Defining genetic interaction

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Sometimes mutations in two genes produce a phenotype that is surprising in light of each mutation's individual effects. This phenomenon, which defines genetic interaction, can reveal functional relationships between genes and pathways. For example, double mutants with surprisingly slow growth define synergistic interactions that can identify compensatory pathways or protein complexes. Recent studies have used four mathematically distinct definitions of genetic interaction (here termed Product, Additive, Log, and Min). Whether this choice holds practical consequences has not been clear, because the definitions yield identical results under some conditions. Here, we show that the choice among alternative definitions can have profound consequences. Although 52% of known synergistic genetic interactions in *Saccharomyces cerevisiae* were inferred according to the Min definition, we find that both Product and Log definitions (shown here to be practically equivalent) are better than Min for identifying functional relationships. Additionally, we show that the Additive and Log definitions, each commonly used in population genetics, lead to differing conclusions related to the selective advantages of sexual reproduction.

epistasis | fitness | gene function

Genetic interactions have long been studied in model organisms as a means of identifying functional relationships among genes or their corresponding gene products, with the nature of these relationships depending on the types of interactions (1–3). Additionally, the extent and nature of genetic interaction are important to theoretical explanations for the selective advantage of sexual reproduction and recombination (4–7). The study of genetic interaction has become increasingly systematic and large-scale, especially in the yeast *Saccharomyces cerevisiae* (6, 8–21). This provides an opportunity to examine properties of different quantitative definitions of genetic interaction and their impact on biological interpretation.

A quantitative genetic interaction definition has two components: a quantitative phenotypic measure and a neutrality function that predicts the phenotype of an organism carrying two noninteracting mutations. Interaction is then defined by deviation of a double-mutant organism's phenotype from the expected neutral phenotype. A double mutant with a more extreme phenotype than expected defines a synergistic (or synthetic) interaction between the corresponding mutations (synthetic lethality, in the extreme case). Alleviating or "diminishing returns" interactions, in which the double-mutant phenotype is less severe than expected, often result when gene products operate in concert or in series within the same pathway. Alleviating interactions arise, for example, when a mutation in one gene impairs the function of a whole pathway, thereby masking the consequence of mutations in additional members of that pathway.

One class of phenotype, fitness, has been central to many large-scale genetic interaction studies. Although fitness was originally measured in terms of population allele frequencies (1, 22, 23), it can also be measured by using growth rates of isogenic microbial cultures. Genetic interaction studies have used different measures of fitness, including: (*i*) the exponential growth rate of the mutant strain relative to that of wild type (4, 9, 15, 19) (the relative-growth-rate measure); (*ii*) the increase in mutant population relative to wild type in one wild-type generation (the relative-population measure) (6); and (*iii*) the number of progeny per mutant organism relative to the number of progeny for wild type in one wild-type generation (the relative-progeny measure) (24). (See supporting information (SI) *Text* for details on these fitness measures.)

Genetic interaction studies have also differed in their choice of neutrality functions, generally using either a multiplicative or a minimum mathematical function. The multiplicative function, which was originally applied to fitness measures defined in terms of allele frequencies, predicts double-mutant fitness to be the product of the corresponding single-mutant fitness values. The multiplicative function can be combined with each of the three fitness measures above to yield three distinct definitions of genetic interaction (4, 6, 15, 19, 24).

A fourth (Min) definition of genetic interaction results from the minimum neutrality function, under which noninteracting mutations are expected to yield the fitness of the less-fit single mutant. Each fitness measure above yields an identical set of genetic interactions under this function. A hypothetical example illustrates one rationale for the Min definition: Two single mutations each disrupt a distinct cellular pathway that limits cell growth, such that one of these mutations is substantially more limiting than the other. The double mutant might then be expected to exhibit the phenotype of the most-limiting single mutant. Five studies using the Min definition (8, 12, 13, 17, 25) have provided the sole source of support for most (52%) of the 13,901 *S. cerevisiae* synthetic genetic interactions in the BioGRID database (ref. 26; www.biogrid.org).

