7 PPIs per protein was similar to the
397 other samples (Table S3, see data at FigShare doi: https://doi.org/10.6084/m9.figshare.19575820).
398 However, this effect was protein-specific and not consistently discriminatory because these different
399 groups overlapped considerably (Figure 4C), implying that genomic context was not a major
400 determinant of PPI rates, and plasmid-related proteins simply had a wider range of PPI levels. To
401 control for the high variation in PPI rates per plasmid-related protein, we found that 19% (65 out of
402 345) of samples with sufficient numbers of plasmid-related proteins and PPIs to test quantitatively had

plasmid-encoded proteins with much higher PPI rates per sample (t-test $p<0.05$), including all six *E. coli* (Figure S16, see data at FigShare doi: https://doi.org/10.6084/m9.figshare.20230299). As an illustration of PPI network structure, a network centred on the SfmC protein in *E. coli* K12 MG1655 showed high levels of connectivity amongst 20 other proteins (Figure S17, Table S4).

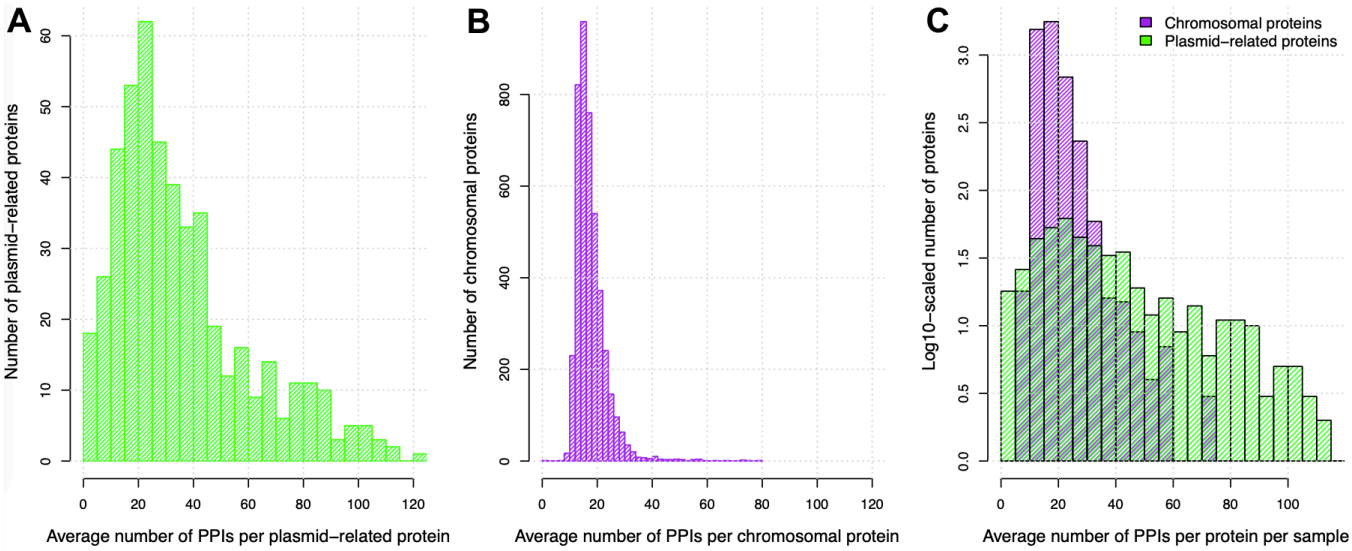


Figure 4. The average number of PPIs per (A) plasmid-related protein (green, 39.0±35.0, mean±SD) was higher than that for (B) chromosomal proteins (purple, 17.6±5.7), but (C) there was no consistent evidence for a consistent difference in PPI rates between these two groups (plasmid-related proteins in green, chromosomal proteins in purple – note log10-scaled y-axis and minor differences in binning per plot).

Nearly all (>99%) plasmid-encoded proteins had a PPI with a chromosomal protein, whereas only 7% of plasmid-encoded proteins had a PPI with another plasmid protein in the 489 samples with at least one plasmid-related PPI. Using the plasmid protein frequency information above, we tested for structure between plasmid-related proteins and chromosomal ones based on the rates of intergroup PPIs in a manner analogous to Wright's F-statistic to quantify the difference between the observed and expected levels of plasmid-chromosome and plasmid-specific PPIs per sample (Wright 1951). We found a relatively higher rate of plasmid-plasmid PPIs compared to plasmid-chromosome PPIs, which in turn were much higher than chromosomal PPIs (Figure S18). This suggested that plasmid-encoded proteins tended to interact with other plasmid proteins more often than would be expected by chance, indicating PPI network separation of plasmid-encoded and chromosomal proteins. Moreover,

Protein-protein interaction networks are generally robust to the loss of plasmid proteins

To examine the effect of plasmid-encoded proteins on PPI network topology, we examined the aPMNLE for all proteins and for chromosomal proteins only, such that the difference between these aPMNLE values indicated plasmid-driven effects (Figure S19). The aPMNLE reflects indirect (or secondary) connections, which may be reduced when plasmid-related proteins are removed if there are no alternate paths between remaining chromosomal proteins. However, if there are many PPI paths between chromosomal proteins, then this high indirect connectivity may indicate robustness within the PPI network such that the elimination of plasmid-related proteins has no effect (plasmid-related proteins thus have few indirect connections). Moreover, if plasmid-related proteins tend toward the PPI network periphery, the total number of indirect connections may not change relative to a larger drop in the numbers of PPIs, again indicating plasmid-related proteins had few indirect connections. In contrast, if plasmid-related proteins are central and mixed among chromosomal proteins in the PPI

network, the number of indirect connections and numbers of PPIs will be closely correlated (here, plasmid-related proteins have proportional indirect connection rates).

A majority of samples (96.3%, 471 out of 489 samples) showed no substantive differences in aPMNLE values between all proteins versus chromosomal ones alone, indicating that plasmid-encoded proteins had no large effects on the PPI network structure even though the plasmid-related proteins contributed large numbers of PPIs, consistent with robust networks. This was consistent with the idea that for these 471 samples the plasmid-encoded proteins overall had comparable indirect connection rates with the chromosomal proteins, but perhaps higher relative PPI rates because no evidence of excessive indirect connection numbers was observed.

3.7% (18 out of 489) samples had an aPMNLE for all proteins (including plasmid-encoded ones) exceeding the chromosomal aPMNLE by two standard deviations, indicating that plasmid-encoded proteins increased the indirect connection rates and that these PPI networks were less robust. No samples had a chromosomal aPMNLE that was two standard deviations above the full network aPMNLE, so no plasmid-encoded protein set strongly increased the rate of indirect connections per sample. When compared to the other 471 samples with plasmid genes, these 18 had altered aPMNLE values for chromosomal proteins (t-test p=4.9e-5) but not for all proteins (p=0.82, Figure S20). Furthermore, if the plasmid-encoded proteins were included for each sample, the number of PPI network connected components grew by 71% for the 471 samples with robust networks, whereas for these 18 samples with less robust networks it grew by 327%, indicating that these plasmid-encoded proteins had high PPI rates, fewer indirect connections and were often disconnected from the main network. We also observed these 18 had fewer genes per sample compared to the larger set of 471 (2,314±1,429 vs 4,013±1,336, mean±SD), perhaps because smaller genomes have fewer PPIs overall and so were less robust to plasmid-related protein removal.

To quantify the extent to which plasmid-encoded proteins increased PPI rates but not indirect connections, we compared the correlation between plasmid-related proteins and PPIs per sample in the 489 samples with plasmid-related PPIs, which were positively correlated (r=0.51, p=1e-143, Figure 5A). However, increasing the number of plasmid-encoded proteins did not have the same magnitude of effect on the number of indirect connections per sample (r=0.06, p=1, Figure 5B). Similarly, the latter was negatively associated with the number of plasmid-related PPIs (r=-0.08, p=0.30, Figure 5C). The partial correlations for each pair of variables were similar (0.86, 0.04, -0.10, respectively). For example, the 69 plasmid-encoded proteins of *Serratia marcescens* subsp. marcescens Db11 had 92 plasmid-plasmid PPIs with one another and an additional 2,214 plasmid-chromosome PPIs with (an average of 33.4 PPIs per plasmid protein), compared to 58,857 chromosomal PPIs among the 4,614 chromosomal proteins (12.8 PPIs per chromosomal protein, Table S4, Figure S21). Overall, this implied that plasmid-related proteins tended to be at the PPI network periphery connected to chromosomal proteins, and not mixed usually with the main chromosomal proteins in the PPI networks.

Plasmid-related proteins are generally peripheral to PPI networks

Given that plasmid-related proteins purported to be highly connected but not central to PPI networks mainly composed of chromosomal proteins, we scaled the numbers of indirect connections by protein. We observed a trimodal pattern of indirect connections per protein. In the 489 samples with plasmid-related PPIs, the chromosomal proteins had more indirect connections per protein than plasmid-encoded proteins (average per sample 3.7±2.1 vs 0.9±0.6, t-test p=5.2e-15). Moreover, the indirect connections per protein in these 489 was positively correlated with the number of PPIs per sample for chromosomal (r=0.61, Figure 6A) but not all proteins (r=0.02) due to the effect of plasmid-encoded proteins, confirming that the latter tended not to be mixed among the PPI networks. The chromosomal proteins of those 489 samples in turn had a lower level of indirect connections per protein compared to the 3,874 samples without plasmid-related PPIs (average per sample 3.7±2.1 vs 5.5±3.1, t-test p=5.2e-15). The latter 3,874 samples had higher levels of indirect connections that were positively correlated with the PPIs per sample like the 489's chromosomal proteins (Figure 6B).

