**INTRODUCTION**

1. **Section Two: How much pleiotropy is there and why is it so hard to find out?**
   1. Long history of debate on this topic.
      1. Is pleiotropy omnigenic/universal or modular? If modular, by learning relationships among traits one might be able to predict which suites of traits are jointly influenced by genetic changes.
      2. Though modularity seems reasonable and many studies suggest cellular systems have a modular organization, GWAS studies detect ungodly amounts of pleiotropy suggesting every gene has the potential to influence every trait.
   2. This debate persists because of challenges defining and measuring phenotypes, and integrating information about phenotypes at different levels of biological organization
      1. There are too many phenotypes, potentially millions or an infinite number depending on how creative you are
      2. Phenotypes are often related, effects cascading through levels of biological organization, introduce genotype-phenotype-phenotype map idea
   3. Conclude by explaining the ‘impossible task’: Quantifying all of these traits and enumerating relationships between them to understand which suites of traits (or whether every trait) will be jointly influenced by a mutation is too much work.
2. **Section three: Clever solutions leveraging barcoding**
   1. We have a new approach to understanding the extent of pleiotropy among adaptive mutations that circumvents the problem. We do not measure any of these phenotypes, instead we repeatedly measure fitness.
   2. Briefly explain the idea in general terms, subtle perturbations, FGM
   3. Explain the large data set we will use to test the idea, and how this is only possible because (1) these strains are barcoded, (2) NGS gives us lots of power to measure fitness, and (3) we cleverly use information about fitness to learn something about phenotypes.
3. **Section four: What do we find? Punchlines**
   1. The number of ‘traits’ affected by an adaptive mutation depends on context
      1. Adaptive mutations influence a limited number of ‘traits’ in environments close to the one in which they are adaptive.
      2. As we perturb the environment farther from this one, we reveal additional pleiotropic effects of adaptive mutations
      3. This suggests resolution to the paradox, how pleiotropy can be pervasive and adaptation can still happen
   2. Our method is really cool.
      1. Talk about all the cool things you could learn using this epic approach.

**RESULTS**

**Section 1: A method to identify fitness-relevant phenotypes *[is this really part of intro??]***

1. Intuition: organism’s phenotype determines its interactions with environment and thus fitness. So by measuring fitness in different environments, we can learn about these interactions at start getting towards the important phenotypes
2. We explicitly treat fitness as a linear combination of these sets of interactions – how many interactions are there? [explain these alongside some simulation examples??]
   1. Overfitting is a common statistical problem. We have two approaches:
      1. With known measurement error, we can simulate all-error matrices and find the largest component of noise. This sets our limit of detection.
         1. This works in simulation – as measurement noise increases, our power to detect small signal diminishes, eventually we don’t detect all of the interactions we simulated
      2. We can use cross-validation. At some point we fit measurement noise, and our predictive power declines.

**Section 2: We detect 9 FRPs from our approach, with 4 we capture A LOT of variation**

1. Explain the data.
   1. Mutants from Levy et al, sequenced in Venkataram…
   2. We can measure things well (within-flask, multiple-flask replicates)
   3. Despite good measurement, we see significant batch effects – could these represent real signal? The problem is we don’t knowwhat was perturbed!!
   4. We added a suite of other conditions as well, to try to intentionally perturb interactions – these range from subtle (within in the range of batch effects) to strong (very different)
   5. Can we use this fitness data to identify differences amongst these mutations? Do they interact similarly or differently to different environments?
2. Hole here? -> talk about real biological signals in the data from coarse-grained approach??
3. Using previously described 2 methods, we can see what we capture using subtle perturbations.
   1. Our approaches tell us that there are 9 components (the first of which is mean fitness)..
   2. With only 4 components, we explain a substantial proportion of the variance – though our methods say that we have the power to detect all 9

**Section 3: We can predict behavior in strong environmental perturbations FRPs in subtle perturbations have significant effects in some strong conditions**

1. We can predict behavior in strong environmental perturbations: 1 component model does very poorly.
2. 4 component model does remarkably well! (again, this is subtle predicting strong) – this means that the major components at least reflect real biology and our space has some meaning!
   1. Point out some specific examples of where we’ve really improved (baffle, 1.8 is captured), benomyl, 7 day
3. 9 component model does minimally better for some conditions (same examples? 1.8% glucose, benomyl, 7day), but has a strong contribution to predictive power for several conditions (1day, suc/raf, 2.5%, 4day, 6day) . This means extra components have biological meaning and they aren’t always “minor” they have sudden, large, contributions in other conditions
4. Our measurement precision could only detect 9 components of interaction with these mutants – there could, of course, be many more such minor components below our level of detection. These could, in turn, contribute to being able to fill in the missing predictive power in some of the stronger conditions.

**Section 4: mutant- and condition-specific pleiotropy**

**Section 5: Our phenotype space uncovers real biological differences amongst these mutants**

Are we showing “real” phenotypes from 4 or 9 components? We detect 9 and think there really are 9… but we also say 4 seems good enough?

1. Gene identity means something
   1. GPB2, PDE2, Diploid
2. Haploids and Diploids have different phenotypes
3. RAS/PKA mutants themselves vary
4. TOR mutants don’t obviously cluster differently from RAS/PKA

What specific mutations/genes are driving this extra detection in the 5 otherwise minor components?

**DISCUSSION**

[not yet in proper order, but some thoughts…]

*[ this approach is a “new tool” through which we can identify fitness-relevant behavior].*

*Some other possible applications:*

*perturbing ecological communities to identify functional classes of behavior and ecological interactions*

*??*

*Possibility of using this approach to* *identify causal lower-level molecular phenotypes (via RNA-seq data or other phenotypic data) ]*

*Evolving to multiple environments?*

*Epistasis? [i.e. how do PDE2/GPB2 double mutants fare in M3,*

There are sort of two related questions:

1. Do all of the mutants do the same thing (i.e. is there “one” way to adapt) and (2) how many things do they do? (i.e. even if they did the same thing, there could be multiple ways to interact with the environment.

We cannot detect the second unless there’s variation in the first. (i.e. if they all have the same interaction profile, we can’t identify how many interactions there are…

How do we reconcile this “build up” approach with the statement that 3 things ex

Do we want to use this “build up” approach?