A direct comparison of the three multiplicative definitions is complicated by the fact that each predicts double-mutant fitness on a different fitness scale. To enable a comparison, each definition can be transformed to use the same relative-growth-rate fitness measure, without altering the resulting set of interactions, by appropriately modifying the neutrality function. Specifically, this is accomplished for definitions that use relative-population and relative-progeny fitness measures by replacing the multiplicative neutrality function with neutrality functions that have additive and logarithmic forms, respectively (see *SI Text*). We will refer to the definitions corresponding to relative-growth-rate, relative-population, and relative-progeny-fitness measures as Product, Additive, and Log. We note that the Additive definition has been used in this form (9), and that other definitions have been applied beyond the four assessed here (see *SI Text*).

It has not been clear whether the choice of genetic interaction definition has any practical consequences. Experiments in *Escherichia coli* (4) produced results for independent mutations that were consistent with both Product and Additive definitions. Similarly, experimental studies in yeast (9) showed results for independent

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Table 1. Gene pairs tested for genetic interaction

	Overlap in number of gene pairs tested			
Study	Study J	Study T	Study P	Study S
Study J (6)	649*	45	56	0
Study T (12)	45	≈747,000*	≈103,000	189
Study P (17)	56	≈103,000	≈348,000*	280
Study S (19)	0	189	280	325*

The number of gene pairs tested by both studies in each pair is shown in off-diagonal values.

*The diagonal shows the number of gene pairs tested systematically by each study considered here.

small fitness-effect mutations that were consistent with the Additive definition but did not rule out other definitions. Indeed, Min, Product, Log, and Additive definitions each yield identical sets of genetic interactions when either mutation alone has wild-type fitness.

To evaluate the impact of definition choice, we applied each of the four definitions in turn to two reference studies, St. Onge *et al.* (19) (Study S) and Jasnos and Korona (6) (Study J), both providing quantitative growth-rate measurements of isogenic wild-type and single- and double-mutant cell populations. Here, we show that the choice of definition can dramatically alter the resulting set of genetic interactions and the extent to which they correspond to shared gene function. Additionally, we show that definition choice may impact previous conclusions related to the selective advantage of sexual reproduction (6).

Results

Assembly of Genetic Interactions and Functional Relationships for Comparison. We examined four systematic studies of genetic interaction. Table 1 summarizes the number of gene pairs examined systematically by each study and the overlap in tested gene pairs between each pair of studies.

The first study, Study J, sought to obtain an unbiased sample of quantitative genetic interactions among deleterious mutations. Among 758 genes with deletions resulting in slow rates of growth (6), the authors randomly sampled 639 gene pairs for measurement of genetic interaction. The second study, Study T, systematically tested 159 "query" genes involved in DNA repair and other specific biological functions (12). Each query mutation was combined with each of ≈4,700 nonessential gene deletions using the synthetic genetic array method, and the presence of synthetic sick or lethal interaction was reported for ≈4,000 gene pairs. The third study, Study P, used the dSLAM method (17, 25) to examine mutations in 74 query genes (involved in DNA replication, checkpoint signaling, and oxidative stress response) (17). Each query mutation was combined with ≈4,700 gene deletions and a competitive growth assay was used to identify ≈4,900 interactions. Synthetic interactions fell into four grades of increasing severity: "SF-slight," "SF," "SL/SF," and "SL". Here, SF indicates synthetic fitness defect and SL indicates synthetic lethality. To ensure that each subset had a sufficient number of interactions, we grouped SF and SF-slight interactions (Study P_{slight}) and SL and SL/SF interactions (Study P_{severe}) in our analyses. The fourth study, Study S, examined a detailed time course of growth for all single and double mutants corresponding to 26 genes related to DNA repair, in the presence and absence of the DNA-damaging agent methyl methanesulfonate (MMS). Each gene pair was classified as being either genetically noninteracting, synergistic (corresponding to synthetic lethality or synthetic fitness defect), or one of five different types of alleviating interaction (19). Among gene pairs examined in Study S, we also identified functional relationships (i.e., gene pairs for which both genes are involved in the same specific cellular process) (see *Methods*).

