Caveats:

The theorem of Shoval et al (Shoval et.al. 2012) shows that under certain assumptions, natural selection under trade-offs leads to a polytope with enrichments. If such a polytope is found in data, it does not automatically mean that it is due to natural selection, because a polytope might arise from other effects. Thus, further tests are needed. We view ParTI not as a way to “prove” that optimality exists in a dataset, but instead as a way to generate hypotheses on what the tradeoffs and tasks faced the evolution of the system. These hypotheses are best if they lead researchers to do new tests or experiments.

In this section we detail some of the effects and approaches used to address them.

1. Independent experimental tests of the function of archetypes.

ParTI provides hypotheses on the functions of archetypes. These can sometimes be experimentally tested. For example, Hausser 2019 used independent experimental data to test hypotheses on the function of archetypes in cancer. They found that drugs that target the predicted functions are more effective on cell lines close to the archetype. They also showed that driver mutations have effects that point to polytope vertices, much more than passenger mutations.

In a recent example, Friedman et. al. (Friedman et. al 2020) used ParTI to discover archetypes in single-cell gene expression of fibroblasts in the cancer microenvironment (non-cancerous cells that support cancer). One of these archetypes had an unexpected MHC expression program for immune interaction. This led the researchers to experimentally test if these fibroblasts activate immune cells in vitro, and found that they did. The same archetypes were then found in human samples and used to establish a potential cancer marker. This is an example of a new experiment done to test an hypothesis raised by ParTI.

In some cases, experimental tests are less important because the archetype functions correspond to previously discussed functions, such as ammonite swimming functions (Tandler et.al 2015), fast-slow life history strategies (Szekely et.al. 2015) or hallmarks of cancer (Hausser et.al 2019).

2. Effects due to phylogeny

Phylogeny effects are a concern especially for data on animal morphology or life history traits. This issue was addressed in ParTI work done since 2013, following a technical comment by Edelaar. The methods use a known phylogenetic tree for the data.

Szekely et al 2015: shuffling test on the known mammalian phylogenetic tree showed that phylogeny cannot explain mass-longevity traits above the level of family. Szekely also listed species that are phylogenetically far but are close on the triangle, and species phylogenetically close that are far on the triangle. This confronts the hypothesis that the polytope is due purely to phylogenetic relatedness. In reality there is a contribution of both phylogeny and selection (Bonsall and Mangel 2004).

Tendler et al 2015: After mass extinctions, in which only 1-2 ammonite genera survive, the ammonites refill the same triangle with statistically similar archetypes. This is a powerful method to address phylogenetic relatedness concerns.

A more recent advance appears in a bioRXiv preprint by Karin et al (2018) on cultural traits (https://doi.org/10.1101/263905). This study used a phylogenetic tree of languages. Karin generated datasets at random by using the phylogenetic tree as a null model (with inferred rates of divergence), rather than by shuffling at random. The enrichments were also tested by adjusting for relatedness.

3. Population structure:

Population structure, for example in a dataset of cancer patients, can in principle cause a polytope with enrichments.

One approach to address this is to test whether the same polytope and enrichments recur in different datasets collected from different populations. For example, similar archetypes were found in two different ParTI publications on breast cancer, one using the Metabric database (Hart et al 2015) and the other TCGA (Hausser et al 2019). The patients who contributed samples to these studies come from different countries. This challenges the hypothesis that population structure is a major factor in these cancer datasets.

4. Data preprocessing and speculations of p-hacking:

It is unfortunately becoming popular for some authors to speculate that others have low standards of statistical analysis, and therefore commit an error called p-hacking.

P-hacking means trying many preprocessing methods and algorithm parameters, until one is tempted to choose certain ones to confirm an hypothesis.

It is thus important to use standard pre-processing methods (if established), and to clarify the choices of data preprocessing and parameters. It is prudent to test the robustness of such choices- a wide range of choices should provide the same results. If possible it is also prudent to use different independent datasets (see https://www.bitss.org/2014/10/31/what-to-do-if-you-are-accused-of-p-hacking/)

Here are two examples or robustness tests from work in our group.

Adler et al; 2019 analyzed single-cell gene expression data of hepatocytes. As established in the scRNA field, data for each cell was normalized to the total expression for that cell. Adler then tried all 4 combinations of log or linear, z-scoring or not. In all four possibilities a highly significant polytope (tetrahedron) was found, with the same enrichments. The paper used the most stringent case (highest p-value).

The fact that certain data can be approximated by a polytope in both log and linear data does not invalidate the theory. Sometimes a polytope is found only in log and not in linear data.

Hausser et al 2019 analyzed bulk tumor transcriptomics data from TCGA. The paper made a conservative choice and used *all of the genes*, with no cutoff. The same preprocessing was applied to 20 cancer types (each with several hundred tumor samples), and a polytope was found for about half of the types. Hausser et al 2019 tested the robustness of the preprocessing, by making a scan of 10 cutoff levels in which some genes were removed. The 10-cutoff-level scan showed that using no cutoff is a robust choice, because the results are not substantially affected by a wide range of cutoff values.

5. Skewed distributions, non-negativity and other effects

If data has skewed distributions, or preprocessing enforces strict boundaries such as using measures of gene expression such as TPM which are proportional to mRNA abundance are thus must be non-negative , one can obtain polytopes that are unrelated to optimality due to edge effects. Thus it is important to use proper null hypotheses with the same skewed distributions, and to be wary of edge effects.

In the textbook on systems biology, for example (Alon 2019), an example of a coin tossing experiment is shown that can generate a triangle. In this case, no significant enrichments can be found because the experiments are independent.

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