**Title Ideas**

1. Using subtle environmental shifts to probe the number of phenotypes that contribute to fitness

2. Adding subtle environmental shifts to Fisher’s Geometric Model reveals…

3. Subtle environmental perturbations reveal the number of fitness-relevant phenotypes

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[add Yuping if we include batch data]

**Abstract**

Tackle this later

Hi Grant! Since you are interested in improving your science writing, I tried to give you advice on how to write this paper in line throughout the paper. But my opinions are just that – opinions! So feel free to disagree. The manuscript will evolve with the data, so some of what I advise may become less relevant as we go. I think this is a good start for now. Happy editing!

**Introduction**: I think the introduction can be thought of as three sections. This is not to say there should be actual sections, or subheadings. It is more a tool to help us write and discuss how to write. There will be transition sentences from one section to the next. The first section is about why our research is important. Here are my ideas for this section, which are not refined (wording sometimes awkward) so please feel free to edit. The second paragraph is critical – it gets to the main question “how many phenotypes are there” and may hint at adaptation, should we decide to include batch effects and focus on adaptation. We will probably want to fine tune the wording of this paragraph once the results section is done so that it corresponds perfectly to what we actually study.

The terms “genotype” and “phenotype” were coined in 1911 by W. Johannsen (Johannsen 1911) to create a distinction between an organism’s characteristics that were inherited and those morphological features that could be observed. Since then, our ability to observe phenotypes has grown by leaps and bounds. We can observe thousands of phenotypes in a single measurement (*e.g.* genome-wide transcriptome or proteome levels [CITE], methylation patterns [CITE], morphometric stuff with mice slicers [CITE Tautz, Frank from Tübingen] and powerful microscopes [CITE Ohya], the location of every gene in nucleus [CITE someone at NW], etc). A major challenge is understanding how all of these phenotypes interact to produce higher-level phenotypes of interest (*e.g.* disease). More specifically, quantitative genetics strives to trace the impact of a genetic change through many levels of molecular-level changes (expression levels, positioning, skeletal) that feedback upon each other in order to ultimately understand how a change at the genotypic level affects organismal fitness.

Complexity at the phenotypic level not only creates challenges for understanding the genetic underpinnings of complex traits, it also highlights questions about the organization of biological systems. Some questions include whether these many interacting phenotypes are organized into modules, such that even though it often appears that genetic changes have effects on many molecular level features (*i.e.* even though pleiotropy appears pervasive), the affected features typically belong to the same module, have levels that influence one another, and can be considered a single unit that orchestrates a single coordinated effect on organismal fitness. In other words, high level questions remain about how many independent phenotypes contribute to organismal fitness. These questions are of practical significance, for example, if many mutations can provide a microbe with drug resistance, each primarily affecting a different gene, but all contributing to drug resistance through the same higher-level phenotypic change, it is a simpler problem to solve than the acquisition of drug resistance through many disparate mechanisms.

Understanding the relationships between phenotypes would also make the study of biology more feasible. Statistical power to identify the genetic variants that contribute to phenotypic variation is diluted when many phenotypes are surveyed. Further, studies that count the number of phenotypes that are affected by a given gene or associated with fitness in a particular environment are upwardly biased if the phenotypes they measure are not independent.

The next section of the introduction is about all the people who have contended with this issue of ‘too many phenotypes’ before us. The point of the section is (1) to convince readers of the importance of our work by revealing the large # of people who tackled this issue, and (2) to synthesize many previous studies into a new understanding of various approaches to this issue.

The point of this section not to describe previous studies. For example, you describe drift load, which is super cool, but our paper is not about drift load. We don’t care if our readers know what drift load is. This section needs to be driven by your opinion of what is important (and perhaps missing) from previous studies, not by the details of the studies themselves. It is very rare to start a sentence and say, “Kinsler et al showed…”. You might want to start a paragraph with an opinion: “The fitness effect of mutations are inherently tied to the genetic backgrounds and environments in which they are measured”. Then expand on this opinion. What is your evidence that this is the case? Why is this the case? Then talk about previous studies in light of this opinion, “previous studies that find conflicting number of phenotypes that contribute to fitness (5 vs. 100) may do so because they focus on different environments”.