Both Study J and Study S reported quantitative measurements for the fitness phenotypes of the mutants they studied. This allowed us to examine alternative genetic interaction definitions. By changing only a single analysis variable, genetic interaction definition, we were thus able to evaluate which definition generates genetic interactions that best correspond to known functional relationships. Furthermore, ≈58% and ≈86% of the gene pairs tested in Study S were also tested in Study T and Study P, respectively (see Table 1), allowing us to assess overlap between these studies.

Neutrality Functions That Place Alternative Definitions on a Common Fitness Scale. To place each definition on a common scale, we hereafter use only the relative-growth-rate fitness measure, combining this fitness measure with different neutrality functions in turn. For a gene pair (x, y), we refer to the fitness of the two single mutants and the double mutant, respectively, as W_x, W_y, and W_{xy}. The neutrality function $E(W_{xy})$, predicting double-mutant fitness for a strain with mutations in noninteracting genes x and y, is defined differently under the Min, Product, Log, and Additive definitions (see *Methods*). A quantitative measure of interaction under each definition is ε, the deviation of observed double-mutant fitness from expectation, i.e., $\varepsilon = W_{xy} - E(W_{xy})$ (1, 15, 16, 19).

Properties Expected of an Ideal Definition for Identifying Functional Relationships. Gene function can be defined at multiple levels of specificity (27). By definition, there are few genes that hold any given specific function, and gene pairs sharing a specific function should then also be rare. Therefore, if interaction (either synthetic or alleviating) is to be an ideal indicator of specific functional relationships, the vast majority of gene pairs should be noninteracting. An ideal definition for interaction should then yield a distribution of observed double-mutant fitness values that closely approximates the expected distribution over most gene pairs. Under an ideal definition, the quantitative measure of interaction ε would then have a tight distribution (indicating low dispersion) that is centered on zero (indicating low bias). This statement requires the use of bias and dispersion measures that are insensitive to the presence of a small "contaminating" fraction of functionally related gene pairs that should deviate from $\varepsilon = 0$.

The Choice of Genetic Interaction Definition Matters. As noted above, Study J examined an unbiased collection of pairs, requiring only that each single mutant be deleterious. In Fig. 1, we plot ε for Study J pairs under each of the four definitions, excluding pairs for which either the single- or double-mutant replicate measurements had a coefficient of variation (standard deviation relative to the mean) >15% (SI Fig. 3). We also excluded pairs for which either single mutation was advantageous; because these cases were incongruous with reported deleterious effects that were initially used to choose the genes (6) and also to restrict our attention to definitions of genetic interaction among mutations that are deleterious as single mutants. This yielded a set of 296 gene pairs. In SI Table 2, we report the median (±standard error of the median) as a measure of central tendency (i.e., bias), and median absolute deviation as a measure of dispersion for ε values underlying the distributions in Fig. 1. These measures are robust to outliers and thus robust to modest contamination of the distribution with functionally related gene pairs. As shown in Fig. 1*A* and SI Table 2, the distribution of ε_{Min} for the 296 gene pairs is negatively biased, whereas $\varepsilon_{Product}$, ε_{Log}, and $\varepsilon_{Additive}$ are each positively biased. Pairwise Kolmogorov–Smirnov tests between the definitions showed that ε_{Min} distribution is significantly different from the $\varepsilon_{Product}$, ε_{Log}, and $\varepsilon_{Additive}$ (see SI Table 3), demonstrating that the choice of genetic interaction definition can significantly impact ε.

