The information content of species: formal definitions of pangenome complexity track with bacterial lifestyle

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Abstract

The information content encoded in the genomes of species varies across the microbial tree of life. Bacterial lifestyle has been shown to drive this information diversity. Challenging environments are linked to information accrual. Pangenome fluidity is often invoked to provide a measure of this genomic diversity. Fluid pangenomes contain genes found only in subsets of species strains. Tighter pangenomes contain more genes that define a shared core among strains. In any global comparative framework, pangenomes must be calculated across all known species. But defining pangenomes is fraught with computational and biological challenges, requiring assembly, annotation, alignment, and phylogenetics of millions of orthologs. Here, we introduce an alternative view that employs agile complexity metrics to quantify the information density of pangenomes. In our framework, ensembles of free-living, motile, and non-pathogenic species have high genomic complexity. Ensemble complexity decreases in species bound to specific hosts. Because we eliminate annotation and alignment, our method is fast enough to evaluate existing species classifications across all known bacterial

genomes. The approach democratizes classification and our results highlight how broad the term “species” has become.

Introduction

Species definitions are starkly different across the microbial tree of life[1]. Microbes come in a variety of flavors. Some species are composed of a few very similar strains, while others have hundreds of strains whose genomes vary substantially in gene content. Pangenomes summarize this variation by outlining a set of core genes shared across strains, and a set of accessory genes found across various subsets. Pangenome size and complexity can therefore vary considerably[2,3].

Pangenome complexity was first defined as the rate of gene accumulation with whole genomes sequenced[4]. When genes are shared uniformly across species strains, this curve saturates. Alternatively, the curve may never reach a clear asymptote. For example, Prochlorococcus has 60,000 genes in its pangenome many of which are restricted to subsets of strains[5], whereas Tuberculosis has a mere 3500, most shared across all strains in the species[6]. Clearly, these organisms employ very different evolutionary strategies. One pangenome seems to be more complex than the other. Yet both are considered species along the same Linnean continuum.

The existing toolset for describing pangenomes only tangentially measures its complexity[7,8]. Traditionally, researchers sequence a set of strains and then assemble, align and annotate them. Tree building and orthology estimation follow. This set of orthologs is then split into the core genome, a set of genes essential to the organism, and the shell, a set of genes found only sporadically. Measures of pangenome fluidity

are driven by this core/shell dichotomy[9]. A tighter core implies a less fluid pangenome as in the case of tuberculosis above. But pangenome fluidity estimation requires a suite of bioinformatics tools that are costly to compute, over-parametrized, and often reference-bound. More importantly, these tools obscure signal from the raw sequence strings themselves.

Complexity is a loaded term. It is often used to simply note the indescribable. But in this paper, we aim to employ formal information theoretic definitions of complexity that operate on sequence ensembles[10,11]. The world is replete with sequence ensembles. Linguistic corpora[12], neural spike trains[13], and the microstates that feed statistical mechanics[14] can all be described as sequence ensembles. We argue that the complexity of pangenomes can also be measured in an information theoretic framework.

In previous work, we've shown that ensemble compression is a good proxy for pangenome complexity[15]. We introduced KHILL, or the effective number of genomes, as a measure of complexity and structure in a viral pangenome. We borrowed the concept from ecology, where Hill numbers are used to compare the effective number of species or the effective number of transects in an environmental sample[16]. We instead measure the effective number of kmers or the effective number of genomes in a pangenome. KHILL can be applied to pangenomes from any species. As a measure of relative entropy, KHILL is in the family of information theoretic measures that employ the Kullback-Leibler Divergence[17].

Entropy itself, a measure of the average amount of information required to describe a variable's state[18], is also a useful tool for measuring complexity, especially

when framed as entropy growth curves[10]. In complex ensembles entropy increases with subsequence (or block) size. But in most ensembles, this increase is limited. A simple system will have ensemble structure whereas complexity often implies less redundancy across block sizes. For example, if a variable contains a highly predictable distribution, like a pangenome with a very tight genomic core, entropy will peak at a low value and at smaller k-mer sizes. But accessory genes increase surprise. Entropy growth curves measure how much surprise remains as you increase the k-mer size. An asymptote indicates full information capture. Continued growth at higher k-mer sizes implies there is more complexity to be learned.

Pangenome fluidity has been shown to vary with bacterial lifestyle[19]. Prochlorococcus is a free-living marine microbe challenged by a constantly shifting environment. It lives in a milieu of candidate genes for horizontal transfer. Its large pangenome could be interpreted as a library of diverse information necessary to survive and flourish in its harsh environment. Tuberculosis is instead restricted to the very controlled environment of its host. Its small pangenome requires only the limited set of genes necessary to flourish in a uniform environment. The large accessory genome of Prochlorococcus tips its complexity measures – KHILL and block entropy – far higher than that for tuberculosis. The accessory genome is therefore the additional information capacity required to compute on a difficult environment.

Here we calculate these two quantities across all genomes of all known microbial species. We show that our pangenome complexity definitions sort species into expected bacterial lifestyles. We argue that unaligned and unannotated information can provide unbiased measures of pangenome complexity. We also demonstrate that the term

“species”, as defined by current binomial classification, must accommodate a very large range of KHILLs.

Results and Discussion

The KHILL distribution of all bacterial species.

We downloaded all available microbial genomes from RefSeq[20] version 223 (73,079 taxa). Figure 1 shows the semi-log KHILL distribution of all species represented by two or more genomes (for a total of 283,097 genomes included in this analysis). We display 10,510 points, each point an NCBI taxon. The red line is an upper bound: a species on this line has a KHILL equal to the number of genomes analyzed. Species sitting on this line have genomes with no information overlap. Most species have a KHILL below five. But some have KHILLs that approach their genome count. The 7095 genomes of Mycobacterium tuberculosis compress to a mere 1.8 effective genomes. The 79 genomes of *Prochlorococcus marinus* yield a KHILL of 21.9, occupying a much larger information space.

We intentionally chose not to filter or screen any of this data. The plot is an unvarnished look at our current, accepted microbial species taxonomy. Clearly, the KHILL distribution of all microbial species follows a power law. The semi-logarithmic nature of our primary visualization allows us to separate some of the detail clumped at the origin. Most species have 10 or fewer genomes and seem to span their entire KHILL range. But most of the low genome count species hover between 1 and 2. Only 21 species have KHILLs higher than 20. These 21 species are represented on average by 82 genomes, a level of compression on par with *Prochlorococcus marinus*. The 28

species with more than one thousand genomes are more uniform and never eclipse a KHILL value of 10, perhaps reflecting the research attention they garner.

The extremes of this distribution reflect both a roadmap for and a demonstration of research priorities. For example, the taxa with the three highest KHILLs are catch-all genus categories (*Flavobacterium sp*, *Pseudomonas sp*, and *Sphingomonas sp*). Poor taxonomic labels assigned to presumably challenging organisms are bound to combine divergent genomes. A genus grab-bag likely obscures reticulated species that require further genomic work. In contrast, species with very high genome counts are among those that make humans most sick. *E. coli* is represented by 26,088 genomes, *K. pneumoniae* by 18,575. But their KHILLs are 4.81 and 3.24, respectively. The more than 25 thousand genomes of *E. coli* compress into just 5 effective units.

While this rate of genome accrual is likely the result of public health initiatives and funding priorities[21], we show that representatives for these species appear to be highly redundant. That is, information is not accumulating at the same rate as genomes in the database. This is not meant to impugn pathogen genome sequencing projects, many of which are looking for subtle genomic signatures in strains or variants causing outbreaks. But low KHILLs among these pathogens imply that their pangenomes have saturated. In terms of their information structure, additional genome sequencing is not shedding any further light on the complexity of the species.

We view either end of this power law distribution as revealing key aspects of the culture of science: relentless classification of even the poorly understood, and relentless investment in the small group of organisms with an outsized impact on human welfare.

Despite these anthropocentric biases, the distribution in Figure 1 contains evidence that KHILL does highlight species with complex pangenomes. *Pseudomonas fluorescens* (KHILL = 45.2), a versatile, free-living, gram-negative bacteria found in soils and water is known to have a large, complex pangenome[22]. And *Buchnera aphidicola* (KHILL = 48.8), an obligate endosymbiont of pea aphids is known to have an unusual amount of genome divergence[23]. These KHILL values suggest pangenome complexity.

In Figure 1, we compress the signal from of all available genomes for each species into a single point. What if we instead calculated a KHILL accumulation curve? Accumulation curves have long been used in ecology to measure diversity in species distributions[24–26]. These curves can also quantify string diversity in sequence libraries[27]. In the next section, we apply this concept to pangenomes.

KHILL accumulation curves

In their seminal work in *Streptococcus agalactiae*, Tettelin et al[4] showed that every new sequenced strain contained new genes. This became the hallmark of an open pangenome. Though their accumulation curve exhibited saturating behavior for five or fewer genomes, thereafter the curve rose linearly with no clear asymptote. Genes seemed unable to demarcate a clear taxonomic boundary.

Still, genes are intuitive functional units for pangenome analysis. Most programs available for pangenomes are built around a gene-centric view of biological information[7,8]. But a reliance on genes requires faith in their annotation, tolerates bias towards model organisms, and relegates non-coding elements. Computing on genes is also costly[28]. And because assigning gene orthology is difficult, most existing

150 pangenome tools require hundreds of CPU hours to analyze thousands of genomes. As an alternative, in Figure 2A, we outline pangenome KHILL accumulation curves.

KHILL decenters the gene and instead focuses on sequence information, distilling signal into genome equivalents. KHILL accumulation curves build out a sequence ensemble. Our sequence ensembles are the set of all genomes that enrich
155 our picture of any given species. Each genome increases the ensemble's complexity, but at differing rates depending on the organism. Like gene-based approaches, if a pangenome is closed, KHILL will eventually flatline. But if new genomes consistently deliver novel information, KHILL will never asymptote. KHILL operates as an information-based, computationally tractable proxy of more cumbersome metrics like
160 pangenome fluidity[9]. The two quantities display a linear relationship (Supplemental Figure 1) but are orders of magnitude apart in terms of computational burden.

Genome size is known to be a confounding factor in orthology-based pangenome analyses[19,29,30]. In Supplemental Figure 2A, we show that the relationship between genome size and pangenome fluidity is linear. The influence of genome size on KHILL,
165 however, is less pronounced (Supplemental Figure 2B). We built linear models of this relationship, separating fitted from residual components. KHILL fitted to genome size flatlines in plots against pangenome fluidity, implying that genome size is not driving the relationship between the two quantities (Supplemental Figure 3B). The KHILL residuals – the component unmodeled by size – correlate with pangenome fluidity (Supplemental
170 Figure 3A).