It can be hard to synthesize previous literature into a unique perspective. Try thinking about the things previous studies have in common or do differently. Which approaches calculate the correlations across traits and which fit data to a model? Which approaches focus on deleterious mutations or single mutations? Which make assumptions about the distributions of mutational effects? Most importantly, focus on the aspects that are most relevant to your own results that you will present later.

Try not to focus only on descriptive statements like, “Most previous studies examine the phenotypes that contribute to fitness by making large genetic perturbations (cite, cite cite).” Try to, once in a while, think deeper and have statements like, “previous studies that examine fitness-relevant phenotypes across diverse genetic backgrounds (cite cite) might tell us about X, but studies that examine phenotypes across more subtle perturbations will tell us about Y.

Overall, this section should be (1) shorter and (2) more inclusive. Add pareto-optimality and antigenic cartography? Add Brauer & Botstein Mol. Evol. Cell? Here are the paragraphs that you had in this section, plus some extra text and comments that might help. Don’t edit these paragraphs. Rethink based on the comments above and rewrite.

To contend with the complexity of phenotype space, a number of dimensional reduction approaches have been used in previous studies. A very common approach is PCA, or variants of PCA like X, Y, Z. These approaches calculate the correlation structure across traits that are measured to find a smaller number of orthogonal phenotypic components. They assume a linear correlation across traits and are useful when trying to understand correlations between traits in an unstratified data set.

A common framework used to model and understand phenotypic evolution that allows for non-linear relationships between traits is Fisher’s Geometric model (Fisher 1930) (see Fig 1A). In this model, traits are represented by orthogonal axes in a *D*-dimensional space, with the number of dimensions, *D,* indicating the number of traits that have independent contribution to fitness. Organisms are represented by points in this space, with their position determined by the organism’s particular combination of trait values. The fitness of an organism in a particular environment is determined by a function of the organism’s position and the position of the optimum combination of traits in that particular environment (hereafter “the optimum”). Thus, evolution can be seen as proceeding through “adaptive walks” from an ancestral organism that occupies some location in phenotype space. Genetic mutations can cause phenotypic changes, moving the mutant to a new location in this space. If this new location is closer to the optimum, then it may increase in frequency and give rise to additional mutations.

Using this general framework, estimating the number of independent traits represented by mutants is equivalent to estimating the number of dimensions in Fisher’s model. Previous work has aimed to measure the dimensionality of this space for mutations in general (and more specifically deleterious mutations), rather than the dimensionality of adaptation. These studies primarily fall into three categories. One class of methods derives the dimensionality of the space from the distribution of fitness effects (Martin & Lenormand 2006) (Fig 1B). Assuming the ancestor is at the optimum, a Gaussian mutation distribution in phenotype space, and a multivariate Gaussian distribution for the fitness function, Martin and Lenormand (Martin & Lenormand 2006) derive the expected distribution of fitness effects (DFE) taking the form of a gamma distribution. One can then derive the effective dimensionality of the system utilizing the moments of the DFE. Using directed mutation and mutation-accumulation techniques, these methods find that phenotypic dimensionality is relatively low (less than three dimensions) for the model organisms considered.

A second class of methods uses drift load (Poon & Otto 2000; Tenaillon et al. 2007). These also assume that populations are relatively close to the optimum, but instead explicitly use distance from an optimum as a metric to quantify the phenotypic dimensionality. Intuitively, this is accomplished by observing that large populations are able to effectively purge most deleterious mutations. However, small populations are unable to remove mildly deleterious mutations that drift to fixation, thus carrying a “drift load”. Moreover, the number of such deleterious mutations available to a population can be worked out as a function of the number of phenotypic dimensions, assuming some mutation distribution. Combining these relationships, this class of methods can then observe the fitness of populations across a gradient of effective population sizes to estimate the effective number of phenotypic dimensions. From this set of measurements, researchers found that there were relatively many dimensions (more than five) for the organisms observed.