The Difference Between Definitions Depends on Single-Mutant Fitness Effects. Differences in the ε distribution for each definition depend highly on single-mutant fitness effects. All definitions exhibit statistically indistinguishable ε distributions (see SI Table 3) when both

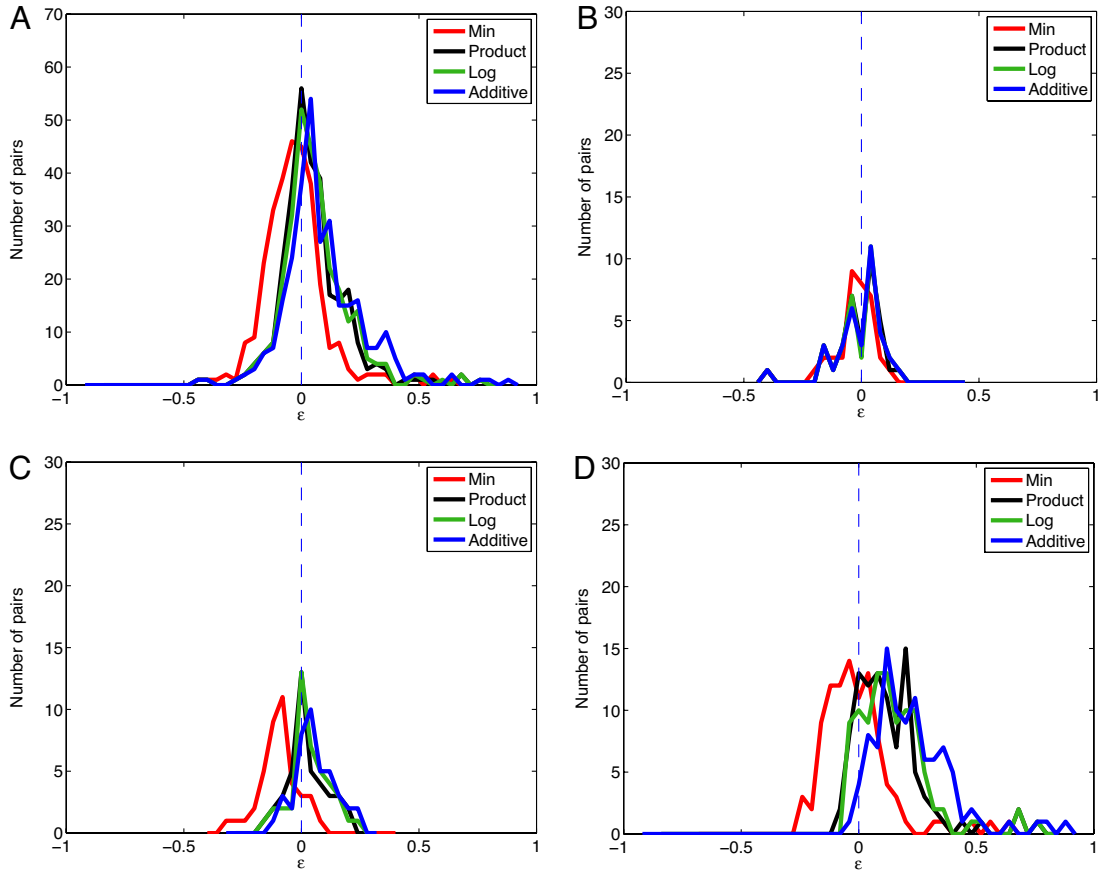

Fig. 1. Different definitions of genetic interaction lead to different distributions of ε (the deviation of the observed double-mutant fitness from expectation). (*A*) Distributions from all reproducibly measured pairs from Study J that involve genes with singly deleterious mutations show the Min definition to have a negative bias and clear differences from other definitions. (*B*) The subset of pairs from *A* involving genes with minor fitness effects shows no significant differences between definitions. (*C*) The subset of pairs from *A* involving genes with moderate fitness effects shows Min to have the most severe bias in ε. (*D*) The subset of pairs from *A* involving at least one extreme fitness defect exhibits a positive shift in bias in ε for definitions.

single mutants have only minor fitness defects (fitness >90% of wild type; Fig. 1*B*), confirming the expectation that the choice of definition matters little under these circumstances.