In Figure 2B, we show KHILL accumulation curves for five pathogenic species. Unlike conclusions drawn in the Tettelin[4] paper, the *Streptococcus agalactiae*

pangenome seems to be closed with an asymptote at about 3 effective genomes. In keeping with its reputation as a clonal organism with no evidence of recombination[31],
175 *Mycobacterium tuberculosis* levels at a KHILL of 1.8. *Staphylococcus aureus* and *Neisseria meningitis* asymptote at KHILLs of 2.4 and 2.6, respectively. *S. agalactiae*, *M. tuberculosis, S. aureus,* and *N. meningitis* all seem to require only about 150 random strains to confirm a closed pangenome. Among the five, only *Helicobacter pylori*, an organism known to exhibit intense recombination[32], is an open pangenome. Even the
180 thousandth *H. pylori* genome adds significant unique information. *H. pylori's* high KHILL suggests a complex pangenome. Differential rates of saturation to various KHILL maxima across these species suggests a new kind of comparative genomics.

KHILL across k-mer sizes

KHILL requires that we set a k-mer size. Shorter k-mers enhance compression
185 but sacrifice combinatorial nuance[15]. Longer k-mers capture more interaction information but grow in computational difficulty. At the maximum theoretical extreme, as k-mers approach the size of the genome, KHILL balloons to the number of input sequences. Entropy also increases with k-mer size[10]. Given a sequence ensemble, calculating entropies and KHILLs with increasing k-mer size spawns new measures for
190 species information content and prescribes a string size required to make that information content bloom (Figure 3).

KHILLs at small k-mer sizes collapse all available information in the ensemble. But as we increase block size, KHILL moves through a steep transition to a second steady state. K-mer sizes beyond this transition increase KHILL at a slower rate (Figure
195 3A). In Figure 3B, we show these curves for our five pathogens. All organisms except *H.*

pylori exhibit dual steady-state dynamics. Though the rate of increase appears to abate, *H. pylori* KHILL continues to increase even beyond a k-mer size of 21.

If an ensemble contains information restricted to only subsets of strains – the hallmark of an open pangenome – we would expect KHILL to continue its increase over larger k-mer sizes. In the case of non-recombining organisms with closed pangenomes, like *M. tuberculosis*, the k-mer ensemble stabilizes across all available strains by a k-mer size of 15. Open pangenomes maintain diverse populations of information restricted to subclades. In these organisms, KHILL will likely never converge regardless of string size.

Block entropy curves

Block entropy curves measure entropy as a function of k-mer size. Larger k-mers generate larger entropies. But in genome ensembles, entropy generally settles into a steady-state after a k-mer size of about 15. K-mer sizes at the block entropy elbow echo those needed to achieve a steady KHILL. In other words, the rate of entropy increase abates just as KHILL transitions between its two steady states (Figure 3C). Diminishing returns with higher string sizes suggests a k-mer optimum. Given an optimal string size, we show that entropy captures the information capacity of our ensemble and KHILL measures its information diversity. The two metrics together join to quantify its complexity.

Block entropy curves are key to complexity analysis in other fields. In physics, they can describe phase transitions in materials at different temperatures[8,11]. In neuroscience, they can measure collective behavior in neural networks[31,32]. We borrow

these concepts directly to develop block entropy complexity in genomics. Like KHILL, block entropy illuminates pangenome structure. After a linear ascent common to most sets of genomes, entropy plateaus to the system's source rate, H_μ. H_μ measures the intrinsic randomness in the sequence ensemble, the noise that achieves no structure regardless of k-mer size[10]. In practice, H_μ is only defined as k-mers approach the size of the genome. For more reasonable and calculable k-mer sizes, we approximate H_μ by measuring the slope of the entropy curve at the largest k-mer sizes we can manage.

The y-intercept of this line is an estimate of the excess entropy (EE), another key quantity in complexity theory[10]. The excess entropy has been called the predictive information[11], the source's memory, or its redundancy. Excess entropy is the system's useful complex structure. H_μ is its inevitable noise. Here, we interpret it as the computational capacity of the system, the capacity of the pangenome to code useful functions for a given environment. As block entropy curves reach a steady state, we approach true values for $H_\mu$and excess entropy. Though much more subtle than KHILL, we show a higher H_μ and higher excess entropy in *H. pylori* than the four other pathogens shown in figure 3D. A higher excess entropy implies higher pangenome complexity.

Different ensembles will saturate at different rates and at different k-mer sizes. The process of synchronizing to the steady-state is also a measure of complexity, and one we can approximate without exploring k-mer sizes in the extreme. The area between the block entropy curve and the line describing H_μ is the amount of information we must learn as we discover the structure of an ensemble (Figure 3C). This quantity is

240 termed transient information (TI)[10]. TI measures our information investment as we probe the dynamics of pangenome evolution. Species with large TIs require more information to discern their internal structure. Like pangenome fluidity, TI trends upwards with genome size (Supplemental Figure 4). A linear model of TI with respect to genome size establishes that the residuals track more closely with pangenome fluidity (Supplemental
245 Figure 5). Genome size is therefore factorable in our view of pangenome evolution. With TI, we show that learning complexity is its own measure of complexity.

Recombination and mutation are two primary drivers of microbial evolution. In Figure 4, we simulate 100 1 Mb genomes for 25 combinations of homologous recombination and mutation rate. Both EE and TI increase as mutation rate and
250 recombination rate increase. In other words, the information capacity and the amount of information required to measure that capacity increase with both modes of sequence change. As we've demonstrated in other studies[28], recombination seems to exert the larger effect.

Block entropy and KHILL track bacterial lifestyle

255 The pathogens we analyze in Figures 2 and 3 are all bacteria subject to one environment: the interstitial spaces of the human body. What if we instead look at free living bacteria? Or obligate intracellular microbes? In Figure 5, we show block entropy and KHILL curves for 10 random genomes of *Pseudomonas fluorescens*, *Rickettsia japonica* and *Staphylococcus aureus*. *P fluorescens* is found in profoundly variable
260 environments[22]. *R. japonica* is non-motile and restricted to living in the cytoplasm of its eukaryotic host[33]. In terms of both block entropy and KHILL, we see a gulf in complexity between these two organisms.

In the complexity literature, the first and second derivatives of the block entropy curve provide information about the excess entropy and the k-mer size that achieves peak predictability, respectively. If excess entropy is the ensemble's store of useful information, peak predictability is the string size most useful to discern that information. For our application, we interpret this as the minimum k-mer size required to retrieve information about the system. The curves in Figure 5 suggest that species with grossly different lifestyles may require very different k-mer sizes to optimize information about their pangenome complexity (Supplemental Figure 6). These curves also hint at a new method to select k-mer sizes that maximize predictability[10], perhaps clarifying size selection procedures in short read alignment and assembly[34,35]. *P fluorescens* requires a k-mer size of 14 to coax out peak information in the ensemble. For *R. japonica* a k-mer size of 12 suffices.

The complexity gap between these two extreme organisms is in keeping with known differences in pangenome diversity and reflects the stark contrast in the environmental challenges they face. We selected *S. aureus* as intermediate between these two lifestyle poles. Remarkably, at a k-mer size of 20, 10 strains of *P fluorescens* contain the information equivalent of over 8 genomes, while all 10 R*. japonica* collapse to a KHILL of 1 across the entire k-mer expanse.

Between the flexible cosmopolitan (*P fluorescens)* and the cytoplasmic shut-in (R*. japonica*), bacteria have settled into several intermediate lifestyle traits. Some are obligate, some facultative. Some move and some don't. Some are pathogens and others, mutualists. Dewar et al[19] recently annotated 126 bacterial species and found that more fluid pangenomes are usually free living, facultative, motile, extracellular, and

non-pathogenic. Variable environments seem to require genomic flexibility. On the other hand, obligate, host-bound pathogens tend to have a streamlined pangenome.

Figure 6 shows KHILL distributions of Dewar et al's 126 species with respect to their pathogenicity. We show that pathogens have a significantly lower KHILL than mutualists, a finding that recapitulates results seen with more expensive metrics like pangenome fluidity. In Figure 7, we show that genome size corrected residuals of transient information also correspond to our intuition regarding pathogenic organisms. Pathogens face homogenous environments. Their pangenomes are often less complex as a result.

KHILL distributions sort with our expectations on every other lifestyle trait annotated by Dewar et al. Bacteria that are host-bound, intracellular, obligate, and/or non-motile all have significantly lower KHILL than their free-living, extracellular, facultative, and motile counterparts (Supplemental Figure 7). Using linear models of both KHILL and TI against genome size, we confirm that it is the residuals of both metrics that undergird our measurement of pangenome complexity (Supplemental Figures 8 and 9). Residuals of transient information agree with our expectations regarding lifestyle but the distinctions between traits are less resolved (Supplemental Figure 10). This is in keeping with the higher resolution of KHILL (Figures 3 and 5). For both motility and host reliance, TI residuals show no distinction between populations.

Conclusion

The information-based techniques we introduce here are lightweight and capable of handling orders of magnitude more data than traditional pan-genomic approaches.

We do this without sacrificing the biological signal we expect. Our metrics track with bacterial lifestyle. Microbes enmeshed in variable environments have higher KHILLs and higher transient information. Both are markers of pangenome complexity, and both are derived from a formal literature that quantifies complex systems. We find that compressibility is a viable comparative strategy. KHILL combines the effects of genetic distance and population heterogeneity. Transient Information is a useful proxy for species information capacity. We use both metrics here to explore bacterial lifestyle, standardize molecular species definitions, highlight tenuous classifications, and confirm pangenome saturation.

Methods

KHILL

The KHILL metric is an adaptation of beta diversity, an ecological concept used to measure species diversity across samples[15,36,37]. In ecological studies, researchers will often divide an area of interest into transects and count the number of species within each sample. The goal is to estimate overall diversity and to assess the efficacy of sampling. If the samples have many species in common at similar occurrence levels, the diversity difference between samples is low, and we calculate a lower effective number of samples or lower Hill number[16]. Hill numbers of this type provide an intuitive metric to compare across environments.

In a genomic context, we shift our frame of reference to measure information diversity. Rather than species, we count k-mers, and rather than transects, we sample genomes. We measure KHILL as

$$D_\beta = \exp\left(\sum_{S=1}^{M} w_s \sum_{i=1}^{N} p_{si}\, ln \frac{p_{si}}{p_i}\right)$$

where *N* is the set of all k-mers, *M*, the set of all genomes, p_{si} is the frequency of a particular k-mer *i* in a particular genome *s,* p_i is the frequency of a particular k-mer *i* across all samples, and w_s weighs all k-mers in sample *s* relative to all individuals counted in the experiment. Whereas in ecology, this quantity measures the effective number of samples, in genomics, the expression yields the effective number of genomes. KHILL summarizes the information diversity of any pangenome. A higher KHILL indicates a diverse set of k-mers, while a lower KHILL implies homogeneity. KHILL varies between 1 and the number of genomes sampled. A KHILL of one implies perfect compression, equivalent genomes. A maximal value signals that the genomes share no strings in common.

Block Entropy

The entropy of an ensemble is the average uncertainty associated with its potential states[18]. Entropy can therefore be used to quantify the amount of information required to describe the state of a variable. We measure entropy as

$$H = -\sum_{i=1}^{N} p_i\, ln\, p_i$$

where *N* is the set of all possible states, *i,* and p_i is the probability of the *i*th state. In fields where observations are organized as a set of linear strings, entropy can be calculated across substrings or blocks of various sizes. Plots of ensemble entropy

against block size contain information about the ensemble's complexity and compression[10].

