Beyond DBGWAS: Exploring de Bruijn Graph in an efficient manner

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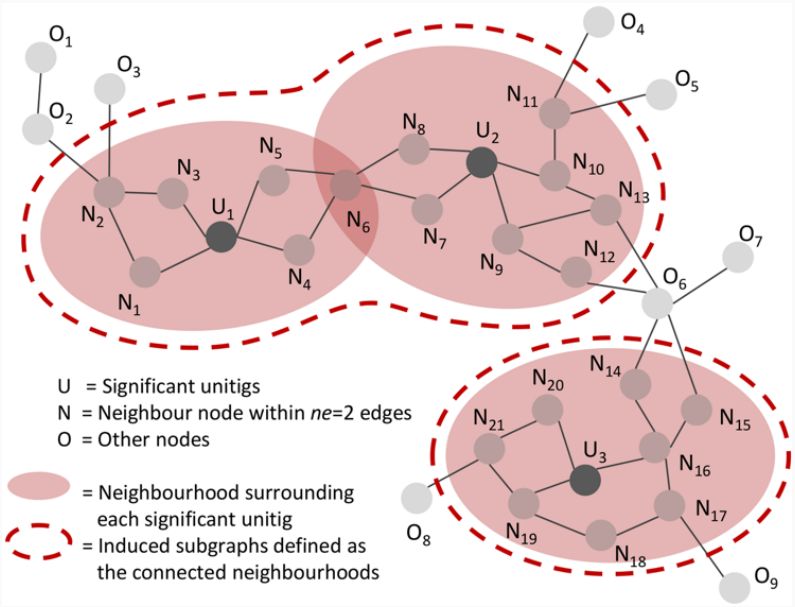
Introduction

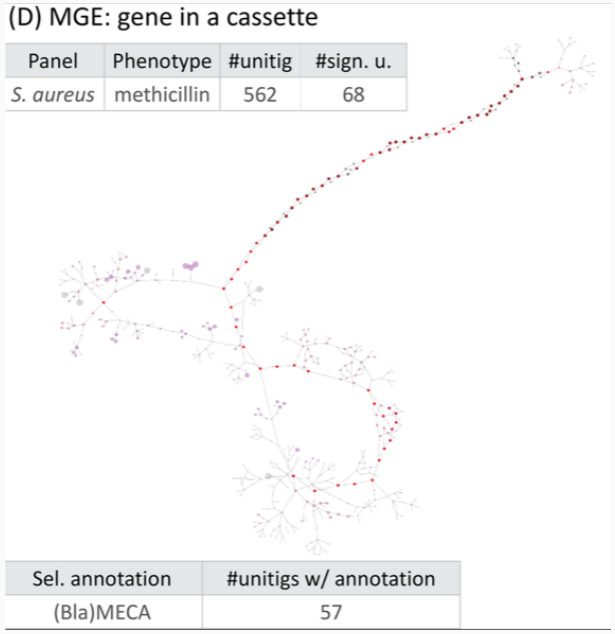
Motivation

Current format of DBGWAS has two limitations:

- Need to select an *nh* parameter to define the neighborhood (a).
- Low power to detect complex structures, as a gene cassette in (b).



(a)



(b)

Figure 1: [Jaillard et al., 2018]

Motivation (continued)