Attempting to reconcile the different measures of phenotypic dimensionally observed by the two classes of methods, Lourenco(Lourenço et al. 2011) conducted analysis that suggests that DFE approaches are downwardly biased by universal pleiotropy assumptions, and that methods using drift load are relatively insensitive to the pleiotropy of mutations. Lourenco proposes a model of “partial pleiotropy” to explain the observed differences, suggesting that single mutations only affect a small subset of traits, but that mutations altogether affect many traits.

A third class of methods utilizes datasets generated to study epistasis (Fig 1C). Assuming a Gaussian mutation distribution around the ancestor and additive effects of mutations in phenotype space, epistasis is expected to be reflected in the nonlinearity of the phenotype to fitness map. Previous studies have used the distribution of angles between pairs of mutations (Weinreich & Knies 2013) and approximate Bayesian computation(Blanquart & Bataillon 2016) on epistasis datasets to understand phenotypic dimensionality.

OK, last we need a section explaining how our method differs from those you just described. The previous section should set this up nicely. Now all you need to say is “Here, we present a model that solves all the issues we just told you about”. But the “issues we just told you about” should be a full paragraph, maybe two, re-visiting the issues and how you solve them. End with a little summary of the results (I know that seems odd, but most introduction sections actually include the conclusion – ever heard this, “tell them what you are going to tell them, then tell them, then tell them what you told them?”).

strict mathematical assumptions to derive tractable mathematical expressions and predictions. Most (if not all) of these assumptions are likely to be violated. It is unlikely that the ancestor is optimally fit, since adaptation is constantly proceeding (Wiser et al. 2013). Moreover, assuming additivity of mutants in phenotype is likely to be wrong. This is particularly clear in the extreme example of pairs of loss of function mutations in the same pathway. Finally, a local Gaussian distribution for mutations in phenotypic space is also unlikely loss of function mutations can drastically change the expression of proteins and thus move a cell’s phenotype very far from the initial set of phenotypes. Additionally, there have been calls for the use of partial pleiotropy in these models to reconcile conflicting conclusions by some of these studies(Lourenço et al. 2011; Bataillon & Bailey 2014).

Something about infinitesimal model? + detectability?

One approach is to instead consider the problem from the other direction – measure changes in fitness across environments to understand which phenotypes are important to fitness in a given environment. There are clear problems with this approach when measuring fitness across very different environments: the environmental contribution to phenotype is likely to have a larger influence and different phenotypes are likely to have very different contributions in these various environments. Instead, consider subtle environmental changes. In such environments, rather than a complete change in the phenotypes important to fitness, there is instead a subtle shift in the relative importance of the phenotypes that influence fitness.

Recent technological advances allow us to measure the fitness of many mutants with high precision and in high-throughput (Levy et al. 2015). In particular, our approach develops the notion of *independent, fitness-relevant phenotypes*, that are affected by genetic variation and have significant and independent contributions to fitness, and uses precise fitness measurements across subtle environmental perturbations to identify these phenotypes (Fig. 1D). These phenotypes are not necessarily measurable traits in the traditional conceptualization of “phenotypes”, rather each fitness-relevant phenotype is likely influenced by complex combinations of measurable (and perhaps currently unmeasurable) features of that organism. We find that our method is able to accurately infer the number of phenotypes relevant to fitness on simulated data. Our ability to detect such phenotypes depends on both the magnitude of measurement error and the relative dispersal of mutants in phenotype space. [Furthermore, we show that we are able to detect particular combinations of mutants and conditions where behavior is inconsistent with the broader collection of mutations. Finally, we apply this method to fitness measurements of a yeast deletion collection, showing *something*.]