For every block entropy curve, we calculate three quantities: H_μ, excess entropy, and transient information. H_μ is the ensemble randomness that remains after all the redundancies of longer and longer sequence blocks have been modelled. The y-intercept of the line chosen to approximate H_μ is the excess entropy, or the intrinsic redundancy of the ensemble. The excess entropy is the non-random information capacity of our ensemble, information that can be captured because of its correlations. Finally, transient information is the amount of information that must be invested to discover H_μ and fill out our excess entropy to its maximum. It is calculated as the area between the block entropy curve and the line describing H_μ.

Genome ensembles with higher excess entropy and higher transient information are generally more complex. In our framework, this complexity is associated with a pangenome's high fraction of accessory genes and a proportionately smaller core. Open pangenomes therefore have higher quantities of useful information (excess entropy) but require more effort (transient information) to decode.

Finally, the block entropy's first derivative is another window into excess entropy. Excess entropy can be calculated by integrating under the curve describing the first derivative of the block entropy. Moreover, the second derivative pinpoints the k-mer size that confers peak predictability in the system. Peak predictability is a useful concept for k-mer based bioinformatics, perhaps defining a lower bound for string sizes optimized to the ensemble in question.

Figure Legends

Figure 1. KHILL compression across all microbial taxa. We show KHILL values for all 10,510 RefSeq microbial taxa with at least two genome sequences as a semi-log plot. The red line plots y = x, the KHILL upper bound. The inset shows that the relationship is a power law distribution. Pathogens dominate the long tail, with tens of thousands of genomes compressing to a few genomic equivalents. High KHILL values are dominated by poorly characterized NCBI taxa and information rich species with complex pangenomes.

Figure 2. Pangenome accumulation curves. We show an alternative measure of the pangenome. Rather than counting the number of unique genes added with each genome in a set, we plot KHILL, a measure of information diversity. An open pan-genome's KHILL will increase at a faster rate than a species with a closed pangenome (Panel A). The rate of increase in KHILL will vary depending on the evolutionary strategy and complexity of the pangenomes in question (Panel B). *H. pylori* is known to be recombinogenic, whereas *M tuberculosis* is clonal. In this framework all species shown are closed pangenomes after about 250 analyzed genomes except for the highly dynamic *H. pylori*.

Figure 3. Varying k-mer size. Increasing k-mer size increases both KHILL and entropy. KHILL increases rapidly at the k-mer size that corresponds to the entropy elbow (A). The block entropy curve (B) yields two important insights into the information content of an ensemble. The y-intercept of the approximate asymptote is the excess entropy, a

quantity often interpreted as the computational capacity of the system. The area between the line tracing this asymptote and the curve itself is the transient information, the information cost required to synchronize to the steady-state entropy of the ensemble. For our five pathogens, we show that all but H. pylori settle into a KHILL maximum (C), and that even small changes in the block entropy curve (D) can lead to large differences in information diversity as reflected by KHILL.

Figure 4. Simulations. We simulated 10 genomes for every recombination rate and mutation rate pair. Higher rates of either form of genomic change increase both the transient information and the excess entropy, two hallmarks of increased ensemble complexity.

Figure 5. Lifestyle extremes. We show block entropy curves (A) and KHILL curves (B) with increasing k-mer sizes across three organisms with very different lifestyles. *P fluorescens* is a cosmopolitan, free living microbe. *R. japonica* is an endosymbiont restricted to the cytoplasm of a single host. These lifestyle differences are reflected in their information capacity (A) and information diversity (B) extremes. *S. aureus* a largely clonal pathogen is an example of an organism that relies on a host for propagation but can survive in the environment for short periods of time.

Figure 6. Pathogens have lower KHILLs. We show that the pathogens among the 126 species curated by Dewar et al have significantly lower KHILLs than mutualists or organisms that both infect their hosts and live independently. Pathogens exist in the controlled space of their hosts, subject to few disturbances. Their low information diversity reflects a lack of accessory genes that might be more functional among organisms that must contend with dynamic environments.

Figure 7. Pathogens have lower transient information. We show that pathogens have lower transient information implying a smaller information capacity relative to non-pathogenic organisms. Here we show transient information residuals after a genome size correction.

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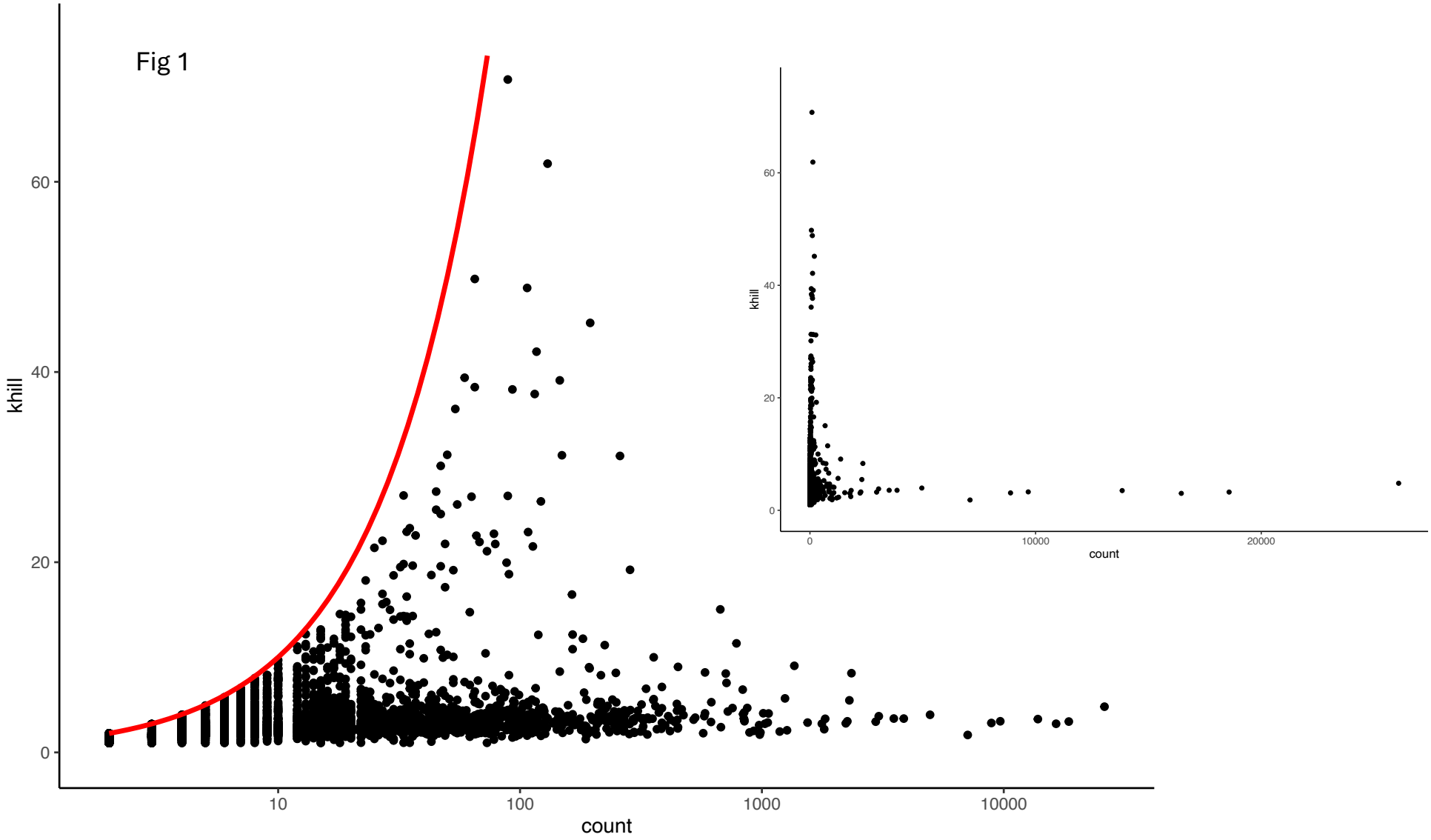
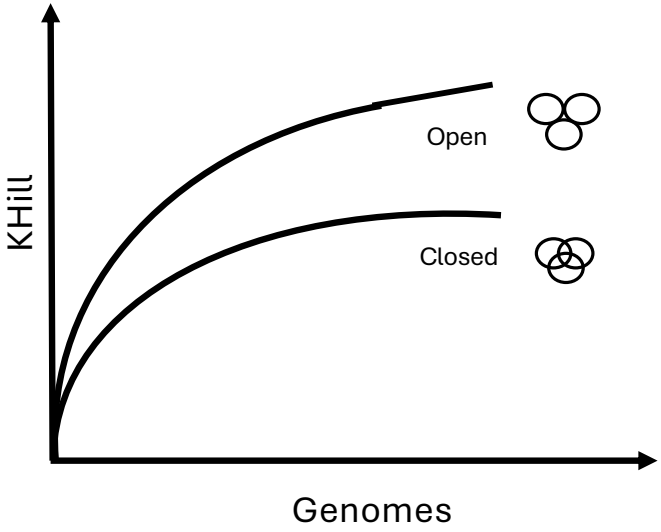


Fig 1
khill
count
0
20
40
60
10
100
1000
10000
khill
count
0
20
40
60
0
10000
20000