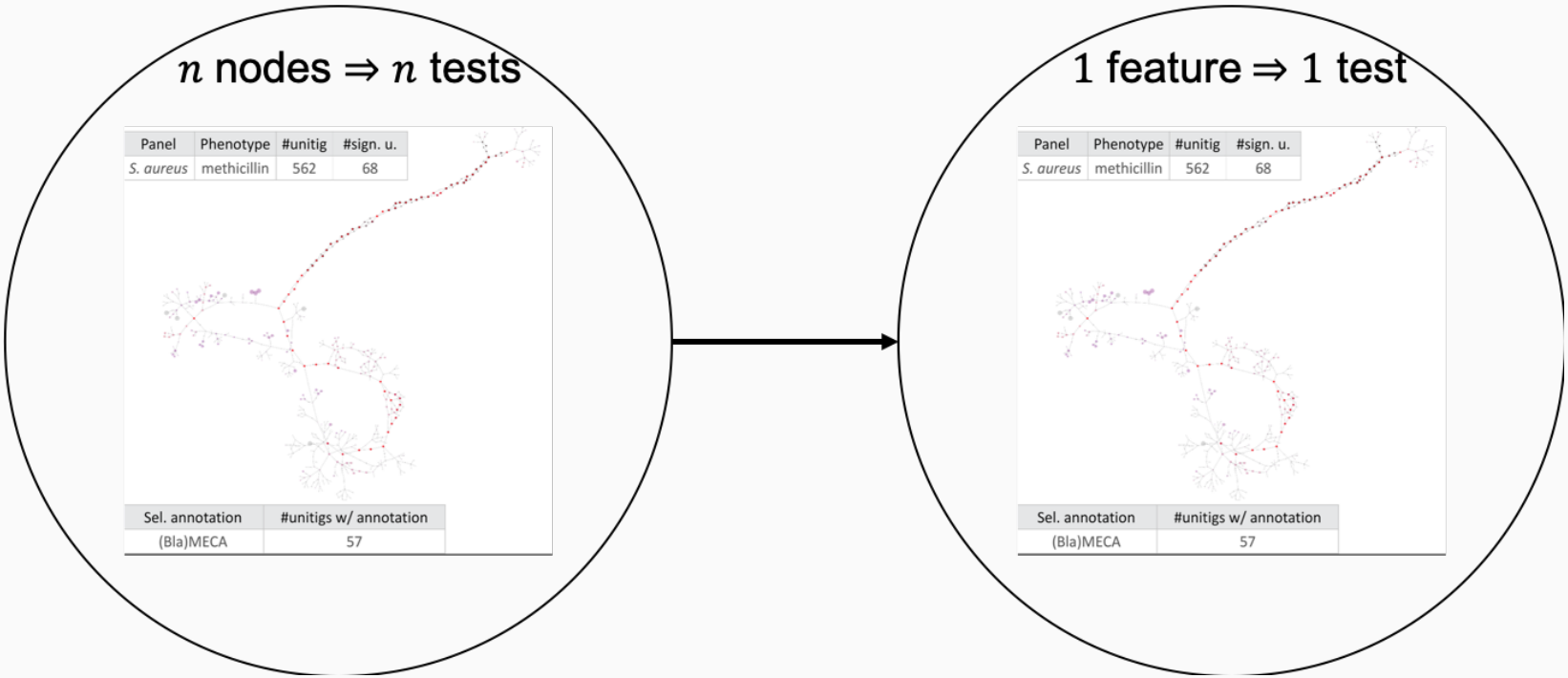
We also want to preserve strong features of DBGWAS

- Correcting for population structure.
- Remain reference-free as long as possible.
- Discover significant SNPs, gene cassettes or even species.
- Good interpretation and visualization tools.

Approach and notation

Approach

Instead of testing at the node level and trying to combine in a heuristic manner, test all possible subgraphs.



Notation

We have a set of n observations $\mathcal{D} = \{\mathcal{G}_i, y_i\}_{i=1}^n$, where

- $\mathcal{G}_i$ is a graph (part of the full k-mer graph)
- y_i is a binary phenotype.

We denote by $\mathcal{G} = U_{i=1}^n \mathcal{G}_i$, the full k-mer graph. For every subgraph $\mathcal{H} \in \mathcal{G}$, we note $z_{i,\mathcal{H}} = (\mathcal{H} \cap \mathcal{G}_i \neq \emptyset)$ and $z_{\mathcal{H}} = (z_{1,\mathcal{H}}, \ldots, z_{n,\mathcal{H}})$.

For all $\mathcal{H} \in \mathcal{G}$, we want to test $z_{\mathcal{H}} \perp Y$.

Tarone's trick

Why use Tarone's trick

Testing all subgraphs in a naive manner is not possible. The number of tests to run is much too large

1. to be computationally tractable.
2. to give reasonable power to any test.

Using Tarone's trick Tarone [1990], we can solve both issues

Tarone's trick in an example

Fisher's exact test for a two-by-two table:

Variable	Favors soccer	Favors rugby	Rows totals
Comes from the south	6	5	10
Comes from the north	8	1	10
Cols Totals	14	6	20

Conditional on the marginals, we have a hyper-geometric distribution and an associated p-value of ≈ 0.16

Minimal p-value and testable hypothesis

Before looking at the data, we can compute the minimal possible p-value. Because we have integer counts, it is not zero. The minimal p-value is obtained with this distribution of the data.