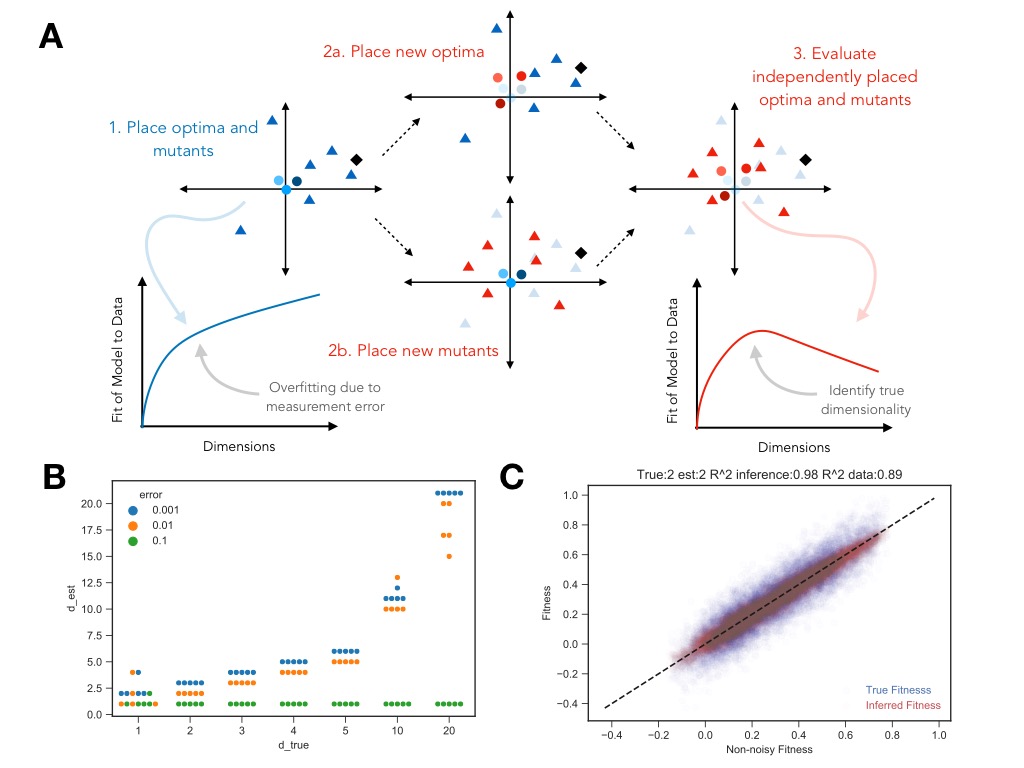
Do we focus on adaptation? All of your questions about this paper are coming back to me. If we focus on adaptation, we should include more on that in this last section of the introduction…but you can kind of go back and forth between this section and your results. Once they crystalize, so will the intro. Because of this evolutionary perspective on the importance of phenotype, identifying phenotype that have an effect on fitness in a given environment is an important area of study. Possibly back to evolution…how have things been shaped into modules such that mutations cannot affect some dimensions as easily as others. Lewontin (Lewontin 1974) further stratified these concepts in the light of natural selection and evolution: an organism’s “genotype” characterizes the information passed down to offspring, and an organism’s “phenotype” is the material that natural selection itself acts on. Our analysis re-defines phenotypes not as things we measure, but as things evolution shaped… Sorry, this is some nonsensical stuff. I was toying around with ending where we started, back on the definition of phenotypes.