Fig 2


KHill
Open
Closed
Genomes

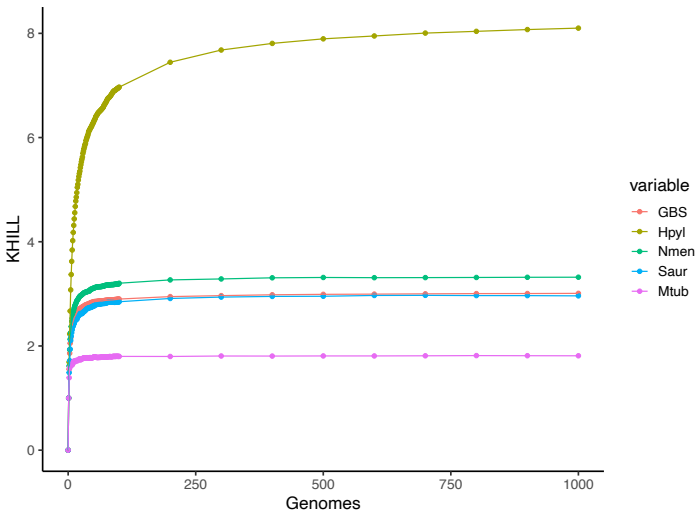

KHILL
8
6
4
2
0
0
250
500
750
1000
Genomes
variable
GBS
Hpyl
Nmen
Saur
Mtub

Fig 3

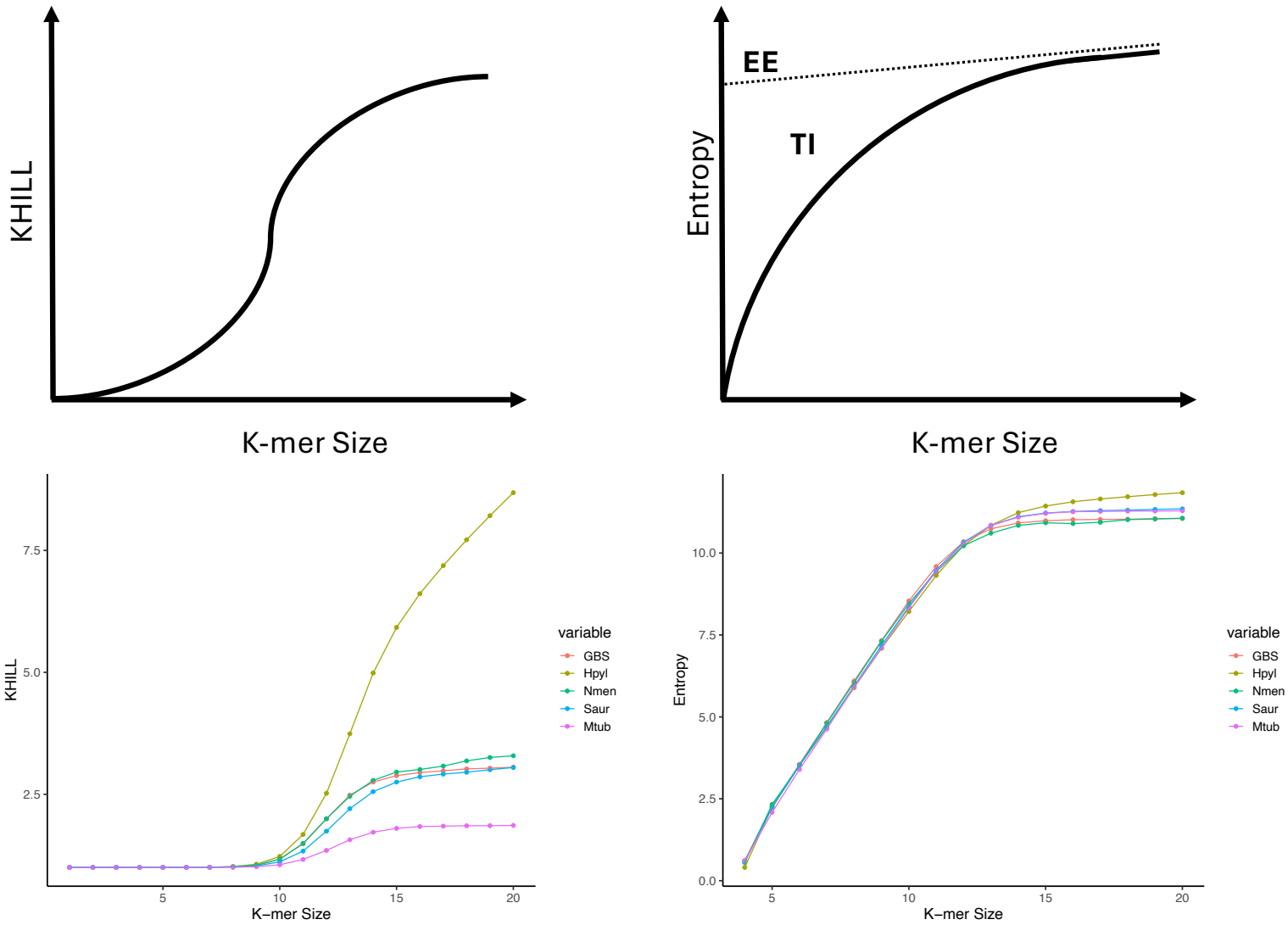
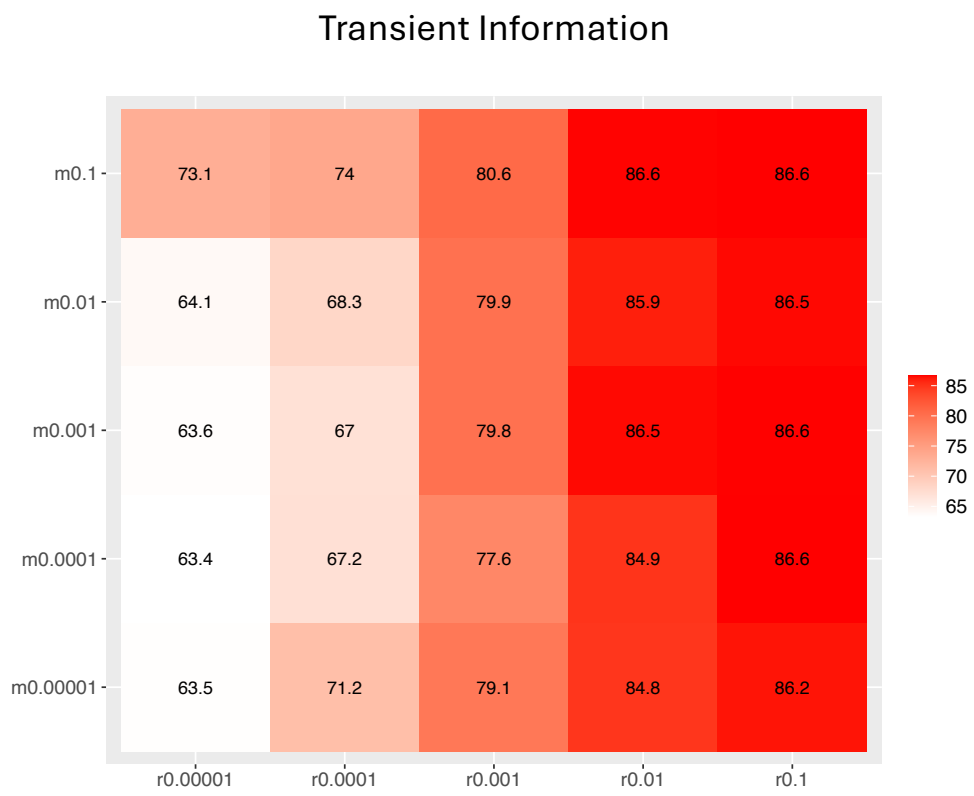

KHILL
K-mer Size
EE
TI
Entropy
K-mer Size
7.5
5.0
2.5
KHILL
5
10
15
20
K−mer Size
variable
GBS
Hpyl
Nmen
Saur
Mtub
10.0
7.5
5.0
2.5
0.0
Entropy
5
10
15
20
K−mer Size
variable
GBS
Hpyl
Nmen
Saur
Mtub

Fig 4


Transient Information
m0.1
m0.01
m0.001
m0.0001
m0.00001
73.1
74
80.6
86.6
86.6
64.1
68.3
79.9
85.9
86.5
63.6
67
79.8
86.5
86.6
63.4
67.2
77.6
84.9
86.6
63.5
71.2
79.1
84.8
86.2
r0.00001
r0.0001
r0.001
r0.01
r0.1
85
80
75
70
65

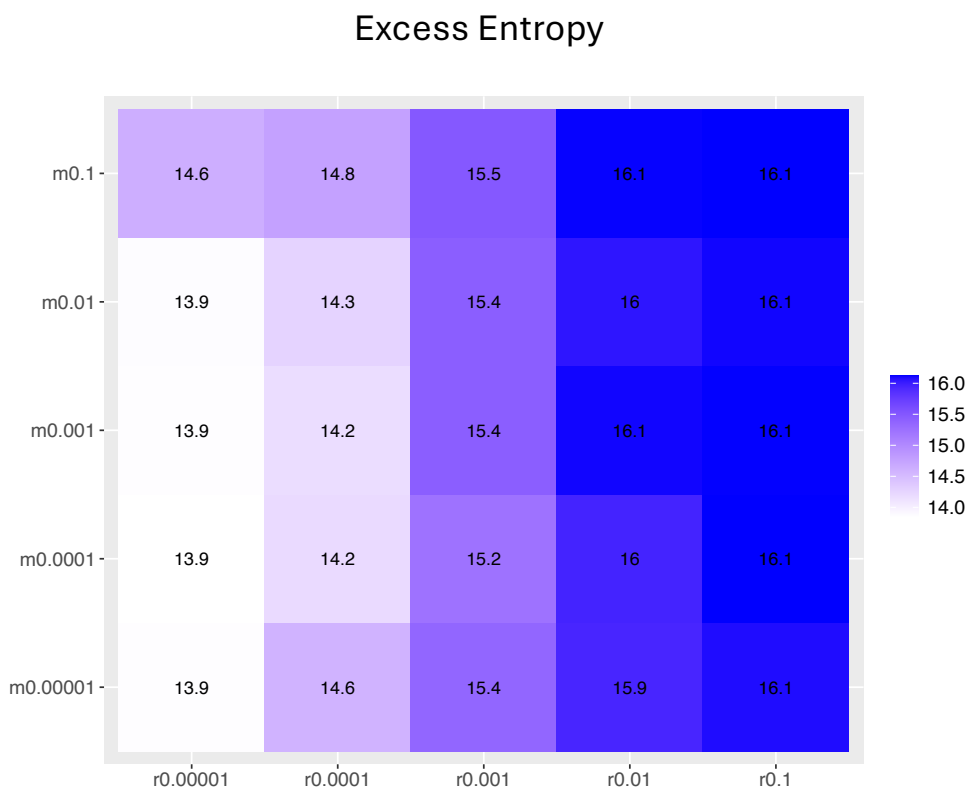

Excess Entropy
m0.1
m0.01
m0.001
m0.0001
m0.00001
14.6
14.8
15.5
16.1
16.1
13.9
14.3
15.4
16
16.1
13.9
14.2
15.4
16.1
16.1
13.9
14.2
15.2
16
16.1
13.9
14.6
15.4
15.9
16.1
r0.00001
r0.0001
r0.001
r0.01
r0.1
16.0
15.5
15.0
14.5
14.0