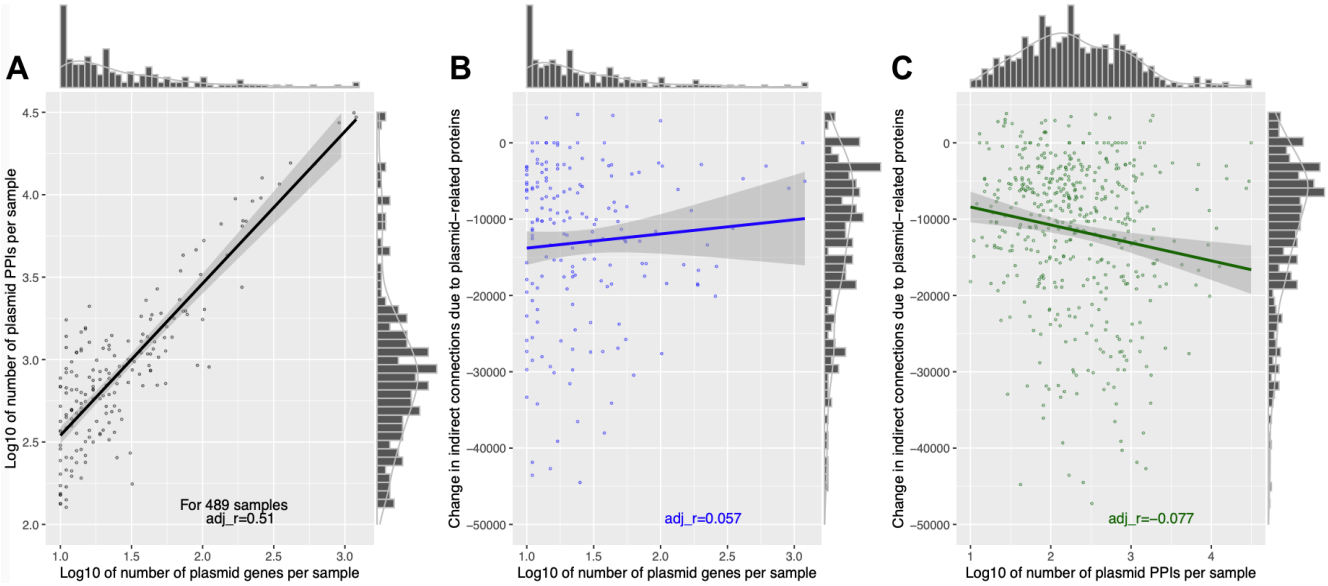


Figure 5. The (A) log10-scaled numbers of plasmid-related proteins per sample (x-axis) was positively correlated with the numbers of plasmid PPIs per sample (y-axis) (black, r=0.51) in the 489 samples with plasmid-related PPIs, but less so with (B) the change in the numbers of indirect connections per sample due to the inclusion of the plasmid-encoded proteins (blue, r=0.06). (C) The log10-scaled numbers of plasmid PPIs per sample (x-axis) was negatively associated with the numbers of indirect connections per sample due to the inclusion of the plasmid-encoded proteins (green, r=-0.08). The lines of best fit for each plot shows the linear correlation of the number of PPIs per sample with the number of indirect connections per protein. The histograms indicate the marginal densities per axis for each dataset.

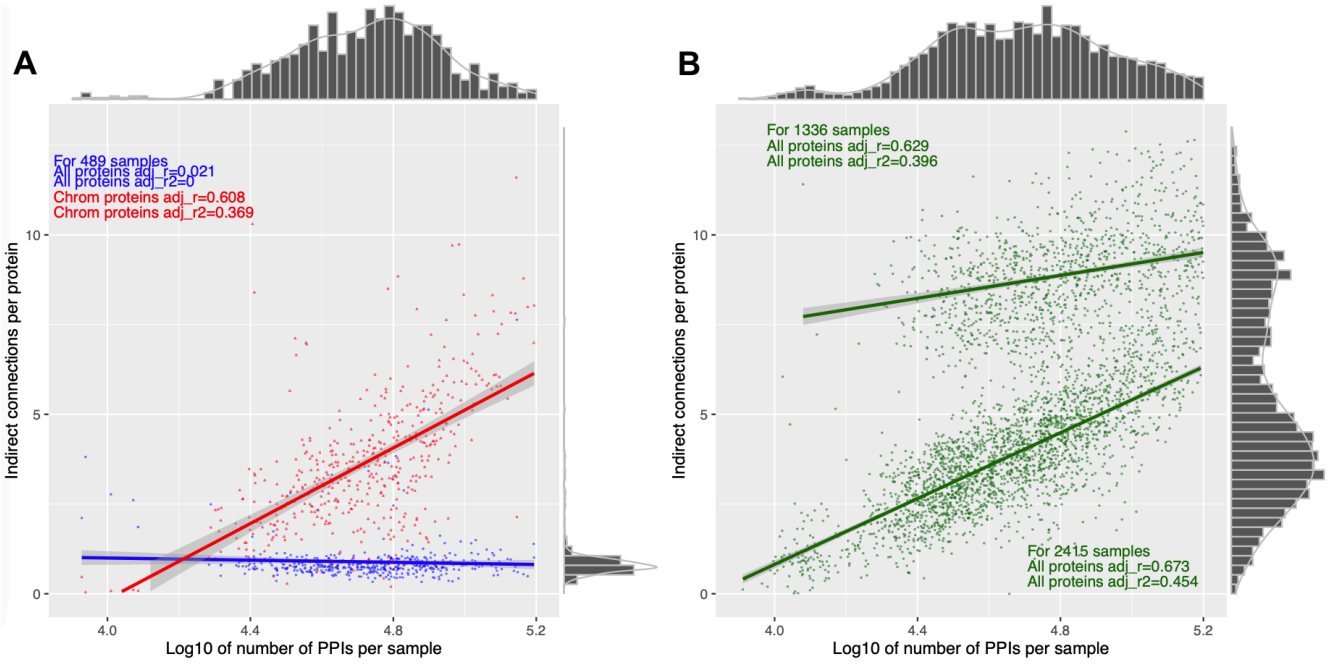
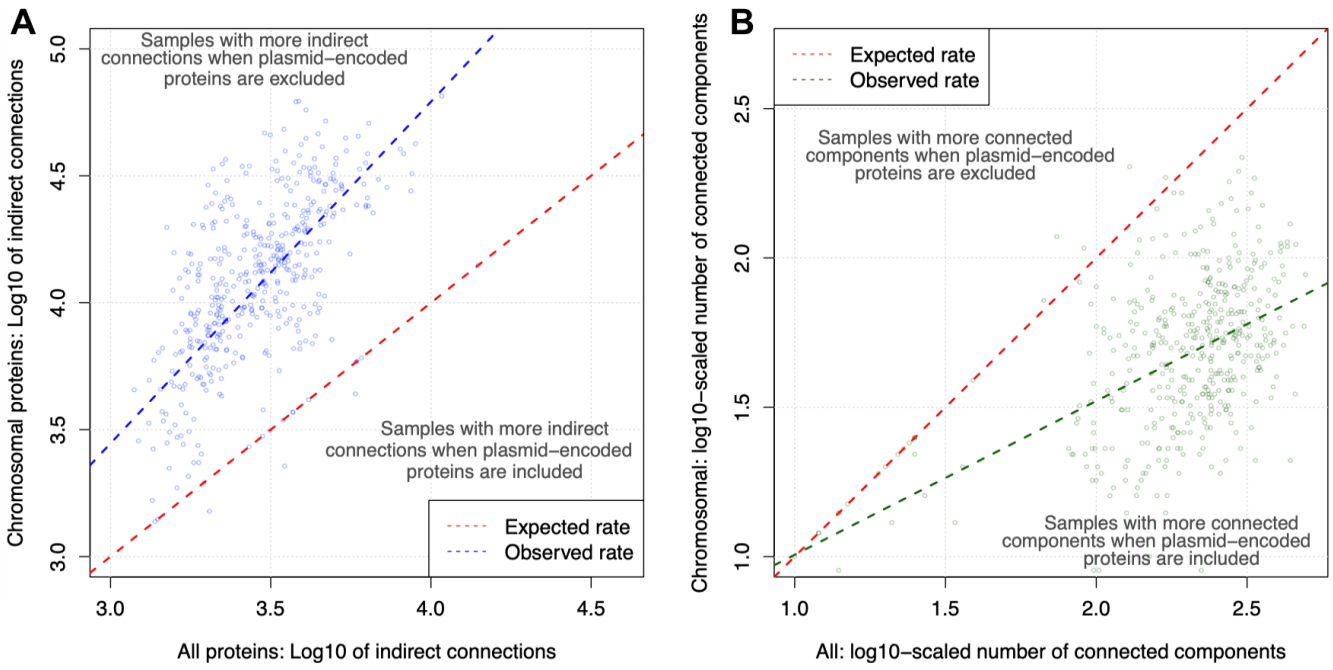


Figure 6. The number of PPIs per sample (x-axis) was positively correlated with the number of indirect connections per protein for (A) chromosomal (red, r=0.61) but not all proteins (including plasmid-encoded ones, blue, r=0.02) in the 489 samples with plasmid-encoded proteins. (B) The other samples with no plasmid-encoded proteins had positive correlations (green, r=0.63 and r=0.67) though with a bimodal pattern (groups of n=2,415 and n=1,336). The lines of best fit for each plot shows the linear correlation of the number of PPIs per sample with the number of indirect connections per protein. The histograms indicate the marginal densities per axis for each dataset.