In contrast, when both single mutants are moderately deleterious (75% < fitness <90%; Fig. 1*C*), the distribution of ε_{Min} among these gene pairs is distinguishable from $\varepsilon_{\text{Product}}$, ε_{Log}, and $\varepsilon_{\text{Additive}}$ distributions (SI Table 3). To determine which definition yields ε values that are closest to 0, we examined distributions of $|\varepsilon|$, the absolute value of ε (see SI Fig. 4*C* and SI Tables 3 and 4). The $|\varepsilon_{\text{Min}}|$ distribution was the furthest from 0 (most biased). Thus, when single-mutant fitness effects are moderate, the Min definition conforms least well to the expectation that genetic interaction should be rare.

When at least one of the single mutants has extreme fitness defects (fitness <75%) and the other is at least moderately deleterious (fitness <90%, Fig. 1*D*), the ε distributions corresponding to different definitions were also highly dissimilar and statistically distinct from one another (SI Table 3). However, in contrast to the results for moderate single-mutant fitness defects, here the Min definition yielded the least bias and dispersion. The Product and Log definitions showed an intermediate positive bias, whereas the Additive definition showed an extreme positive bias. An analysis of $|\varepsilon|$ distributions showed $|\varepsilon_{\text{Product}}|$ and $|\varepsilon_{\text{Log}}|$ distributions to be indistinguishable, whereas all other pairwise comparisons of $|\varepsilon|$ distributions showed significant differences (see SI Fig. 4*d* and SI Tables 3 and 4). These results further illustrate the impact of definition choice.

The positive shift in bias among pairs with extreme single-mutant fitness defects (relative to pairs with moderate single-mutant fitness defects), observed for every definition, was unexpected. We investigated this phenomenon further by performing a similar analysis of bias and dispersion in ε and $|\varepsilon|$ on an independent study, Study S (see SI Figs. 5–8 and SI Tables 5–8). A potential caveat of Study S for this purpose was that it focused on genes involved in DNA repair, and thus is likely to be enriched for genes sharing functional relationships. Study S measured fitness both in the absence and presence of MMS, so that measures of genetic interaction were examined under both conditions. Results from Studies S and J were similar, with a few exceptions. Study S differed from Study J in that for pairs with extreme fitness defects (which are seen only in the presence of MMS), the Product and Log definitions exhibited the lowest bias and dispersion and thus most closely conformed to the expectation that most gene pairs are noninteracting (see SI Fig. 6*c*). A positive shift in ε among gene pairs with extreme single-mutant growth defects (relative to gene pairs with moderate single-mutant growth defects) was observed for all definitions but was considerably smaller for Study S than for Study J for each definition. In *Discussion*, we suggest an artifactual explanation for this phenomenon and discuss differences in the experimental design of Studies J and S that could explain its more pronounced impact on Study J. For the ensuing analyses, we focus on pairs of genes with more moderate fitness defects and on Study S when considering pairs with extreme fitness defects. For these pairs, Product and Log definitions exhibited the least bias and dispersion.

Additive and Log Definitions Demonstrate Different Biases. We sought to determine whether the Additive and Log definitions,

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which predominate in population genetics contexts, have practical differences. Previously published analysis of Study J concluded that quantitative interactions have a significantly positive mean, but this analysis was based only on the Additive definition.** We confirmed this result using all pairs and the 296 pair subset considered here ($\varepsilon_{\text{Additive}}$: mean = 0.092; standard error = 0.0099; $P_{t\ \text{test}} < 10^{-300}$). Under the Log definition, the mean is smaller but still shows significantly positive bias (ε_{Log}: mean = 0.058; standard error = 0.0085; $P_{t\ \text{test}} = 5.3 \times 10^{-11}$). However, we had observed that interaction strength had a significant positive bias (under all definitions) for pairs involving mutations with extreme fitness effects. If such pairs are removed from consideration, $\varepsilon_{\text{Additive}}$ still exhibits significant positive bias (mean = 0.027; standard error = 0.0089; $P_{t\ \text{test}} = 0.0025$); however, the bias of ε_{Log} is no longer significantly positive (mean = 0.016; standard error = 0.0087; $P_{t\ \text{test}} = 0.074$). Thus, the Additive and Log definitions are not practically equivalent in conclusions related to the adaptive value of sex and recombination.