Variable	Favors soccer	Favors rugby	Rows totals
Comes from the south	4	6	10
Comes from the north	10	0	10
Cols Totals	14	6	20

The minimal p-value is ≈ 0.11

Tarone's trick

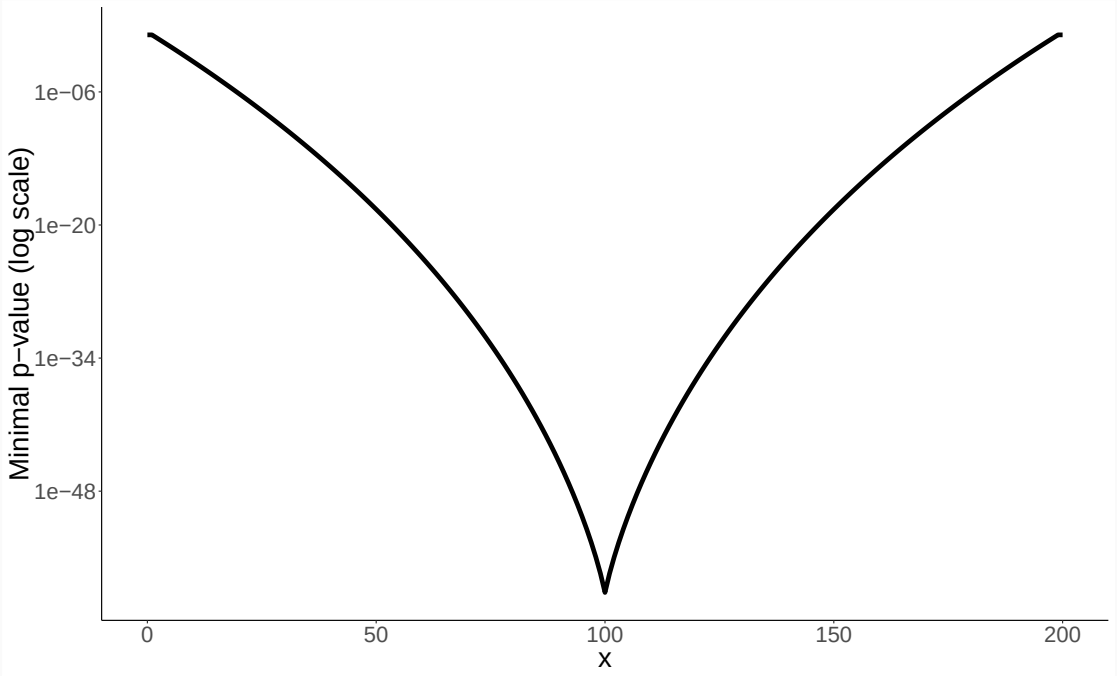
We want to test N hypotheses $h \in \mathbf{H}$. Tarone's trick relies on computing, for various values of $k \in [1, \ldots, N]$, $m(k) = \left|\{h \in \mathbf{H} | p^*(h) \leq \frac{\alpha}{k}\}\right|$. Then we identify $k_0 = \min_k \{k \in [1, \ldots, N] | m(k) \leq k\}$.

We can then define $\mathcal{R} = \{h \in \mathbf{H} | p^*(h) \leq \frac{\alpha}{k_0}\}$ and we only test the hypothesis in $\mathcal{R}$. We can then control the family-wise error rate (FWER) at a level α by rejecting each test $h \in \mathcal{R}$ iif $p(h) \leq \frac{\alpha}{k_0}$.

This has been used for regular GWAS by Llinares-López et al. [2015].

The minimal p-value is strictly increasing (after some point)

If we name $x = \sum_{i=1}^{n} 1\{Y_i = 1\}$, we have



In general, for $x > x' \geq \max(n_1, n_2), p^*(x) > p^*(x')$. So, if a subgraph is not testable, any subgraph that contains it is not testable either. We can use Frequent Subgraph Mining (FSM) algorithms to explore the De Bruijn graph.

Accounting for population structure

K-means on node matrix

We use the node absence / presence matrix $\begin{bmatrix} 0 & 1 & ... \\ 1 & 1 & ... \\ ... & ... & ... \end{bmatrix}$ of n samples by m nodes to run the k-mean algorithm. We can then obtain a categorical variable $c_i \in \{1, \ldots, K\}$ for each sample.

CMH tests with Tarone's trick

Updating previous notation, we now have $\mathcal{D} = \{\mathcal{G}_i, y_i, c_i\}_{i=1}^n$ and we want to test $z_{\mathcal{H}} \perp Y | C$. Tarone's trick works for any test that relies on the discreteness of the data, including the CMH test [Cochran, 1954, Mantel and Haenszel, 1959].