519 Plasmid-related proteins had a median of 4.4-fold fewer indirect connections per sample than
520 chromosomal proteins in the set of 489 samples (Figure 7A). We also observed that additional
521 plasmid-chromosome PPIs constituted 89.7% of all PPIs in these 489, and 93% of plasmid-encoded
522 proteins had no plasmid-related PPIs. To explain this, consider a PPI network where part of it had four
523 chromosomal proteins (A, B, C, D) sharing PPIs A-B, B-C, C-D and D-A, so it has one indirect
524 connection (with a path A-B-C-D, Figure S22). If we added a plasmid protein P to this network such
525 that it had PPIs with all four proteins, the indirect connection across A-B-C-D would be lost but the
526 network would gain four PPIs. An alternative model where A, B, C and D had no PPIs with one
527 another would be less likely because then the addition of P would create PPIs and more indirect
528 connections, which was not observed in our findings. In addition, if A, B, C and D interact with P then
529 it is more likely that they are both functionally related and interact with one another.
530
531 Finally, we assessed the numbers of PPI network connected components for all proteins versus
532 chromosomal ones: the latter had fewer connected components compared to all proteins in the 489
533 samples with plasmid-related PPIs (50.0±34.6 vs 227±95.9, median±SD, Figure 7B). The numbers of
534 proteins per connected component was more strongly associated with the chromosomal proteins than
535 for all proteins in relation to the numbers of PPIs (r=0.35 vs 0.01), chromosome-related PPIs (r=0.35
536 vs 0.00), and proteins per sample (r=0.18 vs 0.00) but not plasmid-related PPIs (r=0.04 vs 0.00, Figure
537 S23) in these 489. Additionally, these 489 samples had far more connected components compared to
538 3,872 samples without plasmid-related PPIs (means 222.4 vs 40.5, t-test p=4.8e-15). This highlighted
539 that when there were more PPIs and proteins present, chromosomal proteins were spread across fewer
540 connected components, whereas plasmid proteins could be found in more separate connected
541 components.
542
543



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546 **Figure 7**. The log10-scaled numbers of (A) indirect connections (blue) and (B) connected components
547 (green) for all (chromosomal and plasmid) proteins (x-axis) related to chromosomal proteins only (y-
548 axis) for the 489 samples with plasmid-related PPIs. The red lines show the expected rate if (A) the
549 numbers of indirect connections for all proteins and chromosomal proteins were identical and (B) the
550 numbers of connected components for all proteins and chromosomal proteins were identical. (A) The
551 blue line shows the correlation between the numbers of indirect connections for all proteins and
552 chromosomal proteins (>98% of samples had more indirect connections when plasmid-related proteins
553 were omitted). (B) The green line shows the correlation between the numbers of connected
554 components for all proteins and chromosomal proteins (>97% of samples had more connected
555 components when plasmid-related proteins were retained).

556 **Discussion**
557
558 Bacterial genome structure has been shaped by HGT more so than gene duplication in many species,
559 such as *E. coli* (Lercher & Pál 2008, Price et al 2008). Mobile plasmid-encoded genes can endow host
560 cells with new phenotypes, including those linked to changed adhesion, AMR and virulence (Lipworth
561 et al 2022), and their fitness effects are associated with specific genetic, regulatory and protein
562 interactions (Hall et al 2017). To further our understanding of how new plasmid-encoded proteins can
563 affect the structure and signalling bacterial PPI networks, we compared the PPI profiles of plasmid-
564 linked and chromosomal proteins across 4,363 representative samples of well-characterised bacteria
565 using data from StringDB to find 491 samples with evidence of plasmid-encoded genes within the
566 same species.
567
568 We found that the number of proteins per sample was correlated with the number of PPIs, as
569 anticipated. This allowed further exploration to compare plasmid-encoded proteins with chromosomal
570 ones. Most plasmid-encoded proteins were rare across bacteria, and the total fraction of the genome
571 they constituted varied extensively. We defined plasmid-related PPIs as PPIs where at least one of the
572 interacting proteins had previously been observed on a contig labelled as a plasmid within the same
573 species, separate from chromosomal PPIs where neither protein was plasmid-linked. Only 11.2% of
574 samples (489 out of 4,363) had plasmid-related proteins and PPIs. Even in these samples, plasmid-
575 related genes made up only 0.65% of all genes detected and thus were rare. As a consequence,
576 plasmid-related PPIs made up only 0.14% of all PPIs, and PPIs that were exclusively plasmid-related
577 were very rare (0.016%).
578
579 Plasmid-encoded proteins covaried with one another depending on the genetic background of the host
580 cell such that the clustering the samples based on their plasmid-encoded gene similarity identified six
581 groups of covarying plasmid-encoded proteins and bacterial samples, including a specific one for *E.*
582 *coli* alone. This aligned with existing expectations of plasmid-host co-evolution (Loftie-Eaton et al
583 2017, Jordt et al 2020). The *Enterobacterales* was the clearly the most variable order, but overall
584 known taxonomical differences were not apparent. Similarly, the PPI rates per protein per sample had
585 even less taxonomical resolution power, showing that the numbers of PPIs per protein was typically a
586 more dynamic feature.
587
588 The complexity hypothesis (Jain et al 1999) asserts that proteins undergoing HGT have fewer PPIs and
589 operational cellular processes rather than not informational ones like transcription or translation
590 (Rivera et al 1998, Jain et al 1999, Jain et al 2002). It has been asserted that proteins with many PPIs
591 are less likely to undergo HGT (Wellner et al 2007, Price et al 2008, Cohen et al 2011), but we found
592 that plasmid-linked proteins had two-fold more PPIs on average. Nonetheless, the predictive power of
593 this across chromosomal versus plasmid-related proteins was low, suggesting that there is no intrinsic
594 difference in PPI rates between these groups of proteins. Nuances to the complexity hypothesis have
595 been found before: firstly, the being in a protein complex versus HGT chances (Wellner et al 2007);
596 secondly, the effect of cellular component state, function complexity and function conservation on
597 adaptive evolution potential (Aris-Brosou 2005); thirdly, HGT-linked genes have higher numbers of
598 regulatory factors (Price et al 2008); and fourthly, PPI connectivity has a large effect for proteins with
599 a similar function (Cohen et al 2011). Notably, many previous studies have focused on conserved
600 orthologs, whose genomic contexts and features may differ from genes that are more mobile.
601 Consequently, operational versus informational processes, complex protein functions, an intracellular
602 location, an ancient essential function, and host-recipient genetic distance may be more dominant
603 factors in determining PPI rates than HGT given our results here. An important caveat is that the
604 general trends we observed may not hold for all genera, families or orders.
605
606 We found that plasmid-related proteins interacted with large numbers of other chromosomal and
607 plasmid proteins. However, plasmid-related proteins contributed much less indirect (aka secondary)
608 connections, unlike chromosomal proteins. We found evidence of PPI network structure between
609 chromosomal and plasmid-encoded proteins, finding that plasmid proteins had a higher rate of plasmid

rather than chromosomal protein partners than expected. In addition, by using the aPMNLE as a measure of indirect connections, most samples with plasmid-related PPIs (>96%) were robust to the loss of plasmid-encoded proteins. The samples with networks more affected by plasmid-related protein loss had smaller genomes, and thus less redundancy in PPI network paths. Altogether, this was consistent with existing assertions that plasmid-encoded proteins tend to be at the periphery of a PPI network (Lercher & Pál 2008, Price et al 2008), perhaps because plasmids seldom encode essential proteins (Wein et al 2021), though gene essentiality depends on the host (Rousset et al 2021).

Our findings were limited by a number of issues. Firstly, our analysis was based on gene name similarity with the assumption that the redundancy in the annotated plasmid gene names would help alleviate the imprecision in gene naming systems, but this did not mitigate fully the limitation that some proteins were likely misclassified as chromosomal or plasmid-encoded. Secondly, many plasmids and bacterial samples are not annotated consistently, so many gene name matches were undoubtedly missed. Thirdly, we assumed that previous studies' labelling of large contigs as plasmids was correct: we made this (and all other) data accessible so that revisions to plasmid annotation could be made in light of the high number of genes (>500) in certain plasmids (see Table S1): sequence-based plasmid detection tools could help with this task. Fourthly, we obtained gene name data per sample from StringDB (Szklarczyk et al 2018, Szklarczyk et al 2021), and yet 5,918 plasmid-encoded genes were not detected in these samples, which highlighted imprecision either in the plasmid annotation, the sample annotation or (most likely) both. Fifthly, we did not perform sequence alignment nor read mapping to confirm at the sequence level matches, which is a next step following this work. Future work is needed to improve plasmid annotation, genome annotation, contig classification as plasmid or chromosomal and conduct gene comparisons at the sequence level. Future investigations of the effects of plasmid-encoded and chromosomal proteins on PPI networks should focus on specific samples that have known plasmid compositions.

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Author Contributions

TD – Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualisation, Project administration. AR – Methodology, Software, Formal analysis, Writing - Review & Editing. All authors reviewed the manuscript.

Competing Interests Statement

The authors declare no competing interests.

Data Availability Statement:

Item	Brief description	FigShare link
Table S1	Plasmid-encoded genes and their associated plasmid link(s)	https://doi.org/10.6084/m9.figshare.19525630
Table S3	Key features of the bacterial samples	https://doi.org/10.6084/m9.figshare.19525708
Figure S2	Heatmap of the bacterial plasmid-encoded genes	https://doi.org/10.6084/m9.figshare.19525453
Figure S3	Dendrogram of the bacteria based on plasmid-encoded gene similarity	https://doi.org/10.6084/m9.figshare.19525435
Figure S5	Heatmap of the bacteria and plasmid-encoded genes alphabetically sorted	https://doi.org/10.6084/m9.figshare.19525465

Data for Figure S5	Presence-absence data with bacteria as rows and genes as columns	https://doi.org/10.6084/m9.figshare.19525471
Figure S8	NMF heatmap of the metasample clustering of plasmid genes	https://doi.org/10.6084/m9.figshare.19525492
Figure S9	NMF heatmap of the mixture coefficient for the plasmid-encoded genes	https://doi.org/10.6084/m9.figshare.19525591
Figure S12	NMF heatmap of the metasample clustering of plasmid PPI rates	https://doi.org/10.6084/m9.figshare.19611276
Figure S13	NMF heatmap of the mixture coefficient for the plasmid-related PPI rates	https://doi.org/10.6084/m9.figshare.19611282
Data for all PPI analysis	4,363 samples' CSV files for all proteins with gene name, plasmid status and the total number of PPIs per sample	https://doi.org/10.6084/m9.figshare.19672527
Data for chrom PPI analysis	4,363 samples' CSV files for each sample showing PPIs, associated genes and StringDB identifiers	shorturl.at/mtFNZ
Data for Figure S16	The numbers of chromosomal & plasmid genes for 343 as PDF image files.	https://doi.org/10.6084/m9.figshare.20230299

Large datasets available from this study on FigShare, showing the item, description and the corresponding DOI link. See the Supplementary Data for more. The code associated with this paper is available on Github at https://github.com/downingtim/mobilome_2022 and https://github.com/arahm/HomologyLive and data files are there at https://figshare.com/projects/Bacteria_mobilome_2022/136720.