**So what is this paper about? What is unique about our model? There are actually a lot of things, My list has things like, (1) we use both environment and genetic perturbations to understand phenotypic complexity (do others?), (2) we do not assume mutation distribution, (3) we study tiny perturbations (single mutations, batch effects), (4) we quantify how much power we have to detect phenotypes with smaller effects on fitness (anisotropy thing), (5) we (might) focus on adaptation. First, decide on the most important items in this list. Then, let them drive section 2 and 3 of the introduction.**

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**Figure 1. Fisher’s geometric model and approaches to infer dimensionality. A.** In Fisher’s geometric model, phenotypes are represented as orthogonal dimensions and mutations are represented as vectors in phenotype space. The fitness of each mutant is determined by its the distance from the origin, relative to the distance from the origin to the ancestral genotype (black diamond). Thus, everything the same distance away from the origin as the ancestor is neutral (on dashed line), and everything closer is adaptive. **B.** Estimating the number of dimensions using the distribution of fitness effects assumes a fixed mutation distribution and that the ancestor is optimally fit. **C.** Estimating the number of dimensions using epistasis data assumes a mutation distribution and also that the effect of mutations on phenotype is additive, i.e. the vector representing a double mutation is the sum of the vectors of the subsequent single mutations. **D.** Our approach. We use subtle environmental perturbations to triangulate mutants and conditions. Relative fitness measurements give us information about the distance between a mutant and the condition’s optimum, relative to the distance between the ancestor and that optimum.

**Results**

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**Figure 2. Cross validation scheme and simulation results. A.** A representation of the cross validation scheme. First, we place a “training” set of optima and mutants in each d-dimensional space. Here, the fit of the model to the data will continuously increase as we add more dimensions. Second, we hold this space constant and place new optima using the information of the mutants in the training set and separately place new mutants using the optima in the training set. Finally, we evaluate the predicted fitness of the “test” set, which consists of the new, independently-placed optima and mutants. The fit of the model with the test set should decrease at some critical number of dimensions, representing where overfitting of the training set occurred, and serving as an estimate for the true underlying dimensionality. **B.** Comparison of the estimated dimensionality and true dimensionality across various simulations with varying levels of error. **C.** Visualization of the fit of the model to true underlying simulated data compared to the “true” fitness with measurement error. Shown in red is the fit of the inferred fitness to the simulated data without noise (). Shown in blue is the fit of the true measured fitness with error to the simulated data without noise ().

The results sections all start with a sub-heading. This is critical real estate. Here are some vague sub headings: “Method” “Simulations”. Think more deeply about the heading.

Maybe a good first step is thinking about the sub headings. Here are some quick ideas for what they could be:

***A model that predicts the number of phenotypes contributing to fitness***

This section includes figure 1

***Our model makes accurate predictions about simulated data***

This section includes figure 2

***Our model makes accurate predictions about real data***

This section includes a figure about the batches

***We can detect fitness-components down to the limits of detection***

This section includes the current figure 3, about dispersion

***Non-subtle perturbations reveal the context-dependent mapping from phenotype to fitness***

This section includes a figure about weird uncles, possibly using simulated data or data from Yuping’s published paper. Maybe include the Hillenmyer data, comparing subtle to less subtle perturbations.

***A model that predicts the number of phenotypes contributing to fitness***

In order to break down the complexity of phenotypes, we imagine a group of single mutants for which we have measured their fitness in many subtly different environments. Each mutant has a phenotype defined by a particular combination of component traits, and a mutant’s fitness in a given environment is determined by the relationship between its component traits and the environment. This mapping from phenotype to fitness is environmentally dependent, with different combinations of traits and different weightings of these traits being more or less important in different environments. Thus, if we subtly change the environment that a mutant is in, then we can subtly change how well-suited each trait is to the environment, and, by extension, how well-adapted the mutant is to this new environment. Consequently, we can identify the number of fitness-relevant traits in the inverse manner, by using a mutant’s variation in fitness across a set of different environmental perturbations to identify how many phenotypes matter to fitness.

One key insight to this process is the use of subtle, rather than strong, environmental perturbations. Subtle perturbations allow us to model mutants as having fixed phenotypes, effectively removing any direct environmental impact on phenotype itself. Moreover, strong perturbations have the potential to reveal new phenotypes that were not relevant to the original environmental condition. Though potentially interesting, such phenotypes do not factor into fitness during the evolution of these strains in a particular condition, and thus, are not informative about the process of adaptation we wish to understand.