Product and Log Definitions Are Equivalent for Deleterious Mutations. In each of several analyses (shown in Fig. 1, SI Figs. 4–8, and SI Tables 2–8), we noted that the Product and Log definitions cannot be statistically distinguished. To examine this observation further, we considered theoretical plots of the difference between $\varepsilon_{\text{Product}}$ and ε_{Log} for the entire deleterious range of W_x and W_y ($0 \leq W \leq 1$) (see SI Figs. 9*d* and 10). These plots show that the $\varepsilon_{\text{Product}}$ and ε_{Log} differ by 0.02 at most over the entire possible range of deleterious single-mutant effects. Thus, although mathematically distinct, Product and Log definitions are numerically practically identical for deleterious mutations. As a result, we consider the Product but not the Log definition in further analyses.

The Product Definition Reveals Functional Relationships Missed by the Min Definition. Although Studies T and P did not define or assess alleviating interactions, Study S reported that the majority of alleviating interactions (under the Product definition and based on growth in the presence of MMS) have a highly specific shared function (see *Methods* for definition of shared function). We repeated this analysis (based on growth in the absence of MMS) for Min, Additive, and Product definitions. The majority of Study S alleviating interactions, as defined under both the Additive and Product definitions, shared a specific function (16 of 26 and 11 of 18, respectively). By contrast, gene pairs defined as alleviating under the Min definition showed little enrichment for functional relationships (one of six). Alleviating interactions under the Additive and Product definitions each showed a significantly greater proportion of functional relationships than that observed under the Min definition ($P = 0.033$ and $P = 0.028$, respectively; exact one-sided binomial test). Furthermore, of the 12 pairs that were noninteracting under the Min definition but interacting under either Additive or Product definitions, 10 (83%) had a functional relationship. This rate is significantly higher than expected ($P = 3.5 \times 10^{-9}$) given the total of only 35 functional links among all 323 pairs tested in Study S. No enrichment for functional links was observed among gene pairs in Study S defined as noninteracting under either the Additive or Product definitions. Thus, noninteraction specific to the Min definition is a strikingly positive predictor of a functional relationship, opposite to the behavior desired of a genetic interaction definition.

Study S Confirms Previous Studies When the Min Definition Is Applied. Synthetic interactions were derived from Study S using both Product and Min definitions in turn (see *Methods*). Because Studies T and P each derived interactions using the Min definition, we first assessed the fraction of interactions that could be confirmed by Study S under the same Min definition (Study S_{Min}).