References

Agyekum A, et al. 2016. blaCTX-M-15 carried by IncF-type plasmids is the dominant ESBL gene in Escherichia coli and Klebsiella pneumoniae at a hospital in Ghana. Diagn Microbiol Infect Dis. 84(4):328-33. doi: 10.1016/j.diagmicrobio.2015.12.010

Ahmer BM, et al. 1999. The virulence plasmid of Salmonella typhimurium is self-transmissible. J. Bacteriol. 181, 1364–1368

Alderliesten JB, et al. 2020. Effect of donor-recipient relatedness on the plasmid conjugation frequency: a meta-analysis. BMC Microbiol. 20(1):135. doi: 10.1186/s12866-020-01825-4

Alonso-Del Valle A, et al. 2021. Variability of plasmid fitness effects contributes to plasmid persistence in bacterial communities. Nat Commun. 12(1):2653. doi: 10.1038/s41467-021-22849-y

Anda, M. et al. 2015. Bacterial clade with the ribosomal RNA operon on a small plasmid rather than the chromosome. Proc. Natl Acad. Sci. USA 112, 14343–14347.

Andam CP, Gogarten JP. 2011. Biased gene transfer in microbial evolution. Nat. Rev. Microbiol. 9, 543–555

Aris-Brosou S. 2005. Determinants of adaptive evolution at the molecular level: the extended complexity hypothesis. Mol Biol Evol. 22(2):200-9. doi: 10.1093/molbev/msi00

Babu M, et al. 2006. Evolutionary dynamics of prokaryotic transcriptional regulatory networks. Journal of molecular biology 358:614-633

Baltrus DA. 2013. Exploring the costs of horizontal gene transfer. Trends Ecol Evol. 28(8):489-95. doi: 10.1016/j.tree.2013.04.002

Bevan ER, et al. 2021. Molecular characterization of plasmids encoding blaCTX-M from faecal Escherichia coli in travellers returning to the UK from South Asia. J Hosp Infect. 114:134-143. doi: 10.1016/j.jhin.2021.03.030

Brinkmann H, et al. 2018. Horizontal operon transfer, plasmids, and the evolution of photosynthesis in Rhodobacteraceae. ISME J. 12, 1994–2010.

687 Brito IL, et al. 2016. Mobile genes in the human microbiome are structured from global to individual
688 scales. Nature. 2016 535(7612):435–439
689 Brockhurst MA, Harrison E, Hall JPJ, Richards T, McNally A, MacLean C. 2019. The Ecology and
690 Evolution of Pangenomes. Curr Biol. 29(20):R1094-R1103. doi: 10.1016/j.cub.2019.08.012
691 Brooks L, Kaze M, Sistrom M. A Curated, Comprehensive Database of Plasmid Sequences. Microbiol
692 Resour Announc. 2019 8(1):e01325-18. doi: 10.1128/MRA.01325-18
693 Canosi U, Lüder G, Trautner TA. SPP1-mediated plasmid transduction. J Virol. 1982 44(2):431-6. doi:
694 10.1128/JVI.44.2.431-436.1982
695 Carattoli, A. 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother.
696 53, 2227–2238.
697 Chamberlain S, et al. 2020. taxize: Taxonomic information from around the web. R package version
698 0.9.98, https://github.com/ropensci/taxize.
699 Chen H. 2022. VennDiagram: Generate High-Resolution Venn and Euler Plots. R package version
700 1.7.3. https://CRAN.R-project.org/package=VennDiagram
701 Coelho LP, et al. 2021. Towards the biogeography of prokaryotic genes. Nature doi:
702 https://doi.org/10.1038/s41586-021-04233-4
703 Cohen O, et al. 2011. The complexity hypothesis revisited: connectivity rather than function
704 constitutes a barrier to horizontal gene transfer. Mol. Biol. Evol. 28, 1481–1489
705 Cohen O, Gophna U, Pupko T. 2011. The complexity hypothesis revisited: connectivity rather than
706 function constitutes a barrier to horizontal gene transfer. Mol Biol Evol. 28(4):1481-9. doi:
707 10.1093/molbev/msq333
708 De Gelder L, Ponciano JM, Joyce P, Top EM. 2007. Stability of a promiscuous plasmid in different
709 hosts: no guarantee for a long-term relationship. Microbiology (Reading). 153(Pt 2):452-463.
710 doi: 10.1099/mic.0.2006/001784-0
711 Decano AG, Downing T. 2019. An Escherichia coli ST131 pangenome atlas reveals population
712 structure and evolution across 4,071 isolates. Scientific Reports doi: 10.1038/s41598-019-
713 54004-5
714 Decano AG, et al. 2019. Complete assembly of Escherichia coli ST131 genomes using long reads
715 demonstrates antibiotic resistance gene variation within diverse plasmid and chromosomal
716 contexts. mSphere doi: 10.1128/mSphere.00130-19
717 Decano AG, et al. 2020. Plasmids shape the diverse accessory resistomes of Escherichia coli ST131.
718 Access Microbiology doi: 10.1099/acmi.0.000179
719 Dewar AE, et al. 2021. Plasmids do not consistently stabilize cooperation across bacteria but may
720 promote broad pathogen host-range. Nat Ecol Evol. 5(12):1624-1636. doi: 10.1038/s41559-
721 021-01573-2
722 Downing T. 2015. Tackling drug resistant infection outbreaks of global pandemic Escherichia coli
723 ST131 using evolutionary and epidemiological genomics. Microorganisms 3(2):236-267.
724 Dunn S, Carrilero L, Brockhurst M, McNally A. 2021. Limited and Strain-Specific Transcriptional and
725 Growth Responses to Acquisition of a Multidrug Resistance Plasmid in Genetically Diverse
726 Escherichia coli Lineages. mSystems. 6(2):e00083-21. doi: 10.1128/mSystems.00083-21
727 Erdmann S, Tschitschko B, Zhong L, Raftery MJ, Cavicchioli R. A plasmid from an Antarctic
728 haloarchaeon uses specialized membrane vesicles to disseminate and infect plasmid-free cells.
729 Nat Microbiol. 2017 2(10):1446-1455. doi: 10.1038/s41564-017-0009-2
730 Forsberg KJ, et al. 2014. Bacterial phylogeny structures soil resistomes across habitats. Nature
731 509:612-616
732 Galata V, Fehlmann T, Backes C, Keller A. PLSDB: a resource of complete bacterial plasmids.
733 Nucleic Acids Res. 2019 47(D1):D195-D202. doi: 10.1093/nar/gky1050
734 Gama JA, Kloos J, Johnsen PJ, Samuelsen Ø. 2020. Host dependent maintenance of a blaNDM-1-
735 encoding plasmid in clinical Escherichia coli isolates. Sci Rep. 10(1):9332. doi:
736 10.1038/s41598-020-66239-8
737 Galili T. 2015. dendextend: an R package for visualizing, adjusting, and comparing trees of
738 hierarchical clustering. Bioinformatics. doi:10.1093/bioinformatics/btv428
739 Gaujoux R, Seoighe C. 2010. A flexible R package for nonnegative matrix factorization. BMC
740 Bioinformatics 11:367

741 Hall JPJ, Brockhurst MA, Harrison E. 2017. Sampling the mobile gene pool: innovation via horizontal
742 gene transfer in bacteria. Philos Trans R Soc Lond B Biol Sci. 372(1735):20160424. doi:
743 10.1098/rstb.2016.0424
744 Hall JPJ, Wright RCT, Guymer D, Harrison E, Brockhurst MA. 2020. Extremely fast amelioration of
745 plasmid fitness costs by multiple functionally diverse pathways. Microbiology (Reading).
746 166(1):56-62. doi: 10.1099/mic.0.000862
747 Harrison E, Brockhurst MA. 2012. Plasmid-mediated horizontal gene transfer is a coevolutionary
748 process. Trends Microbiol. 2012 20(6):262-7. doi: 10.1016/j.tim.2012.04.003
749 Harrison E, Guymer D, Spiers AJ, Paterson S, Brockhurst MA. 2015. Parallel compensatory evolution
750 stabilizes plasmids across the parasitism-mutualism continuum. Curr Biol. 25(15):2034-9. doi:
751 10.1016/j.cub.2015.06.024
752 Ho J, et al. 2020. Systematic review of human gut resistome studies revealed variable definitions and
753 approaches. Gut Microbes. 12(1):1700755. doi: 10.1080/19490976.2019.1700755
754 Huang W, et al. 2017. Emergence and Evolution of Multidrug-Resistant Klebsiella pneumoniae with
755 both blaKPC and blaCTX-M Integrated in the Chromosome. Antimicrob Agents Chemother.
756 61(7):e00076-17. doi: 10.1128/AAC.00076-17
757 Huber VJ, et al. 2015. Orchestrating high-throughput genomic analysis with Bioconductor. W. Huber,
758 V.J. Nature Methods 12:115.
759 Ihaka R, Gentleman R. 1996. R: A Language for Data Analysis and Graphics. Journal of
760 Computational and Graphical Statistics. 1996 (5)3:299-314.
761 Irrgang A, et al. 2017. CTX-M-15-Producing E. coli Isolates from Food Products in Germany Are
762 Mainly Associated with an IncF-Type Plasmid and Belong to Two Predominant Clonal E. coli
763 Lineages. Front Microbiol. 8:2318. doi: 10.3389/fmicb.2017.02318
764 Jain R, Rivera MC, Lake JA. 1999. Horizontal gene transfer among genomes: the complexity
765 hypothesis. Proc Natl Acad Sci U S A. 96(7):3801–3806.
766 Jain R, Rivera MC, Moore JE, Lake JA. 2002. Horizontal gene transfer in microbial genome evolution.
767 Theor Popul Biol. 61(4):489-95. doi: 10.1006/tpbi.2002.1596
768 Jordt H, Stalder T, Kosterlitz O, Ponciano JM, Top EM, Kerr B. 2020. Coevolution of host-plasmid
769 pairs facilitates the emergence of novel multidrug resistance. Nat Ecol Evol. 4(6):863-869. doi:
770 10.1038/s41559-020-1170-1
771 Kamil Slowikowski. 2021. ggrepel: Automatically Position Non-Overlapping Text Labels with
772 'ggplot2'. R package version 0.9.1. https://CRAN.R-project.org/package=ggrepel
773 Kassambara A, Mundt F. 2020. factoextra: Extract and Visualize the Results of Multivariate Data
774 Analyses. R package version 1.0.7. https://CRAN.R-project.org/package=factoextra
775 Kottara A, Hall JPJ, Harrison E, Brockhurst MA. 2018. Variable plasmid fitness effects and mobile
776 genetic element dynamics across Pseudomonas species. FEMS Microbiol Ecol. 94(1):fix172.
777 doi: 10.1093/femsec/fix172
778 Lee DD, Seung HS. 1999. Learning the parts of objects by non-negative matrix factorization. Nature
779 401:788–791.
780 Lee MC, Marx CJ. 2012. Repeated, selection-driven genome reduction of accessory genes in
781 experimental populations. PLoS Genet. 8(5):e1002651. doi: 10.1371/journal.pgen.1002651
782 Lehtinen S, et al. 2021. Evolutionary mechanisms that determine which bacterial genes are carried on
783 plasmids. Evol. Lett. 5, 290–301
784 Lercher MJ, Pál C. 2008. Integration of horizontally transferred genes into regulatory interaction
785 networks takes many million years. Mol. Biol. Evol. 25:559
786 Lipworth S, et al. 2022. The mobilome associated with Gram-negative bloodstream infections: A
787 large-scale observational hybrid sequencing based study. MedRxiv doi:
788 https://doi.org/10.1101/2022.04.03.22273290
789 Loftie-Eaton W, et al. 2017. Compensatory mutations improve general permissiveness to antibiotic
790 resistance plasmids. Nat Ecol Evol. 1(9):1354-1363. doi: 10.1038/s41559-017-0243-2
791 Ludden C, Decano AG, et al. 2020. Genomic surveillance of Escherichia coli ST131 identifies local
792 expansion and serial replacement of subclones. Microbial Genomics doi:
793 https://doi.org/10.1099/mgen.0.000352