However, we loose the increasing property of the initial p-value.

Defining an envelope

We define $\tilde{p}^*(\mathcal{H}) \equiv \min_{\mathcal{H}' \supseteq \mathcal{H}} p^*(\mathcal{H}')$. Then we recover the wanted property. Papaxanthos et al. [2016] proved that the envelope can be computed in $O(k \log k)$.

We need to modify the FSM algorithm to prune the graph based on the envelope, instead of the frequency (*work in progress*).

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Appendix

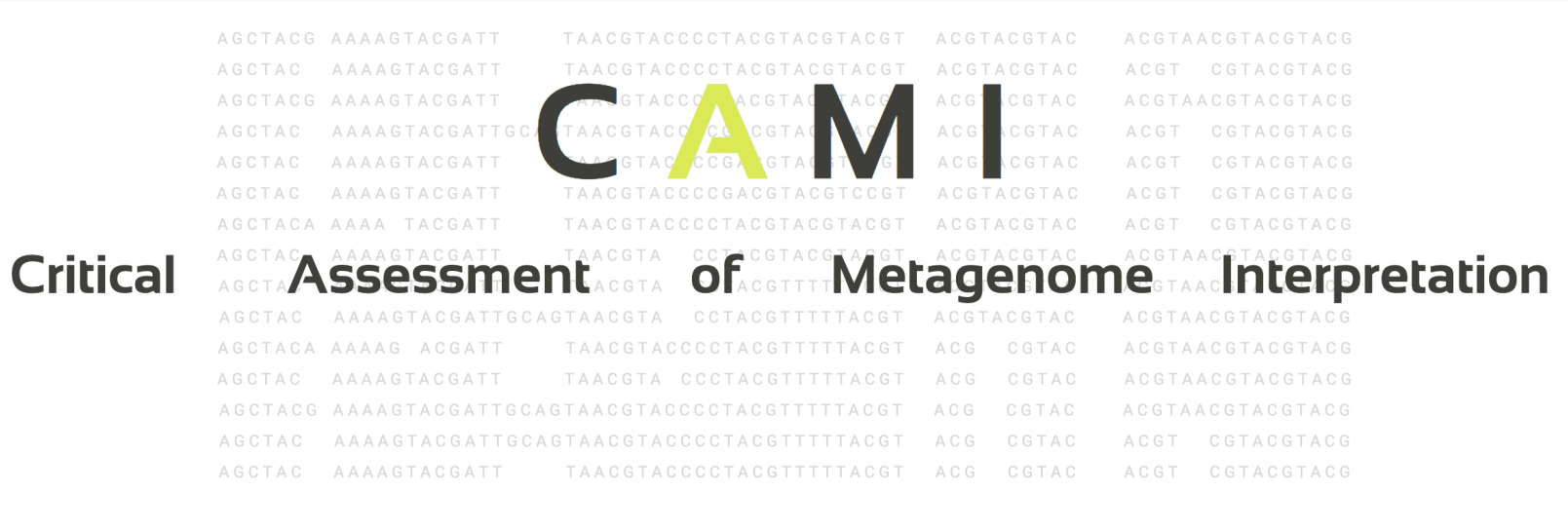
Musing on multiple testings

We note $\mathcal{R} = \{h \in \mathbf{H} | p^*(h) \leq \frac{\alpha}{k_0}\}$

- We proved that, if $|\mathcal{R}| \leq \sqrt{n}$, then Tarone's trick with FWER is less conservative than the FDR, with the same α level.
- As Gilbert [2005] pointed out, controlling the FDR on $\mathcal{R}$ controls the FDR on $\mathbf{H}$.
- Actually, there are no reason to use the same test to define $\mathcal{R}$ and then to test on $\mathcal{R}$. We still control the FDR (or the FWER) on $\mathbf{H}$.

Datasets

- Single species from the original publication: 282 bacterial genomes of Pseudomonas aeruginosa along with their drug (amikacin) resistance/sensitivity phenotype. Many results have been validated in the lab.
- Simulated metagenomics data from CAMI [Sczyrba et al., 2017]. The contigs are known, the genes will be revealed at some point.



Datasets (Continued)

- Simulate data from real datasets. Randomly add contigs from a hold-hover sample with probability $\pi = \pi(y_i)$.
- Real diabetes datasets from Karlsson et al. [2013], Wang et al. [2012]. Many results are known.