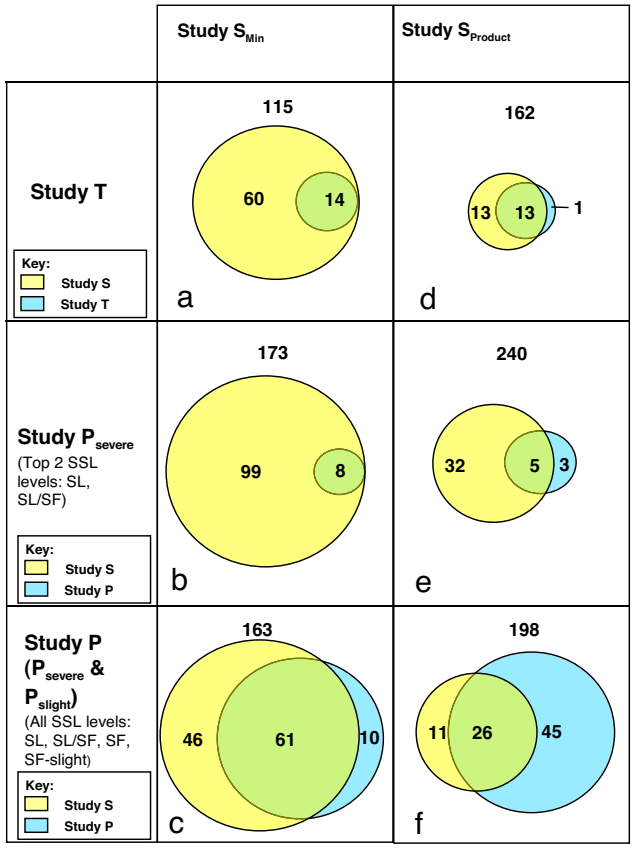

Fig. 2. Comparison of Study S synthetic interactions with those of Study T and Study P. *a–f* contain a Venn diagram characterizing agreement between two sets of interactions, with the sum of numbers equaling the number of pairs tested in both compared studies. *a–c* compare synthetic genetic interactions derived from Study S using the Min definition (Study S_{Min}) with those of *a* Study T (12); (*b*) Study P_{severe} (17) (Study P considering only the two more severe synthetic interaction levels) and (*c*) Study P (17) (interactions at all levels of severity). Note that all 14 interactions of Study T and all eight of the Study P_{severe} interactions were confirmed, confirmation rates of 100% with a 95% C.I. of 76.7–100% and 63.1–100%, respectively. Furthermore, 53 of the 63 Study P_{slight} interactions were also identified by Study S_{Min} (confirmation rate 84.2% with a 95% C.I. of 72.7–92.1%). *d–f* compare synthetic interactions derived from Study S using the Product definition (Study S_{Product}) with those derived from (*d*) Study T, (*e*) Study P_{severe}, and (*f*) Study P. In these comparisons, 13 of 14 Study T interactions were confirmed by Study S_{Product} (confirmation rate 92.9%, with a 95% C.I. of 66.1–99.8%). By contrast, five of eight Study P_{severe} were confirmed (confirmation rate 62.5%, with a 95% C.I. of 24.5–91.5%), and only 21 of the 63 Study P_{slight} synthetic interactions were confirmed (confirmation rate 33.3%, with a 95% C.I. of 22–46.3%).

As shown in Fig. 2 *a–c*, Study S_{Min} interactions largely confirm interactions from both Studies T and P despite differences among these studies in terms of both double-mutant construction and growth-measurement techniques. The Min definition applied to Study S identified many additional synthetic interactions that were not identified in Study T or Study P (60 and 46, respectively). This could be due to the high resolution of growth measurements in Study S and a resulting increase in sensitivity to small differences in fitness between single and double mutants.

Genetic Interaction Networks from Min and Product Definitions Differ Greatly. We next derived interactions from Study S using the Product definition (Study S_{Product}). Study T had 13 of its 14 interactions confirmed by Study S_{Product}, whereas the rate of interaction confirmation for Study P was considerably lower (Fig. 2 *d–f*).

**Note that ε as defined in Study J differs from our Additive ε definition by a scaling factor. The same conclusions are reached using the definition of a ε from Study J.

This was primarily because only 21 of the 63 Study P_{slight} were confirmed. Thus, those favoring the Product definition may wish to exclude Study P_{slight} interactions altogether. Removal of the Study P_{slight} category (containing 51% of Study P interactions) would have a substantial impact and would call into question a previous conclusion that Study P demonstrated heightened sensitivity over previous studies (17). Thus, it is apparent that the network of interactions derived from large-scale studies hinges on the definition of interaction.

Why were Study T interactions confirmed by $S_{Product}$ more frequently than those of Study P_{slight}? Study T may have used a more conservative criterion when scoring a double mutant as less fit than the minimum fitness of the single mutants. The values of $\varepsilon_{Product}$ for the 13 interactions found in both Studies T and $S_{Product}$ had large deviations from zero (see SI Fig. 11*a*), supporting this idea. That Study P reported 92% more interactions than Study T among pairs tested in both studies (see SI Fig. 12) is also consistent with this idea. Furthermore, the decrease in confirmation rate in Study $S_{Product}$ for Study P_{slight} interactions is clearly explained by the change in interaction definition. The $\varepsilon_{Product}$ values of the unconfirmed Study P_{slight} interactions clearly cluster about the noninteracting region under the Product definition, i.e., where $W_{xy} = W_x \cdot W_y$ (SI Fig. 11*c*). A majority of these interactions were confirmed when growth data were interpreted by using the Min rather than the Product definition of interaction.