Fig 5

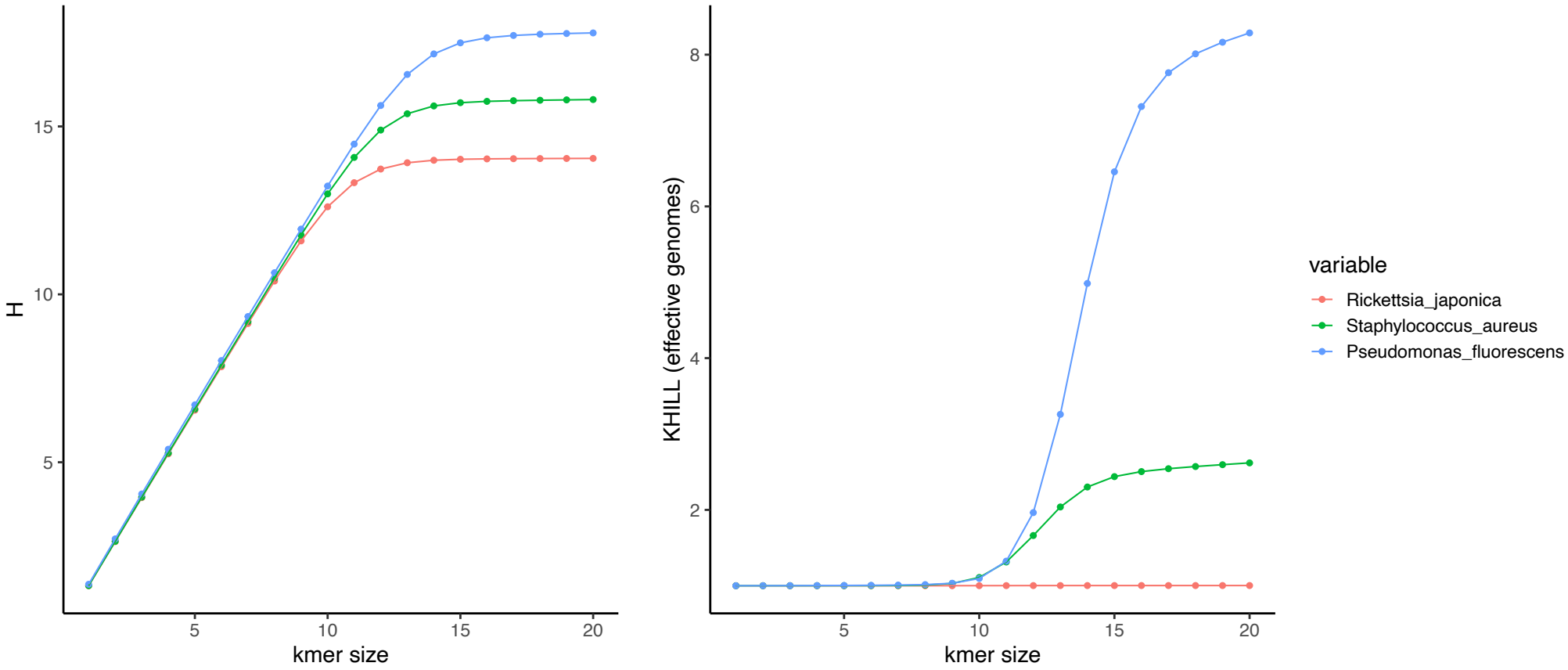

H
15
10
5
5
10
15
20
kmer size
KHILL (effective genomes)
8
6
4
2
5
10
15
20
kmer size
variable
Rickettsia_japonica
Staphylococcus_aureus
Pseudomonas_fluorescens

Fig 6

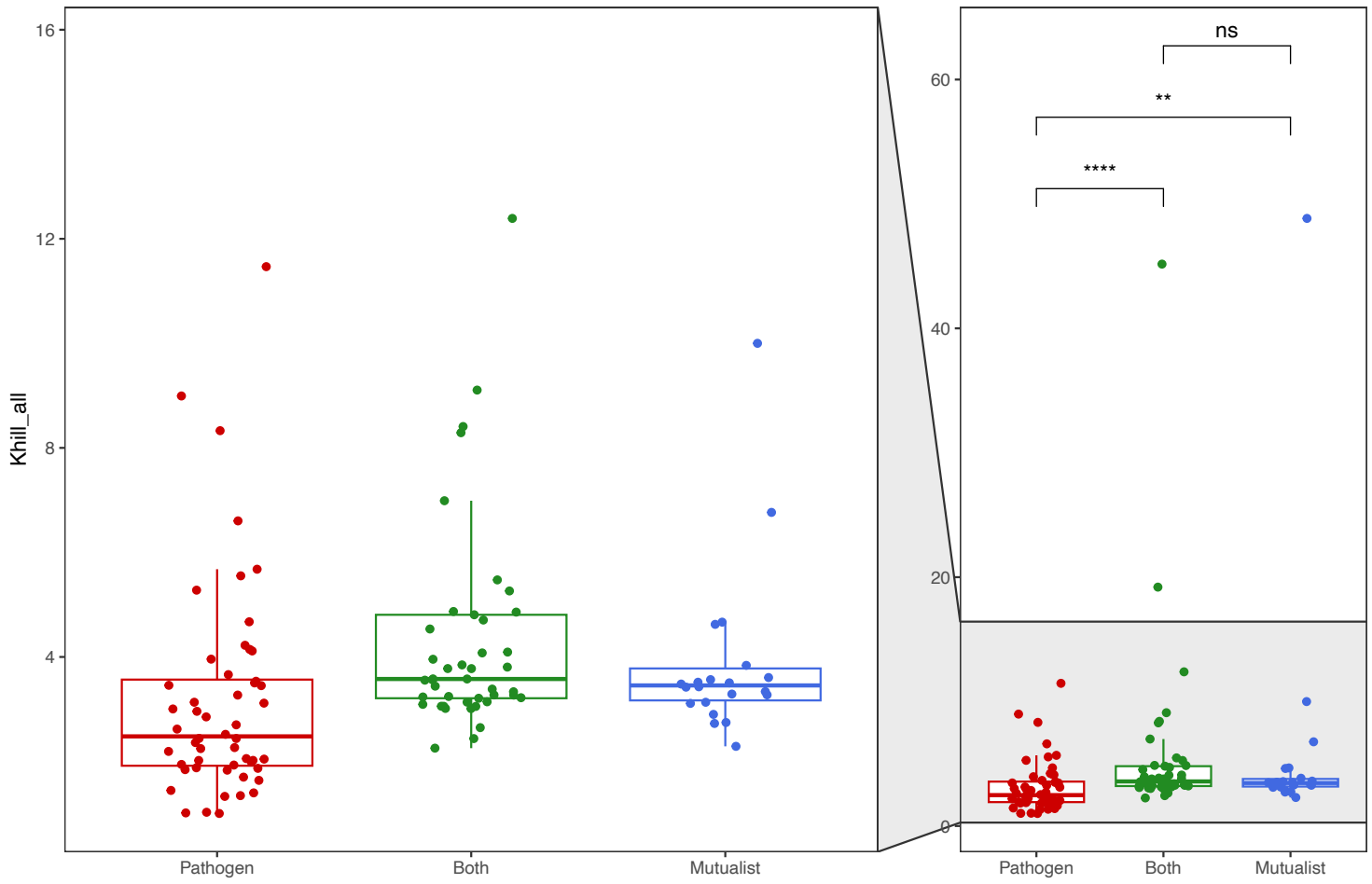
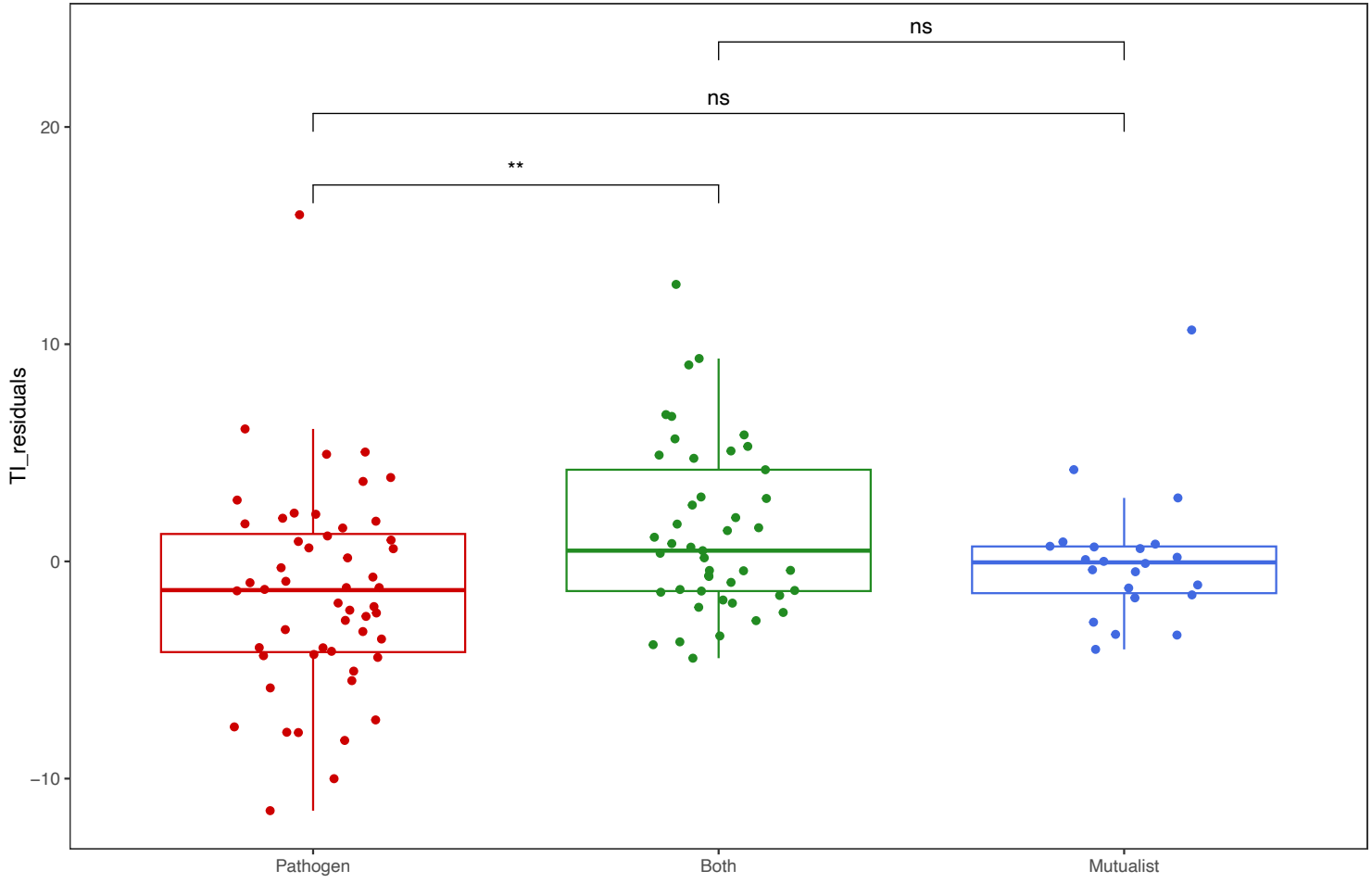
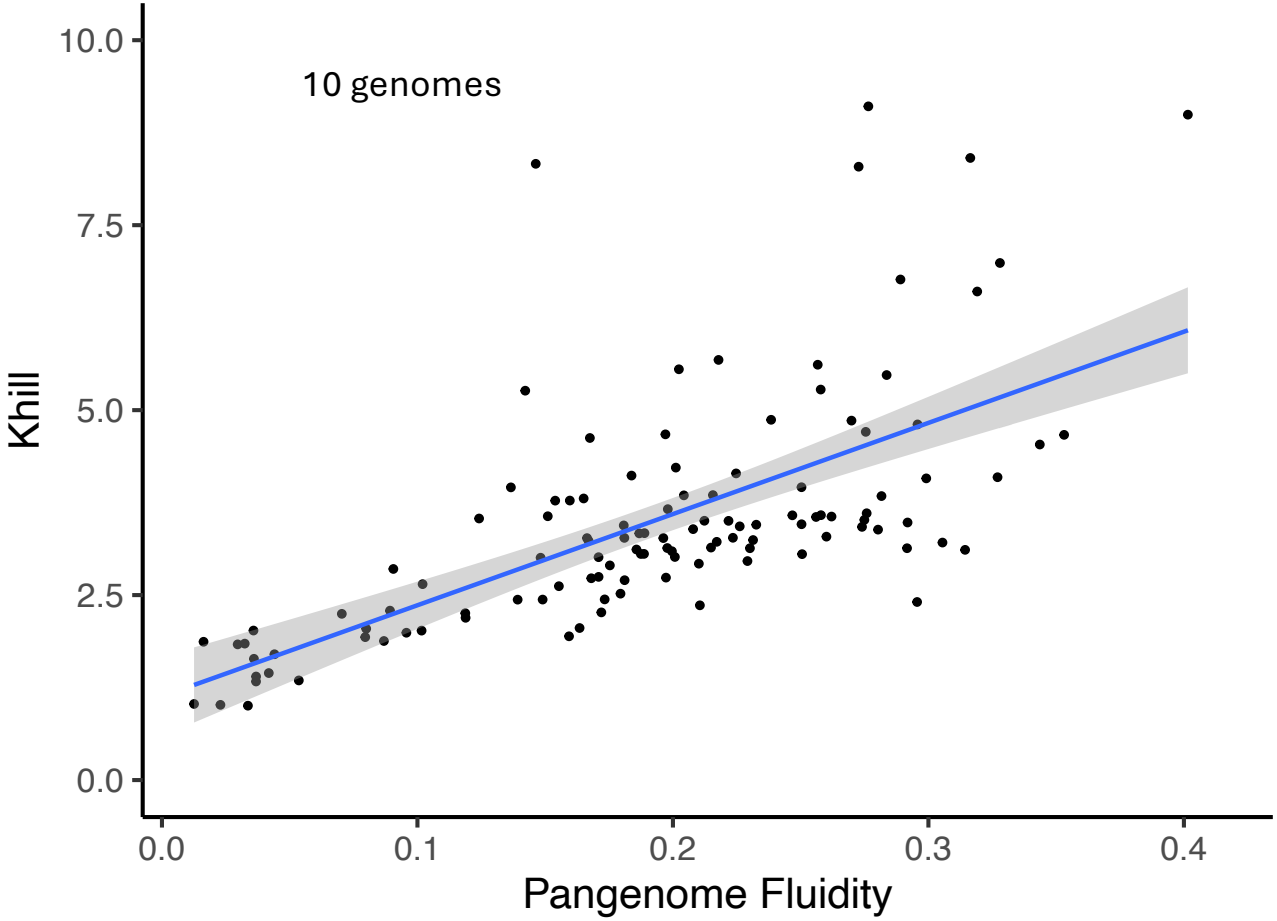