Manzano-Marin A, et al. 2020. Serial horizontal transfer of vitamin-biosynthetic genes enables the establishment of new nutritional symbionts in aphids' di-symbiotic systems. ISME J. 14, 259–273.

Modi RI, Wilke CM, Rosenzweig RF, Adams J. 1991. Plasmid macro-evolution: selection of deletions during adaptation in a nutrient-limited environment. Genetica. 84(3):195-202. doi: 10.1007/BF00127247

Müller K, Wickham H. 2021. tibble: Simple Data Frames. R package version 3.1.6. https://CRAN.R-project.org/package=tibble

Nakamura Y, et al. 2004. Biased biological functions of horizontally transferred genes in prokaryotic genomes. Nat. Genet. 36:760–76

Niehus R, et al. 2015. Migration and horizontal gene transfer divide microbial genomes into multiple niches. Nat. Commun. 6, 8924

Norman A, Hansen LH, Sørensen SJ. 2009. Conjugative plasmids: vessels of the communal gene pool. Philos Trans R Soc Lond B Biol Sci. 364(1527):2275-89. doi: 10.1098/rstb.2009.0037

Novick A, Doolittle WF. 2020. Horizontal persistence and the complexity hypothesis. Biology & Philosophy 35:2.

Pilla G, Tang CM. 2018. Going around in circles: Virulence plasmids in enteric pathogens. Nat. Rev. Microbiol. 16, 484–495.

Porse A, et al. 2018. Biochemical mechanisms determine the functional compatibility of heterologous genes. Nat Commun. 9(1):522. doi:10.1038/s41467-018-02944-3

Porse A, Schønning K, Munck C, Sommer MO. 2016. Survival and Evolution of a Large Multidrug Resistance Plasmid in New Clinical Bacterial Hosts. Mol Biol Evol. 33(11):2860-2873. doi: 10.1093/molbev/msw163

Price MN, Dehal PS, Arkin AP. 2008. Horizontal gene transfer and the evolution of transcriptional regulation in Escherichia coli. Genome Biol. 9(1):R4. doi: 10.1186/gb-2008-9-1-r4

Puigbo P, Wolf YI, Koonin EV. 2010. The tree and net components of prokaryote evolution. Genome Biol. Evol. 2, 745–756

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org

RStudio Team. 2022. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com

Rahm A. HomologyLive; 2019 https://github.com/arahm/HomologyLive

Rivera MC, Jain R, Moore JE, Lake JA. 1998. Genomic evidence for two functionally distinct gene classes, Proc Natl Acad Sci U S A, vol. 95 (pg. 6239-6244)

Rousset F, et al. 2021. The impact of genetic diversity on gene essentiality within the Escherichia coli species. Nat Microbiol. 6(3):301-312. doi: 10.1038/s41564-020-00839-y

Rusakovica J, et al. 2014. Probabilistic latent semantic analysis applied to whole bacterial genomes identifies common genomic features. J Integr Bioinform. 11(2):243. doi: 10.2390/biecoll-jib-2014-243

San Millan A, MacLean RC. Fitness Costs of Plasmids: a Limit to Plasmid Transmission. Microbiol Spectr. 2017 5(5). doi: 10.1128/microbiolspec.MTBP-0016-2017

San Millan A, et al. 2014. Positive selection and compensatory adaptation interact to stabilize non-transmissible plasmids. Nat Commun. 5:5208. doi: 10.1038/ncomms6208

San Millan A. 2018. Evolution of plasmid-mediated antibiotic resistance in the clinical context. Trends Microbiol. 26, 978–985

Shawa M, et al. 2021. Novel chromosomal insertions of ISEcp1-blaCTX-M-15 and diverse antimicrobial resistance genes in Zambian clinical isolates of Enterobacter cloacae and Escherichia coli. Antimicrob Resist Infect Control. 10(1):79. doi: 10.1186/s13756-021-00941-8

Sheppard RJ, Beddis AE, Barraclough TG. 2020. The role of hosts, plasmids and environment in determining plasmid transfer rates: A meta-analysis. Plasmid. 108:102489. doi: 10.1016/j.plasmid.2020.102489

Smillie CS, et al. Ecology drives a global network of gene exchange connecting the human microbiome. Nature. 2011 480(7376):241–4

Soucy SM, et al. 2015. Horizontal gene transfer: building the web of life. Nat. Rev. Genet. 16, 472–482

Stalder T, Rogers LM, Renfrow C, Yano H, Smith Z, Top EM. Emerging patterns of plasmid-host coevolution that stabilize antibiotic resistance. Sci Rep. 2017 7(1):4853. doi: 10.1038/s41598-017-04662-0

Stasiak G, et al. 2014. Functional relationships between plasmids and their significance for metabolism and symbiotic performance of Rhizobium leguminosarum bv. trifolii. J. Appl. Genet. 55, 515–527

Szklarczyk D, et al. 2018. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic acids research 47.D1: D607-D613.

Szklarczyk D, et al. 2021. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 49(D1):D605-D612. doi: 10.1093/nar/gkaa1074

The LinBox Group Exact linear algebra over the Integers and finite rings, version 1.1.6; 2008

Touchon M, et al. 2020. Phylogenetic background and habitat drive the genetic diversification of Escherichia coli. PLoS Genet. 16(6):e1008866. doi: 10.1371/journal.pgen.1008866

Vietoris L. Über den höheren Zusammenhang kompakter Räume und eine Klasse von zusammenhangstreuen Abbildungen. Mathematische Annalen 1927; 97:454–472

Wang, X, et al. 2018. Comparative symbiotic plasmid analysis indicates that symbiosis gene ancestor type affects plasmid genetic evolution. Lett. Appl. Microbiol. 67, 22–31.

Wein T, et al. Essential gene acquisition destabilizes plasmid inheritance. PLoS Genet. 2021 17(7):e1009656. doi: 10.1371/journal.pgen.1009656

Wein T, Dagan T. Plasmid evolution. Curr Biol. 2020 30(19):R1158-R1163. doi: 10.1016/j.cub.2020.07.003

Wellner A, Lurie MN, Gophna U. Complexity, connectivity, and duplicability as barriers to lateral gene transfer. Genome Biol. 2007;8(8):R156. doi: 10.1186/gb-2007-8-8-r156

Wickham H, Bryan J. 2022. readxl: Read Excel Files. R package version 1.4.0. https://CRAN.R-project.org/package=readxl

Wickham H, et al. 2019. Welcome to the tidyverse. Journal of Open Source Software, 4(43), 1686, https://doi.org/10.21105/joss.01686

Wickham H, François R, Henry L, Müller K. 2022. dplyr: A Grammar of Data Manipulation. R package version 1.0.8. https://CRAN.R-project.org/package=dplyr

Wickham H, Girlich M. 2022. tidyr: Tidy Messy Data. R package version 1.2.0. https://CRAN.R-project.org/package=tidyr

Wickham H, Hester J, Bryan J. 2022. readr: Read Rectangular Text Data. R package version 2.1.2. https://CRAN.R-project.org/package=readr

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wickham H. 2021. forcats: Tools for Working with Categorical Variables (Factors). R package version 0.5.1. https://CRAN.R-project.org/package=forcats

Wickham H. 2019. stringr: Simple, Consistent Wrappers for Common String Operations. R package version 1.4.0. https://CRAN.R-project.org/package=stringr