An analogous and geometric representation of this framework is Fisher’s Geometric Model (FGM) (Fisher 1930). In this model, organisms are represented as fixed points in a *D*-dimensional phenotype space, with each dimension represented traits that are independent with respect to their effect on fitness. Fitness in a given environment is a function of this phenotype space. A commonly used function is the Gaussian, where there exists an optimal combination of traits in that environment, and fitness decreases with the distance from this optimum. Different environments are represented by different fitness functions, and in the Gaussian case, by different locations of optima and/or different widths (Figure 1). In this geometric approach, identifying the number of fitness-relevant traits is equivalent to inferring the number of “dimensions” if Fisher’s representation. Moreover, subtle environmental changes are depicted by small changes in the locations of the optima for each condition, making our

To infer the number of dimensions represented by the adaptive mutants, we use two independent approaches. The first relies on an explicit use of Fisher’s model and

The first relies on singular-value decomposition (SVD) to represent fitness data of mutants across many conditions in a low-dimensional form. We select the num

The second approach explicitly steps from Fisher’s model and relies on

***Our model makes accurate predictions about simulated data***

Now you can say, whether we make assumption associated with SVD (eg a linear mapping onto fitness) or with FGM (fitness in a given environment is a Gaussian function) we predict the correct number of dimensions in simulated data (Fig 2BC).

*P1: short overview of simulations*

To test our method for estimating the number of phenotypes from fitness data, we perform a simulation study. First, we simulate data by placing an ancestor and mutants in a phenotype space. We generate fitness data for these mutants buy placing optima for many environments and calculating fitness based on the distances of these mutants from the optima.

[To test our method for estimating the phenotypic space from fitness data, we perform a simulation study. First, we simulate data that fits our phenotypic model and then feed the corresponding data into our method to infer the phenotype space and number of fitness-relevant phenotypes. For simplicity and consistency with previous literature, we start with a Gaussian function of distance. Perhaps use the following to justify why you perform the simulations in the way you do, rather than saying, ‘for simplicity’: Because we are interested in detecting the relevant phenotypic differences that lead to differences in fitness, a relevant fitness function must have the property that traits with no differences between organisms cannot factor into the relative fitness differences between these organisms. An exponential function of squared distance (of which the Gaussian is) is only class of functions with this property (see SI).]

*short overview of inference techniques*

We take two independent approaches to identify the number of fitness-relevant phenotypes. The first explicitly uses the framework of Fisher’s model and uses optimization techniques to solve for the best fitting phenotype space and dimensionality for the simulated fitness data. The second approach uses singular-value decomposition (SVD) to identify the number of factors represented in the fitness data. [do we need a figure for the two methods?]

*overfitting, inference procedure method comparison*

Both the SVD and FGM approaches face the common problem of overfitting –

*Signal/noise ratio*

Our inference procedure accurately estimates the correct number of dimensions (Fig. 2B). Generally, our estimates match the simulated true underlying dimensionality. In addition to our models estimating the true dimensionality of simulated data well, it is also able to accurately estimate the relative fitness of test data (Fig. 2C), fitting the underlying simulated data better than the noisy measurements passed into the model. This means that our cross validation scheme properly accounts for measurement error and finds the true underlying fitness values.

***Our model makes accurate predictions about real data***

*now that we know it’s important for noise to be low on simulated data, how low is our noise for rel data?*

*It’s low! (or not so much)*

Perhaps wait to work on this section until we are sure we will include it.

***We can detect fitness-components down to the limits of detection***

Measurement error limits our ability to detect particular fitness-relevant phenotypes (Fig 2B) – in the extreme case where measurement error is very high (\sigma\_m^2 = 0.1, corresponding to X% of the mean relative fitness value), our method is unable to detect more than one phenotype. This drives the question of how sensitive this method is to measurement error in general, and what aspects of data limit our ability to detect particular phenotypes.