Khill_all
16
12
8
4
Pathogen
Both
Mutualist
60
40
20
0
ns
**
****
Pathogen
Both
Mutualist

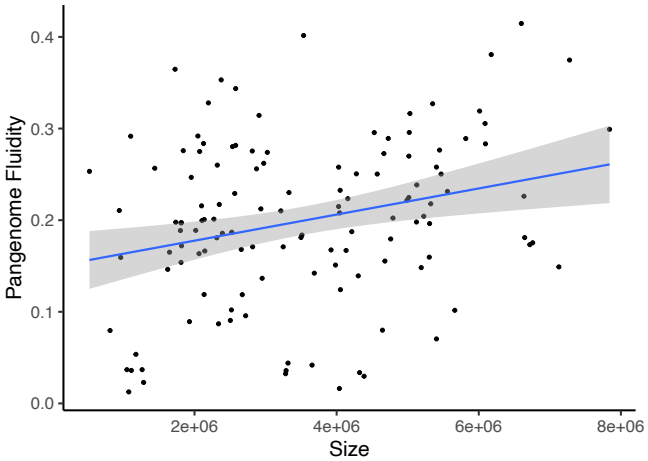
Fig 7

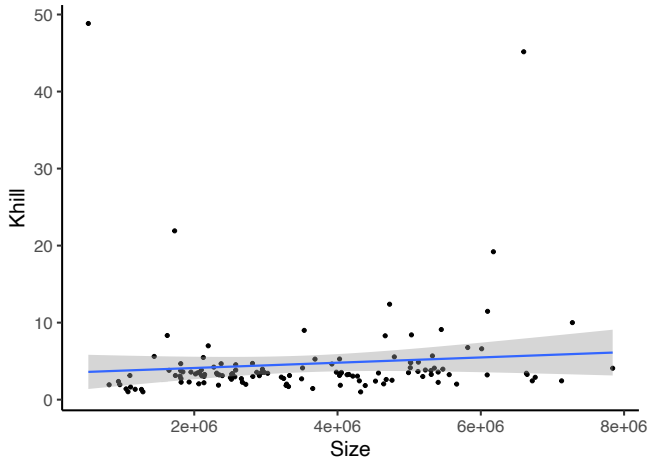


SuppFig 1

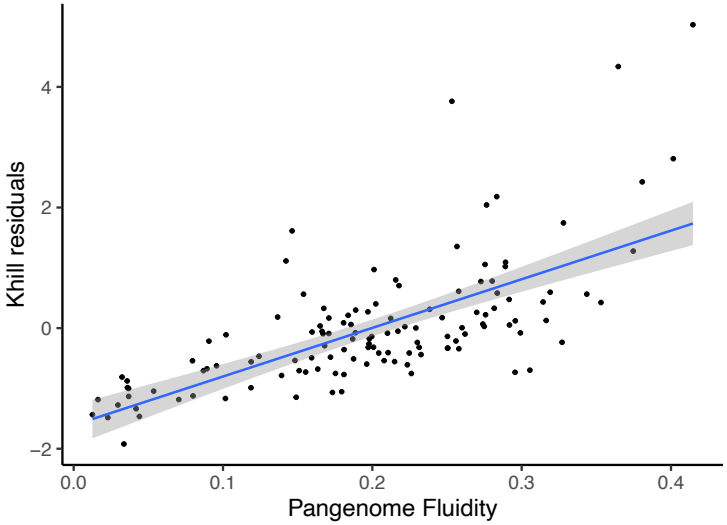

10 genomes
Khill
10.0
7.5
5.0
2.5
0.0
0.0
0.1
0.2
0.3
0.4
Pangenome Fluidity

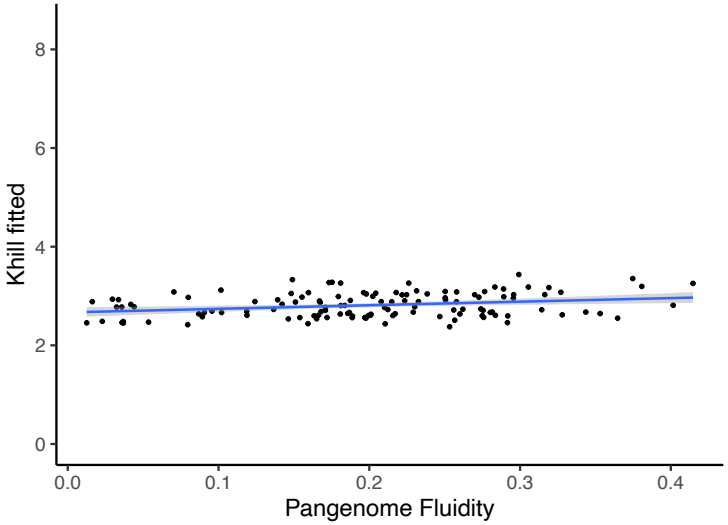
SuppFig 2


Pangenome Fluidity
0.0
0.1
0.2
0.3
0.4
2e+06
4e+06
6e+06
8e+06
Size

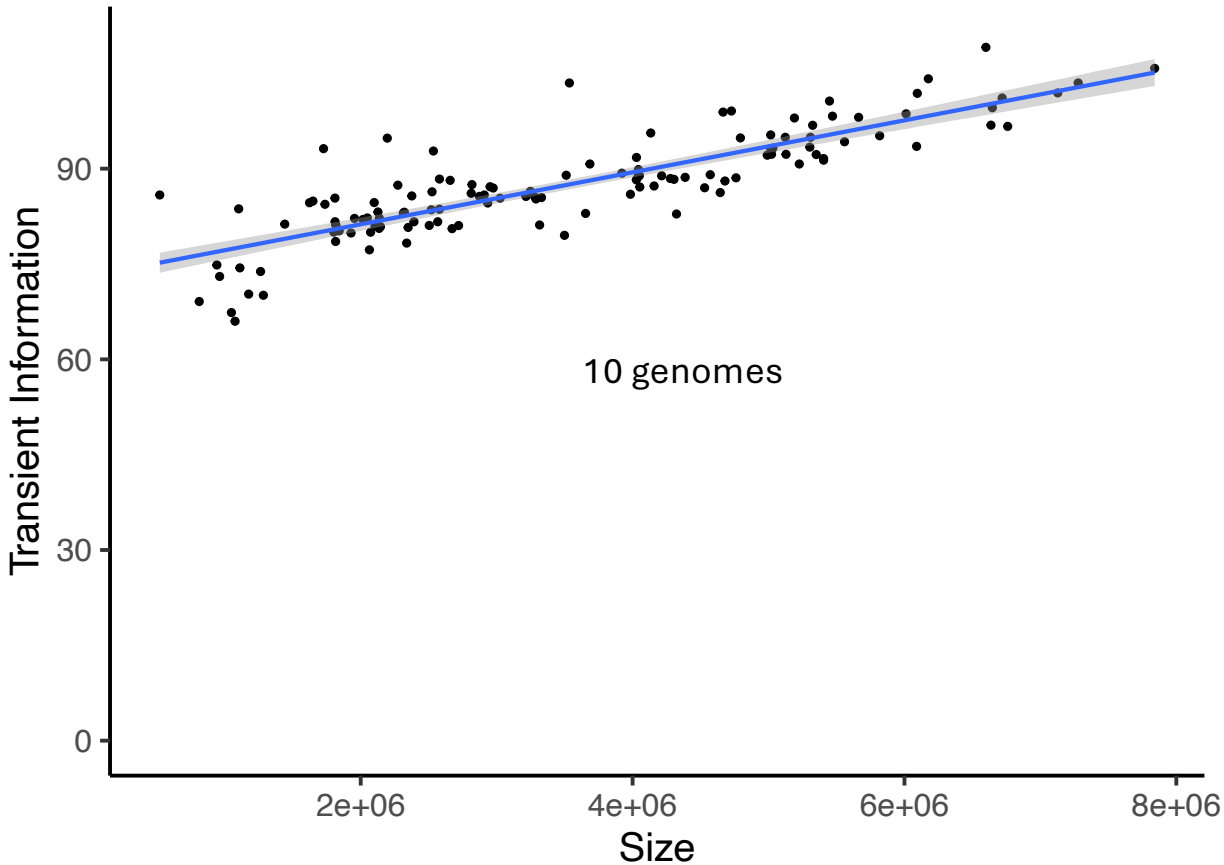

KhIll
0
10
20
30
40
50
2e+06
4e+06
6e+06
8e+06
Size

10 genomes



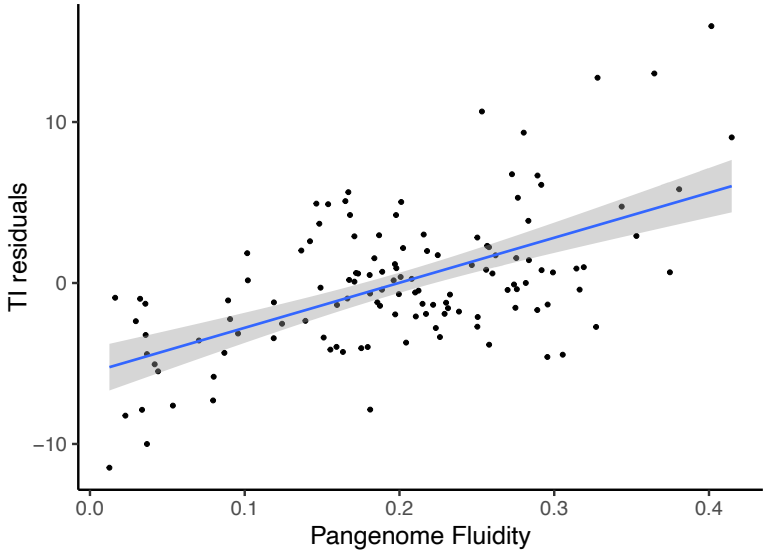


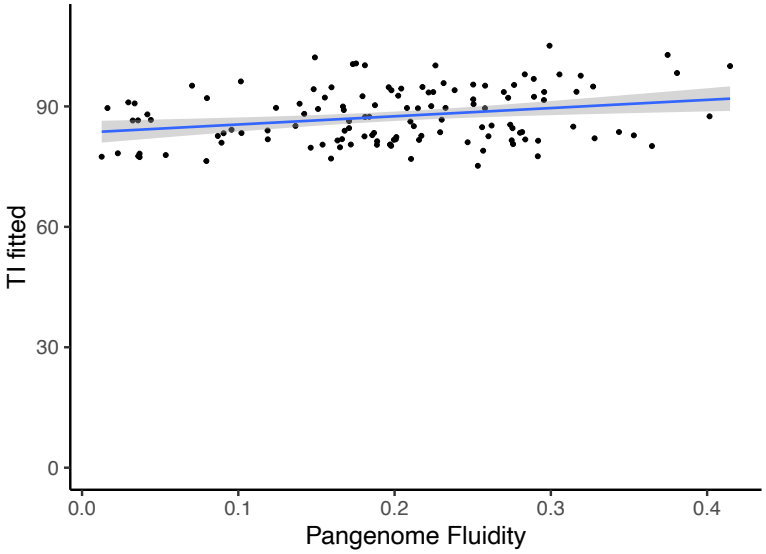
SuppFig 3