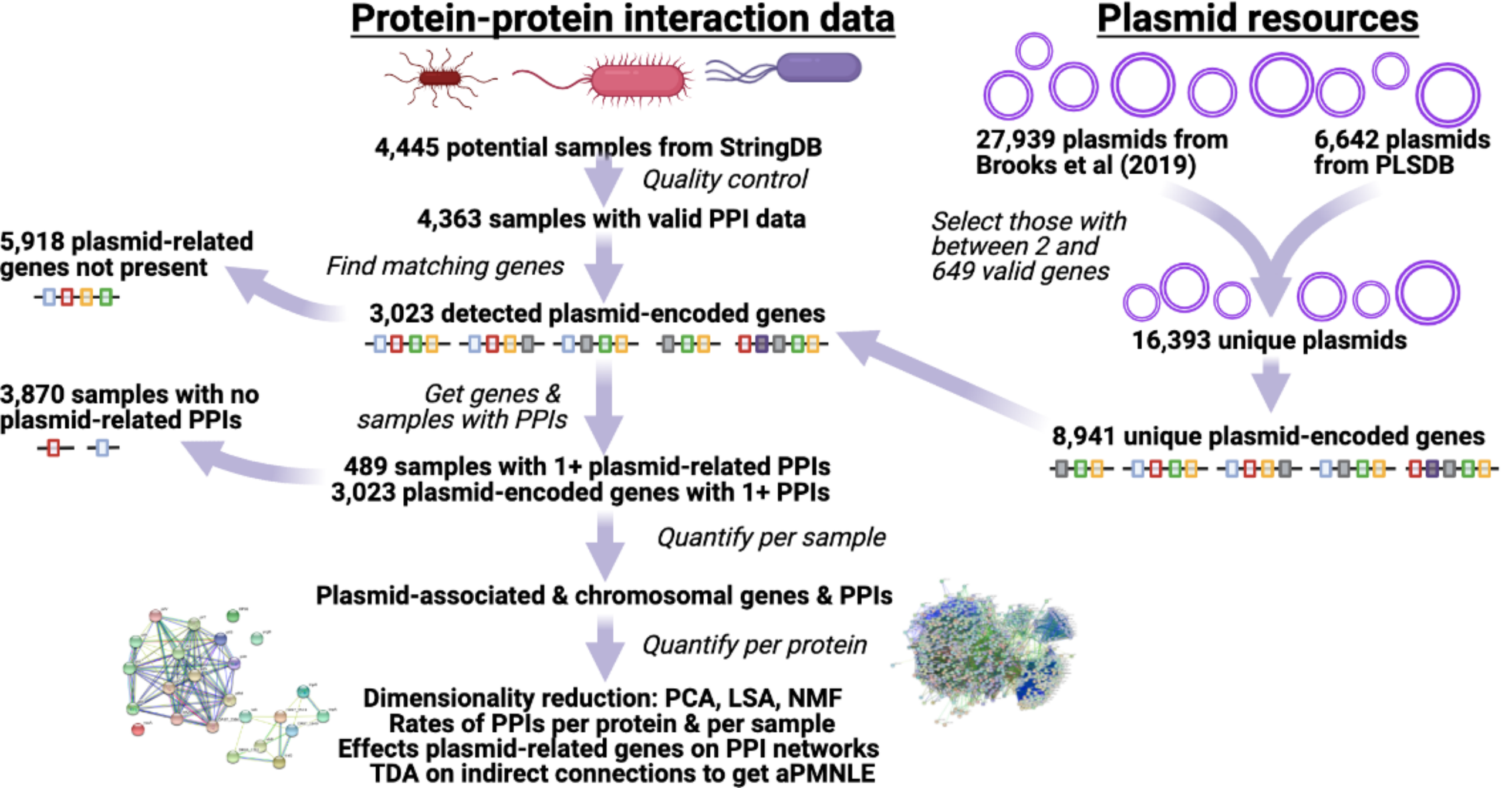
Winter DJ. 2017. rentrez: an R package for the NCBI eUtils API The R Journal 9(2):520-526

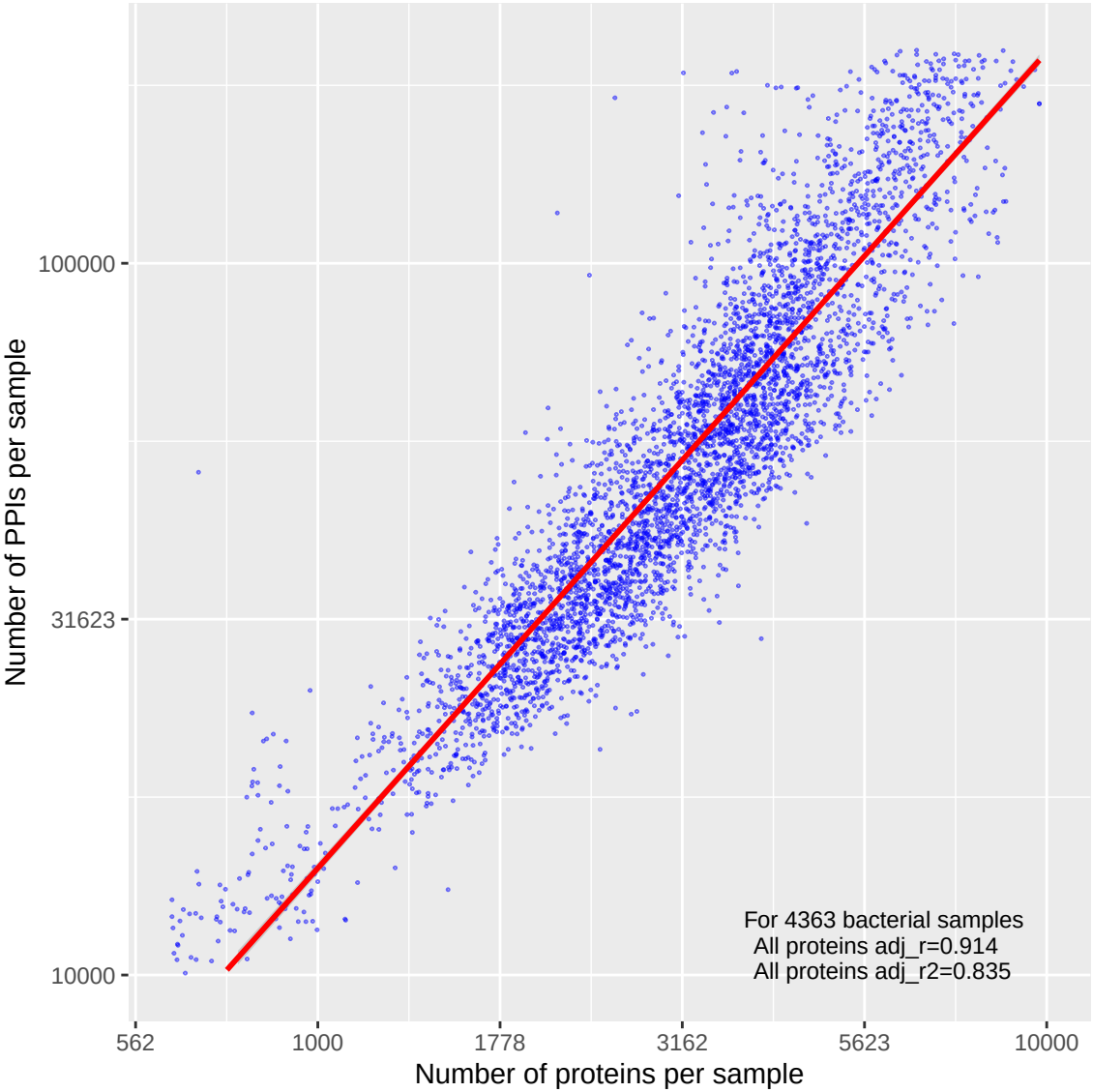
Wright S. 1951. The Genetical Structure of Populations. Annals of Eugenics 15:323-354. doi: http://dx.doi.org/10.1111/j.1469-1809.1949.tb02451.x

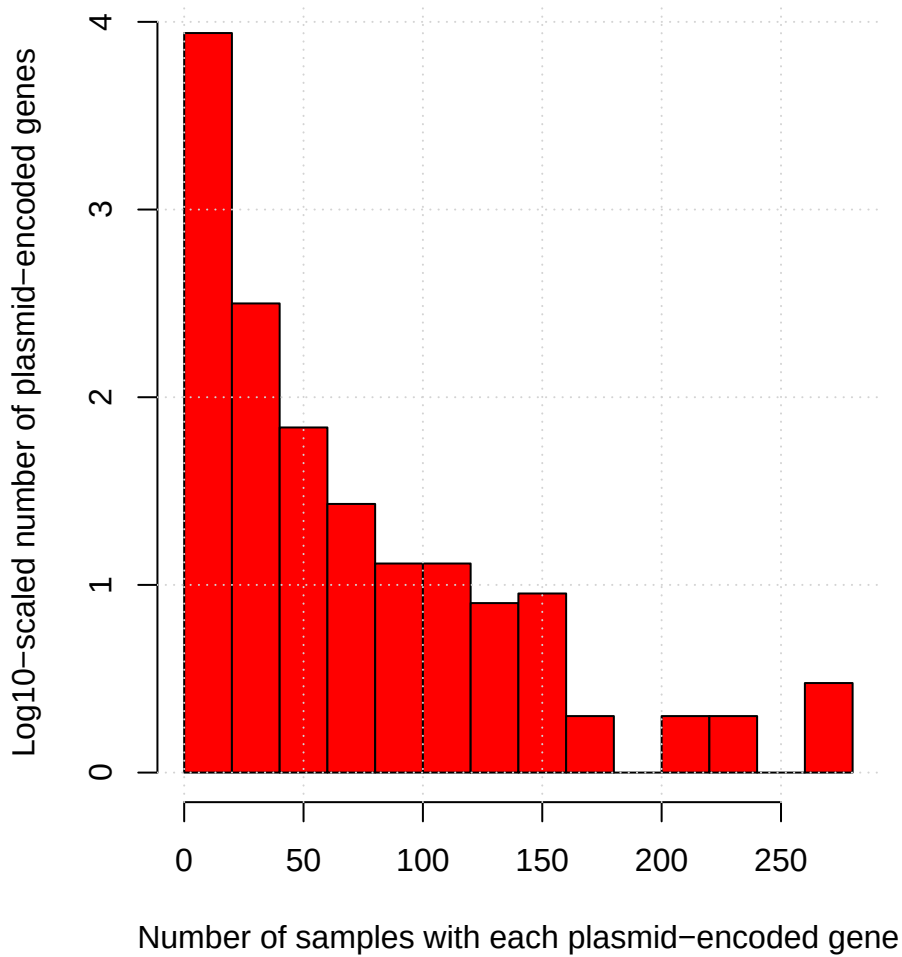
Yoon EJ, et al. 2020. Beneficial Chromosomal Integration of the Genes for CTX-M Extended-Spectrum β-Lactamase in *Klebsiella pneumoniae* for Stable Propagation. mSystems. 5(5):e00459-20. doi: 10.1128/mSystems.00459-20