The ability to detect the influence depends on the dispersion of mutants and optima in trait space, precision of measurement, and the combination of the two. If mutants and optima are less dispersed (mutants have similar phenotypes and conditions have subtle differences), then more precise measurements are needed (and vice versa). Because our model assumes that each trait contributes equally to fitness, traits that have lower contribution (in un-rescaled space) will also have the effect of lowering the dispersion of mutants for that trait in rescaled space. In particular, if mutants are uniformly distributed around the ancestor, but traits have varying levels of importance to fitness (Fig. 3A), then the re-scaling of the phenotype space in units of fitness will lower the dispersion of mutants. Similarly, if mutations change some traits more than others, this dispersal could also affect detectability (Fig. 3B).

We assess detectability by simulating cases where the true dimensionality is 3, but the third dimension has either reduced influence on fitness (Fig. 3D) or mutations affect this dimension less strongly (Fig. 3E). Both of these have a similar effect to the accuracy of inference: the less dispersed the third dimension is, the less detectable it is. This effect is exacerbated when measurement error is higher, indicating that the ability for our inference to detect a dimension depends on both measurement error and dispersal. [derive a measure for dispersal, show that this captures everything in Fig. 3F – 3C will be schematic for the measure if needed to explain clearly.]

*[need a section on the results of non-subtle perturbations?, possibly here?]*

***Non-subtle environmental perturbations reveal the context dependent mapping from phenotype to fitness***

*R3a. Identification of “peculiar” behavior of particular combinations of mutants + conditions (weird uncles)*

Our method for inferring the space inherently relies on how the collection of mutants behave over a collection of conditions. How well can we detect the behavior of particular mutants and conditions? That is, is our method only good for inferring the commonalities but not particular behavior, and if so, how can we know that (and which) particular behaviors are not picked up?

*Result 4: Application to Hillenmeyer data?*

**Discussion**

[awesome discussion of this paper + upcoming amazing paper on 1bigbatch]

**Methods**

We consider an explicit model of phenotypic evolution analogous to Fisher’s Geometric Model (Fisher 1930). Phenotypes are depicted as orthogonal axes in a *D*-dimensional space, with the number of dimensions, *D,* representing the number of traits possibly relevant in this space. Organisms are depicted as points in this *D*-dimensional space, with their position determined by the combination of phenotypes represented by that particular organism. An organism’s absolute fitness in a particular environment is determined by a function of its distance from an optimal phenotype.

Our implementation of the model makes some inherent assumptions about the fitness function that determines fitness in each environment. First, it assumes that each trait contributes independently to fitness in any given environment – this can be done by transforming the space if considering a single optimum (Martin & Lenormand 2006), but is not generally true if the interactions between traits differ between conditions. Second, each trait is rescaled such that they have equal effect on fitness in a given condition. We assume this scaling holds for all conditions. Furthermore, we assume that absolute fitness in a given environment is a Gaussian function of the distance between an organism’s location in phenotype space and the location of the optimum for that particular environment:

where is the height, is the variance, represents theth coordinate of theth mutant, represents the th coordinate of the th optimum. This function is typically assumed for many investigations of Fisher’s model [cite a bunch] due to analytical tractability. Because we are interested in detecting the relevant phenotypic differences that lead to differences in fitness, a relevant fitness function must have the property that traits with no differences between organisms cannot factor into the relative fitness differences between these organisms. An exponential function of squared distance (of which the Gaussian is) is only class of functions with this property (see SI).

Our model makes several assumptions about phenotype space and fitness in phenotype space. The main key assumption is that of subtle environmental perturbations – more specifically, this assumption assumes that mutants have a fixed location in phenotype space (we ignore any environmental contribution to phenotype) and is constant across environmental condition and dimension (ignoring any specific, correlated effect of phenotypes on fitness in a given environment).