Transient Information
90
60
30
0
10 genomes
2e+06
4e+06
6e+06
8e+06
Size

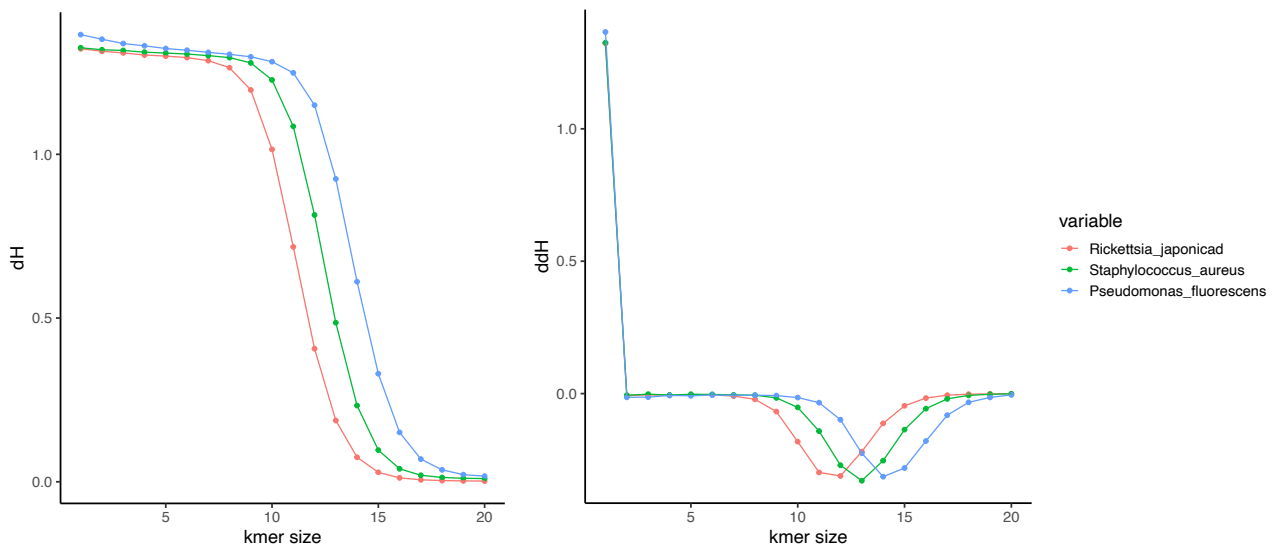
SuppFig 4

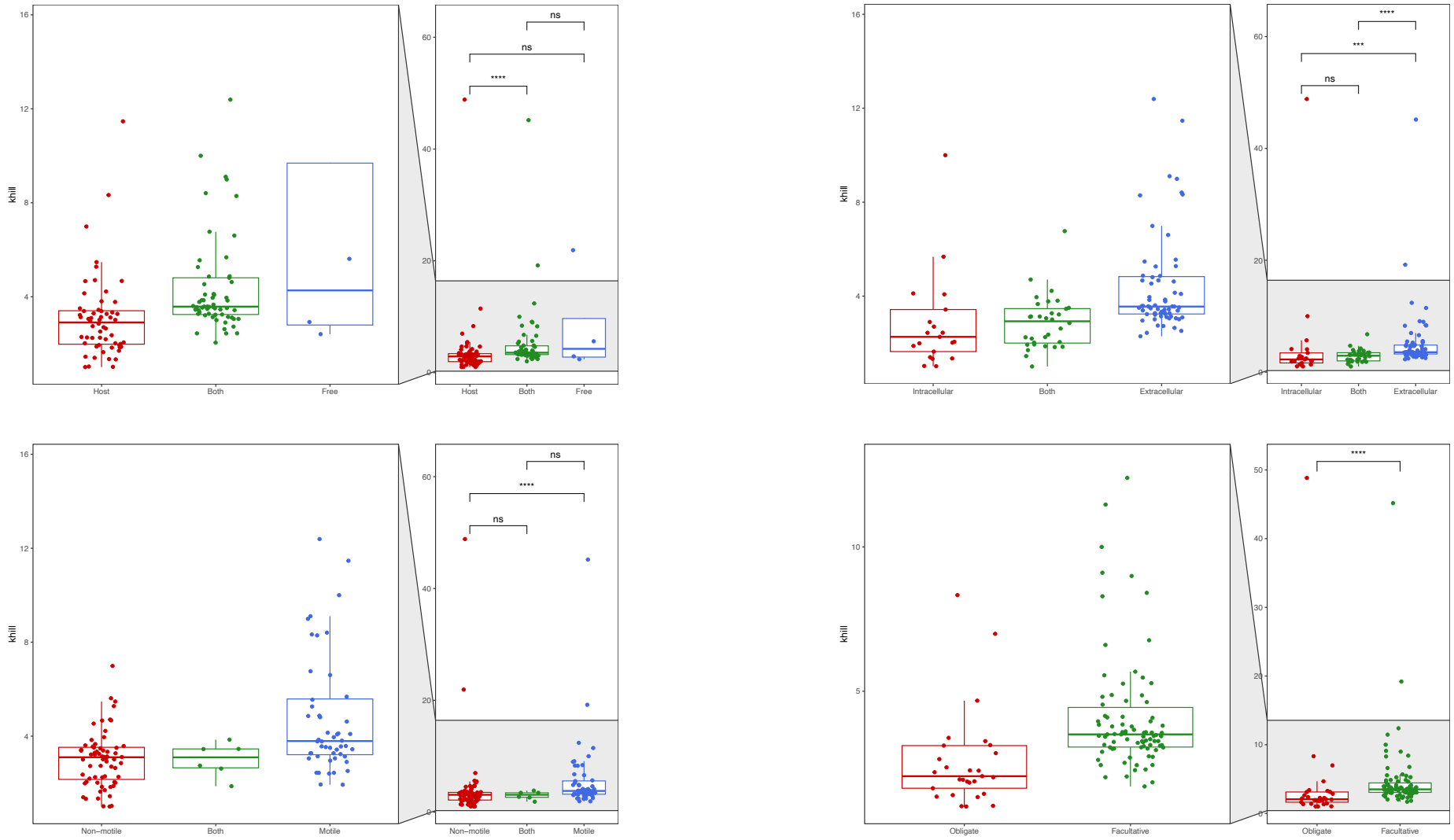
10 genomes SuppFig 5



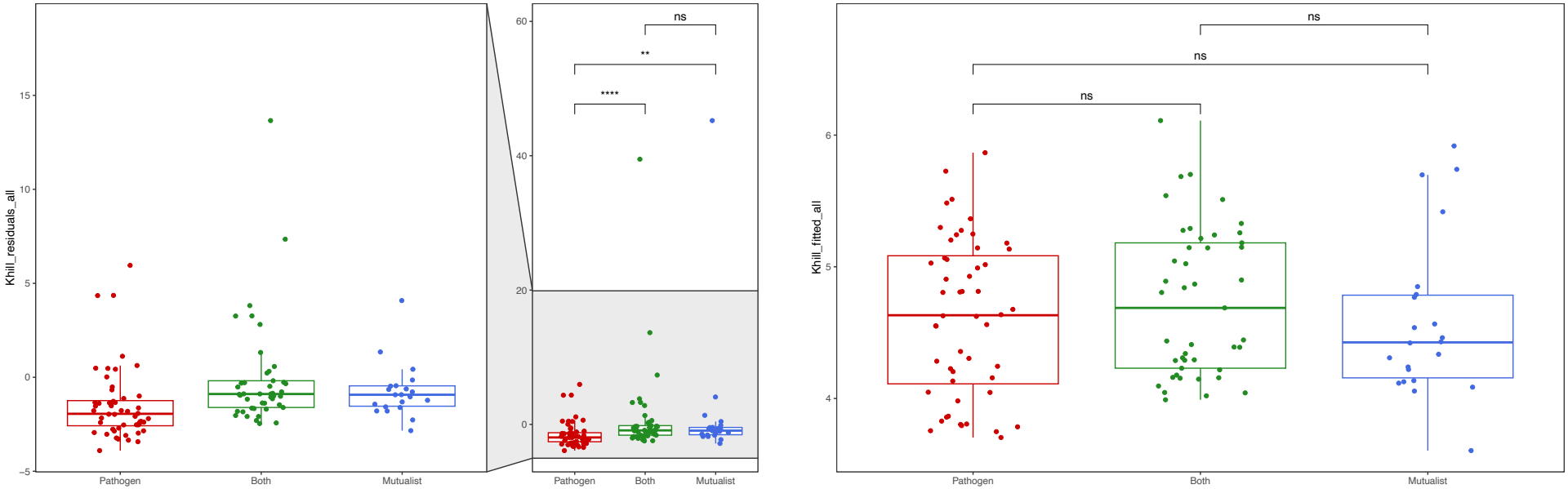


SuppFig 6

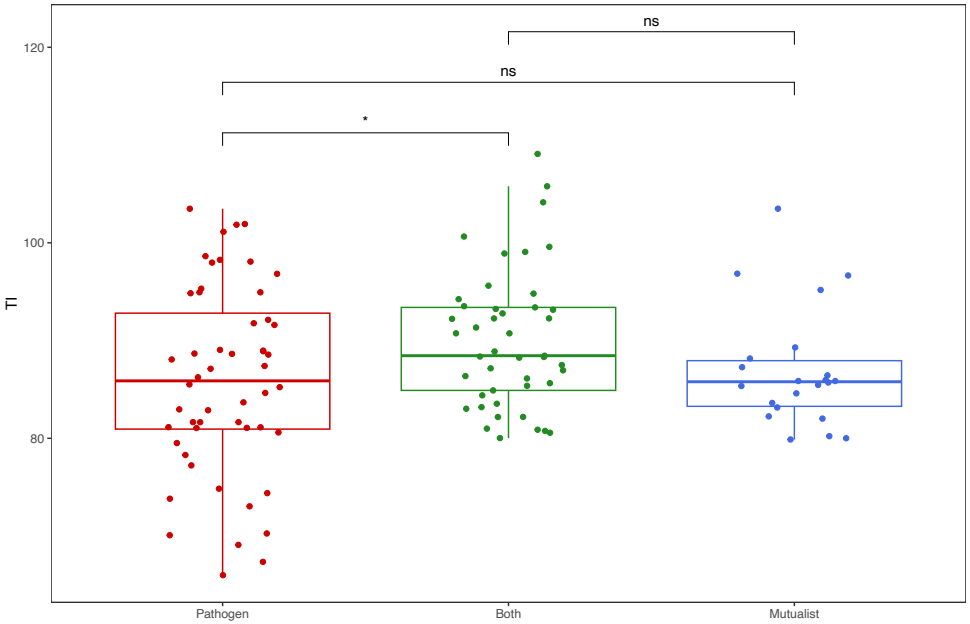

dH
1.0
0.5
0.0
5
10
15
20
kmer size
ddH
1.0
0.5
0.0
5
10
15
20
kmer size
variable
Rickettsia_japonicad
Staphylococcus_aureus
Pseudomonas_fluorescens

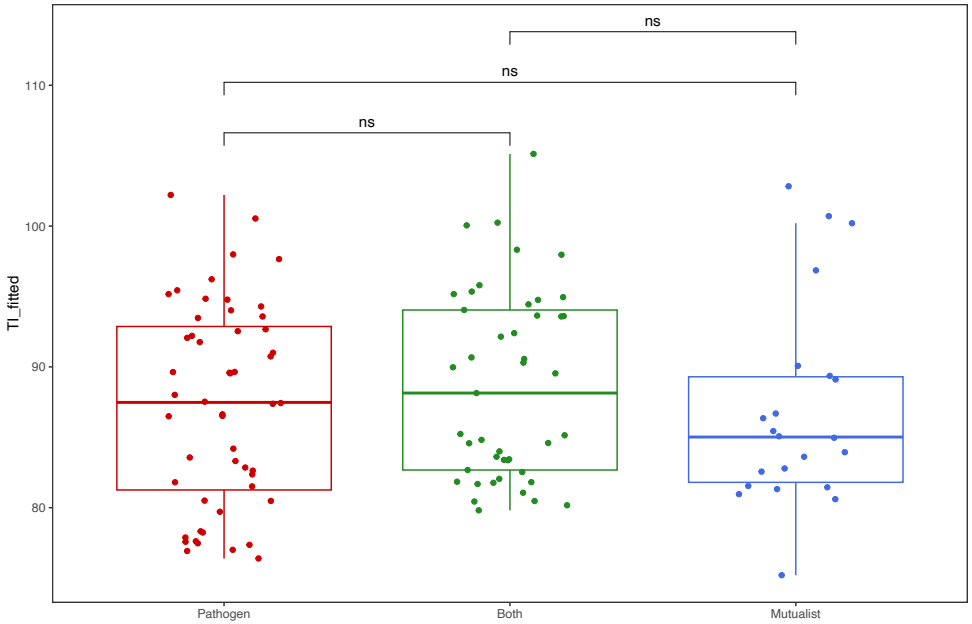

khill
Host
Both
Free
ns
ns
****
Intracellular
Both
Extracellular
****
***
ns
Non−motile
Both
Motile
ns
****
ns
Obligate
Facultative
****

SuppFig 7