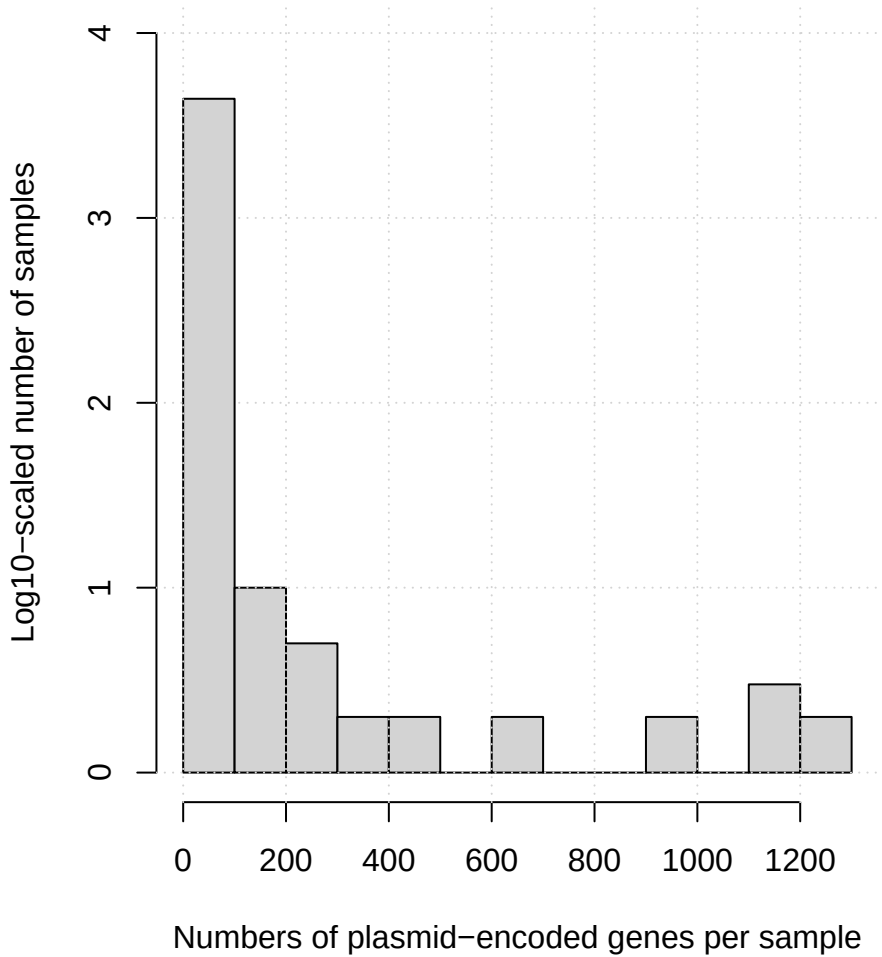
Zhang X, et al. Improvement in the efficiency of natural transformation of *Haemophilus parasuis* by shuttle-plasmid methylation. Plasmid. 2018 98:8-14. doi: 10.1016/j.plasmid.2018.07.001

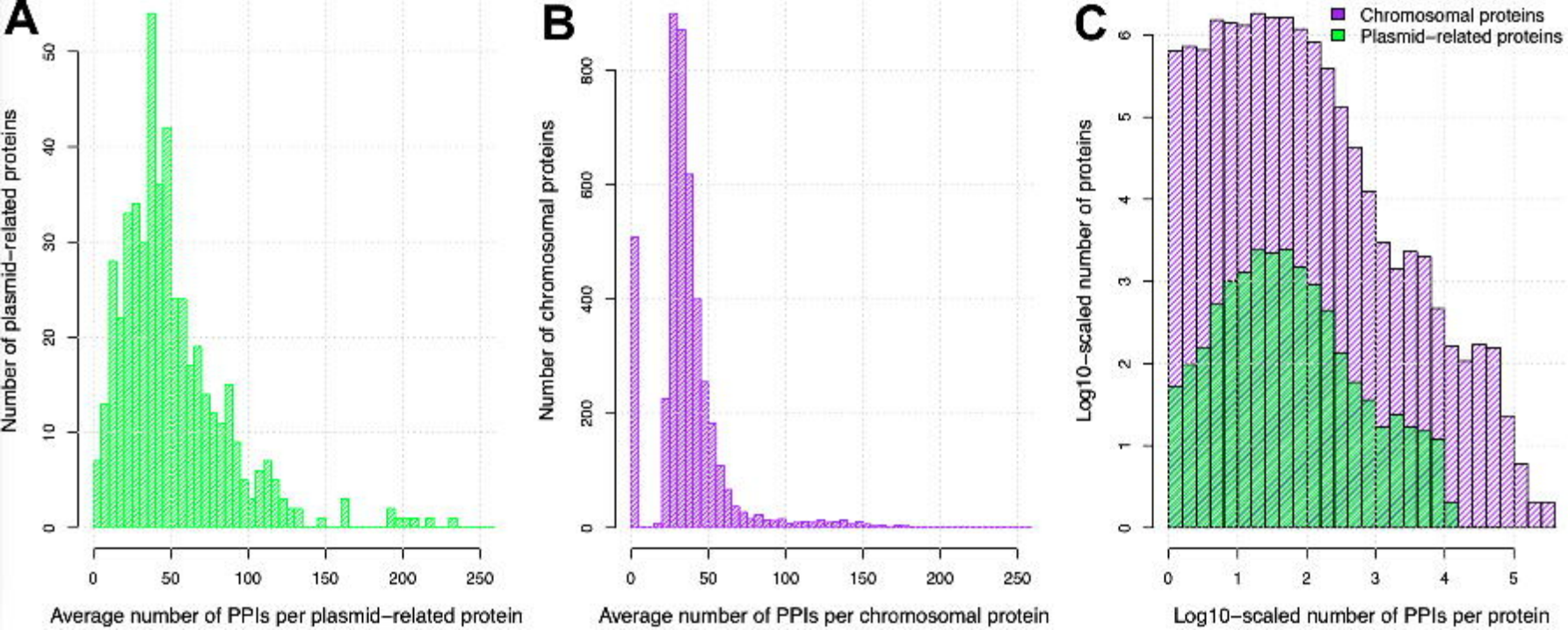
Zhou H, et al. 2021. Functions predict horizontal gene transfer and the emergence of antibiotic resistance. Sci Adv. 7(43):eabj5056. doi: 10.1126/sciadv.abj5056.

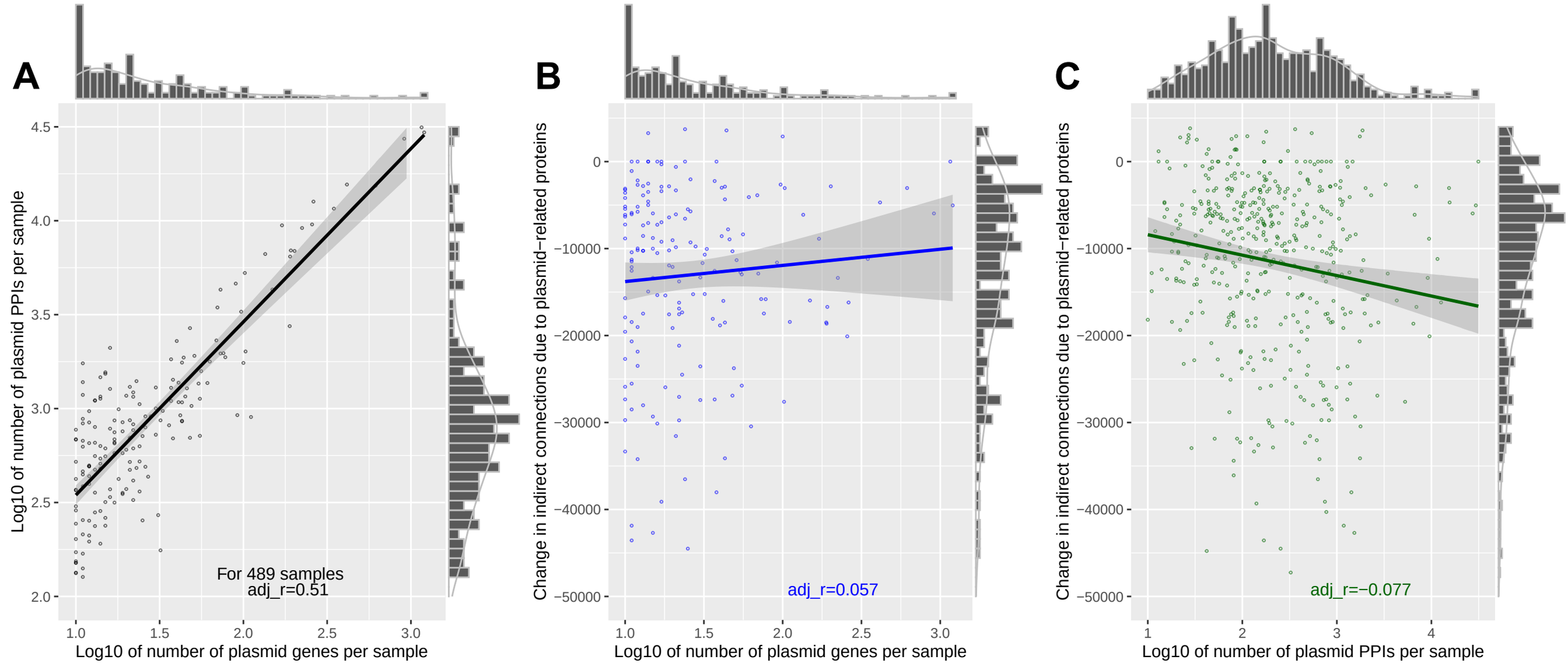

Protein-protein interaction data
Plasmid resources
4,445 potential samples from StringDB
Quality control
4,363 samples with valid PPI data
Find matching genes
3,023 detected plasmid-encoded genes
5,918 plasmid-related genes not present
Get genes & samples with PPIs
489 samples with 1+ plasmid-related PPIs
3,023 plasmid-encoded genes with 1+ PPIs
3,870 samples with no plasmid-related PPIs
Quantify per sample
Plasmid-associated & chromosomal genes & PPIs
Quantify per protein
Dimensionality reduction: PCA, LSA, NMF
Rates of PPIs per protein & per sample
Effects plasmid-related genes on PPI networks
TDA on indirect connections to get aPMNLE
27,939 plasmids from Brooks et al (2019)
6,642 plasmids from PLSDB
Select those with between 2 and 649 valid genes
16,393 unique plasmids
8,941 unique plasmid-encoded genes