*Simulation Methods*

To test our method for estimating the phenotypic space from fitness data, we perform a simulation study. First, we simulate data that fits our phenotypic model and then feed the corresponding data into our method to infer the phenotype space and number of fitness-relevant phenotypes. For simplicity and consistency with previous literature, we start with a Gaussian function of distance.

We use simulated data to test our method for estimating phenotype space from fitness data. First, we simulate data that fits our phenotypic model and feed this data into our method to infer the phenotype space. To do this, we use two independent simulation techniques. The first simulation technique is a classical Fisher’s Geometric Model simulation: an ancestor’s location is randomly selected in *D*-dimensional space. Mutations are generated according to a multivariate normal distribution, centered at the ancestor with covariance matrix . Locations of optima are uniformly sampled from the *D*-ball with radius *r*. Relative fitness for each mutant in each condition is calculated according to a Gaussian function, centered at the optimum with selection in each dimension according to covariance matrix (as in eqn. 1). Random Gaussian noise is added to these fitness values to account for measurement error.

The second technique simulates an *D*−dimensional phenotype space by randomly placing the ancestor on the surface of the n−ball of unit distance. Then, we randomly generate “mutants” by sampling uniformly within the n−ball, to simulate only adaptive mutations. Finally, we randomly sample locations for the optima for new conditions in a ball of smaller radius, to simulate subtle environmental perturbations. We now have a set of mutants and conditions, for which we can generate relative fitness data by calculating a fitness function on this phenotype space for each mutant in each condition.

*Estimation Methods*

To infer the phenotypic space of our model from a set of mutants, we use an optimization technique on the relative fitness values of the set of mutants across subtle environmental perturbations. For a given number of traits *D*, we aim to find the positions of mutants, optima, and the ancestor that best fits our relative fitness data. We do this by finding the parameters that minimize the function:

where ,, and represent the locations of the mutants, optima, and ancestor, respectively. is the measured relative fitness of mutant in condition with a measure of the measurement uncertainty (see SI).

[section on other dimensionality reduction and why we’re different? (in a conceptual way) – if convincing don’t need to put comparison in the methods? ] The primary difference between our approach and other “dimensionality reduction” techniques like Principle Component Analysis (PCA) and Multidimensional Scaling (MDS) is that these methods find a low-dimensional representation of data based on distances between objects in the same class (in this case the mutants). Instead, we use relative fitness, which is a measure of distance between 2 classes of objects (mutants and optima). [CITE] [why SVD is different – linear (and doesn’t have the nice properties discussed above), not scaled in terms of fitness which makes D=1 mean fitness]

We use a global optimization technique to find the parameter values minimize this score for each number of dimensions. To estimate the number of dimensions and avoid overfitting, we use a 5-fold bi-cross validation scheme (Fig. 2) analogous to that used for Singular Value Decomposition(Owen & Perry 2009). We divide our data into 5 distinct sets or “folds” (250 mutants into 5 groups of 50 and 50 conditions into 5 groups of 10). For each fold of the data, we exclude a set of mutants and conditions to use as a test set and first use the computational method to estimate the best space for the remaining set of data (See step 1 of Fig 2A.) for each value of D (the number of dimensions) we are interested in. If we were to only do this, we would expect the fit to continually increase as we increase the number of dimensions, because we will begin to fit measurement noise. Next, we fix this space and use only the locations of the original set of mutants as information to find the best location for the conditions in the test set (step 2a of Fig 2A), and separately, use the locations of the original set of conditions to find the best location for the mutants in the test set (step 2b). Finally, we evaluate the relative fitness values predicted from the estimated locations of the mutants and conditions in the test set that were placed independently (step 3). The model that has the best fit of the test set’s relative fitness values to the measured values is the correct number of dimensions that was not over-fit to the original data. This is repeated for each of the 5 test sets, and we pick the model with the best average predictive ability across all 5 folds.

[transition paragraph?]

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