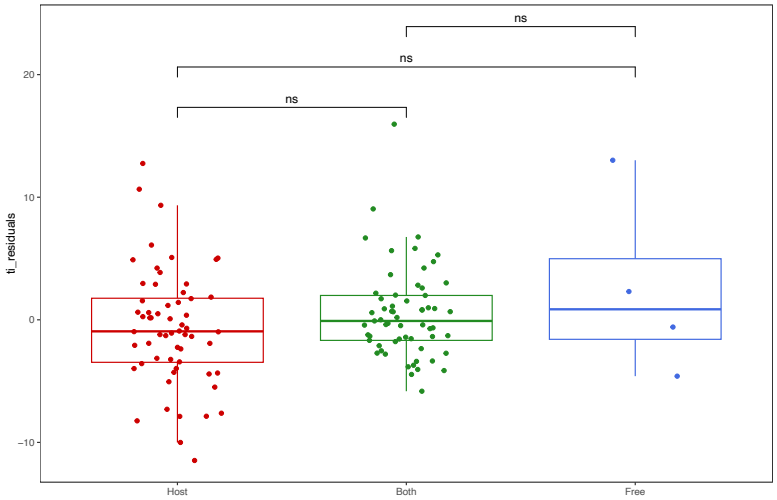

Khill_residuals_all
15
10
5
0
−5
Pathogen
Both
Mutualist
60
40
20
0
ns
**
****
Khill_fitted_all
6
5
4
ns
ns
ns

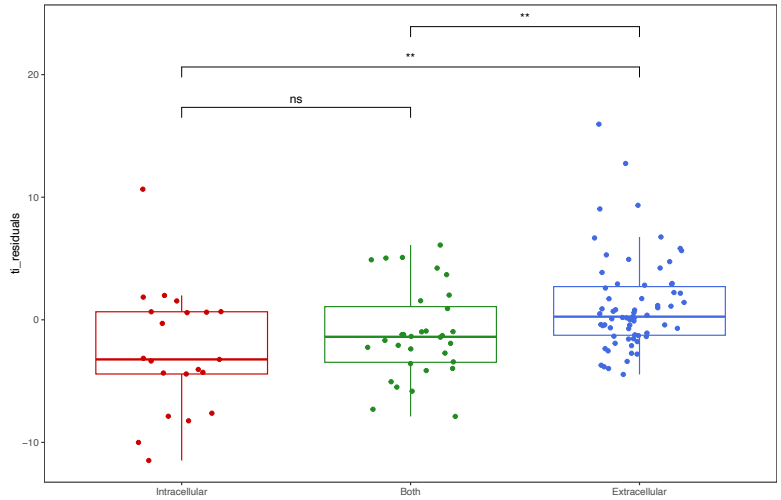
SuppFig 8

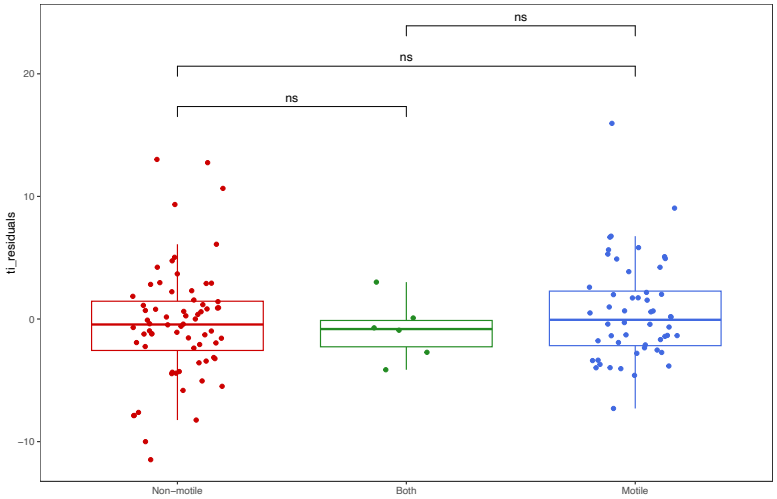

ns
ns
*
120
100
80
TI
Pathogen
Both
Mutualist

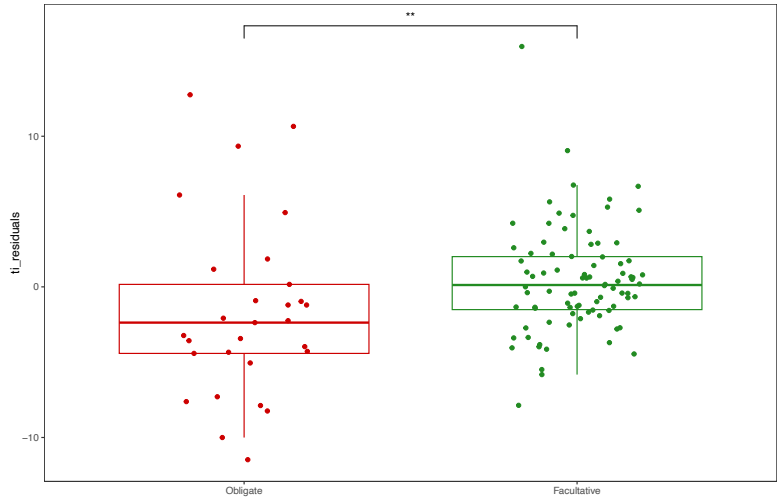

ns
ns
ns
110
100
90
80
TI_fitted
Pathogen
Both
Mutualist

SuppFig 9


ns
ns
ns
ti_residuals
20
10
0
−10
Host
Both
Free


**
**
ns
ti_residuals
20
10
0
−10
Intracellular
Both
Extracellular


ns
ns
ns
ti_residuals
20
10
0
−10
Non−motile
Both
Motile


**
ti_residuals
10
0
−10
Obligate
Facultative

SuppFig 10