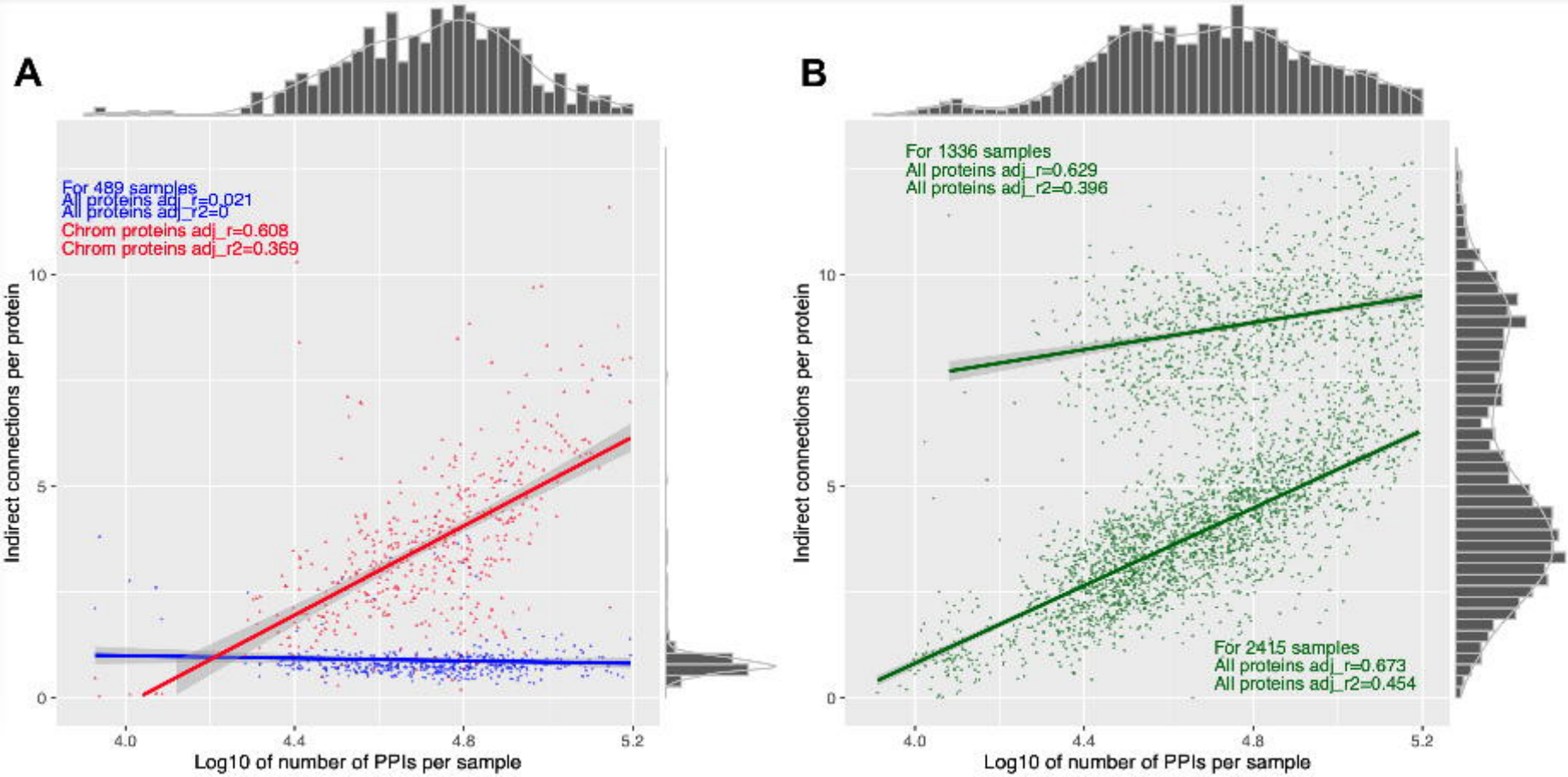

For 4363 bacterial samples
All proteins adj_r=0.914
All proteins adj_r2=0.835
Number of PPIs per sample
100000
31623
10000
562
1000
1778
3162
5623
10000
Number of proteins per sample

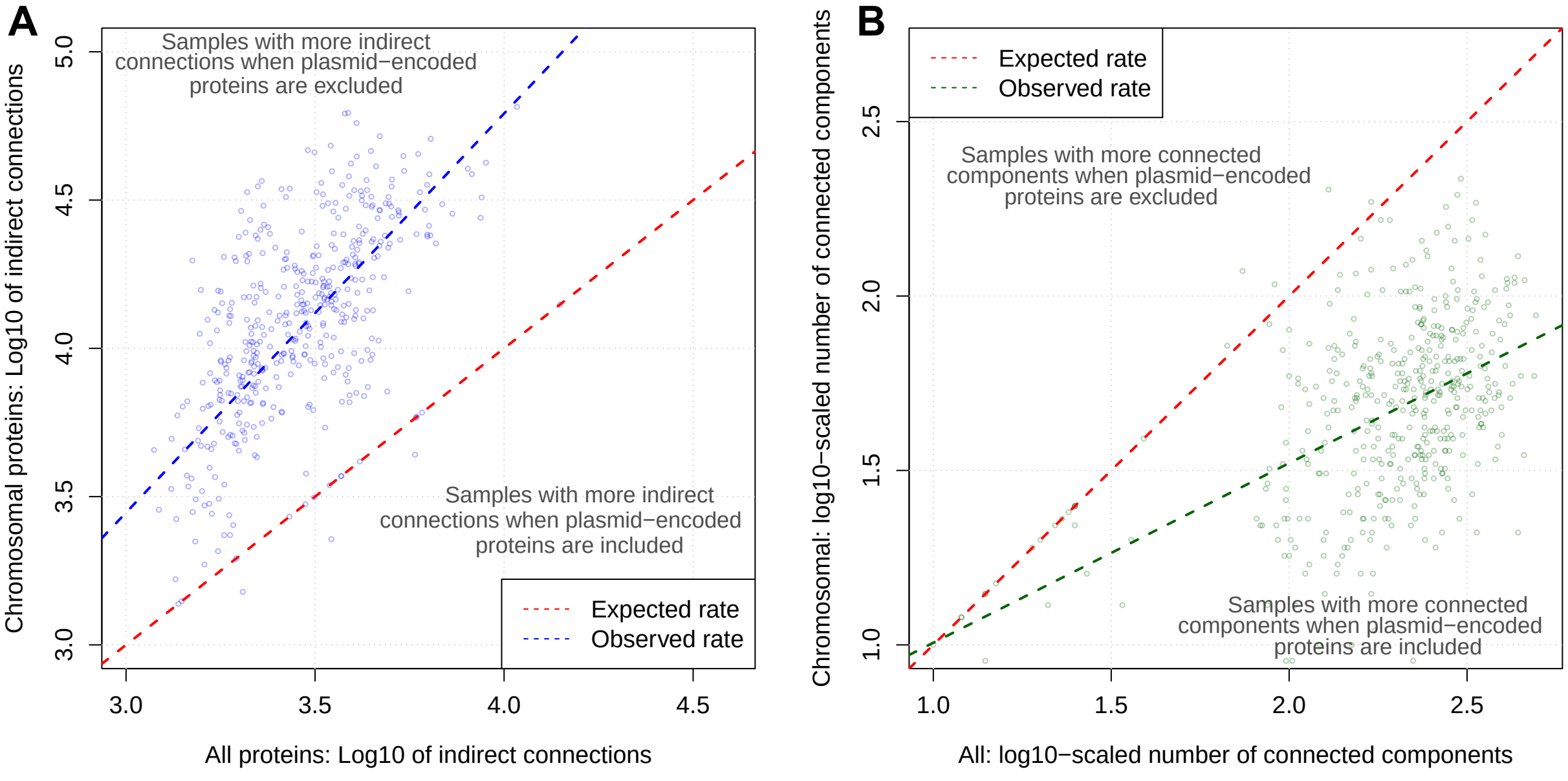

Log10−scaled number of plasmid−encoded genes
0
1
2
3
4
0
50
100
150
200
250
Number of samples with each plasmid−encoded gene


Log10−scaled number of samples
0
1
2
3
4
0
200
400
600
800
1000
1200
Numbers of plasmid−encoded genes per sample


A
Number of plasmid−related proteins
0
10
20
30
40
50
0
50
100
150
200
250
Average number of PPIs per plasmid−related protein
B
Number of chromosomal proteins
0
200
400
600
800
0
50
100
150
200
250
Average number of PPIs per chromosomal protein
C
Chromosomal proteins
Plasmid−related proteins
Log10−scaled number of proteins
0
1
2
3
4
5
6
0
1
2
3
4
5
Log10−scaled number of PPIs per protein


A
Log10 of number of plasmid PPIs per sample
4.5
4.0
3.5
3.0
2.5
2.0
For 489 samples
adj_r=0.51
1.0
1.5
2.0
2.5
3.0
Log10 of number of plasmid genes per sample
B
Change in indirect connections due to plasmid−related proteins
0
−10000
−20000
−30000
−40000
−50000
adj_r=0.057
1.0
1.5
2.0
2.5
3.0
Log10 of number of plasmid genes per sample
C
Change in indirect connections due to plasmid−related proteins
0
−10000
−20000
−30000
−40000
−50000
adj_r=−0.077
1
2
3
4
Log10 of number of plasmid PPIs per sample


A
For 489 samples
All proteins adj_r=0.021
All proteins adj_r2=0
Chrom proteins adj_r=0.608
Chrom proteins adj_r2=0.369
Indirect connections per protein
Log10 of number of PPIs per sample
B
For 1336 samples
All proteins adj_r=0.629
All proteins adj_r2=0.396
For 2415 samples
All proteins adj_r=0.673
All proteins adj_r2=0.454
Indirect connections per protein
Log10 of number of PPIs per sample


A
Samples with more indirect connections when plasmid−encoded proteins are excluded
Samples with more indirect connections when plasmid−encoded proteins are included
Expected rate
Observed rate
Chromosomal proteins: Log10 of indirect connections
All proteins: Log10 of indirect connections
3.0
3.5
4.0
4.5
5.0
B
Expected rate
Observed rate
Samples with more connected components when plasmid−encoded proteins are excluded
Samples with more connected components when plasmid−encoded proteins are included
Chromosomal: log10−scaled number of connected components
All: log10−scaled number of connected components
1.0
1.5
2.0
2.5