Discussion

The Min, Product, Log, and Additive definitions of genetic interaction provide different predictions for the fitness of a strain harboring a combination of mutations in functionally unrelated genes. Although the definitions agree under certain circumstances, they often diverge dramatically. We have demonstrated both theoretically and empirically that differences among definitions can have profound effects on the interpretation and conclusions of large-scale genetic interaction studies.

Which definition for genetic interaction (or equivalently, noninteraction) is the most appropriate? If one wishes genetic interaction to indicate close functional relationships between genes and makes the reasonable assumption that most gene pairs do not have a close functional relationship, then it follows that most gene pairs should not interact, and thus that most double-mutant fitness values will conform to the neutral expectation. Study J (6) was best suited for assessing this question, given that it did not focus on any function in particular but rather chose random pairs among genes with deleterious single-mutant effects. We examined the distribution of ε, the deviation of the expected double-mutant phenotype from the observed double mutant phenotype, and found the Product and Log definitions to be closest to this ideal in general. Additionally, we showed that the Log and Product definitions are practically equivalent when both single mutants are deleterious.

For pairs of genes in Study J with more extreme single-mutant defects, the Min definition came closest to the idealized distribution (Fig. 1*D*). For this collection of gene pairs and for each definition, ε was shifted in the positive direction with respect to gene pairs with more moderate single-mutant defects. Thus, there was a tendency for doubly mutant strains to be more fit than expected, when the expected fitness defect is extreme. Pairs for this range of single-mutant effects were also examined for Study S in the presence of MMS, and the positive shift in ε was much smaller for each definition. For example, the positive shift under the Product definition was 0.029 vs. 0.097 for Studies S and J, respectively. Furthermore, the Study S analysis showed ε distributions to be more ideal under the Product definition than under either Min or Additive definitions for all sets of gene pairs with distinguishable ε distributions (see SI Figs. 5–8 and SI Tables 5–8). The more extreme positive shift in ε for Study J (relative to Study S) under every definition of genetic interaction may be explained by compensatory mutations that have arisen more frequently in Study J strains (see *SI Text* for a more detailed discussion). Thus, we place more reliance on conclusions from pairs involving moderate single-mutant defects.

To evaluate the practical consequences of definition choice, we also compared synthetic interactions from Studies T and P (which used the Min definition) with those identified in Study S under both Min and Product definitions (Fig. 2). We found that Study S_{Min} largely confirmed Study T and P interactions, indicating that the data underlying Studies T and P were consistent. In contrast, changing a single variable of the analysis of Study S, genetic interaction definition, was enough to introduce substantial disagreement with Study P, and particularly the weaker Study P_{slight} synthetic interactions. In particular, we found that the Product definition rejected ≈66% of these weak interactions (95% C.I. of 53.7–78%). This large rejection rate demonstrates that the choice of genetic interaction definition can substantially affect the classification of gene pairs. Indeed, definition choice could strongly impact the totality of our knowledge of *S. cerevisiae* genetic interaction, given that 52% (of 13,901 total) synthetic interactions are supported solely by studies using the Min definition (8, 12, 13, 17, 25).

The Min definition is clearly not ideal for defining alleviating interactions. "Masking" interactions, which for other definitions can be defined by a double-mutant phenotype that equals the phenotype of the single mutant with the most severe phenotype (19), must be classified as neutral under the Min definition. Among the 35 functionally linked pairs in Study S, six were found to be masking interactions by the Product definition but were classified as noninteracting by the Min definition. Thus, the Min definition will miss functional links associated with masking interactions, and these are estimated to be 17% (95% C.I.: 6.5–33.7%) of all functional links. Another subtype of alleviating interaction, "co-equal" interaction, is defined by single- and double-mutant strains that exhibit fitness values that are indistinguishable from one another (19). Although the Min definition would define such pairs as noninteracting, 9 of 10 such pairs observed in Study S corresponded to reported protein interactions (19). The classification of these categories as neutral explains why pairs that are noninteracting in Study S_{Min} (but not $S_{Product}$ or $S_{Additive}$) are highly enriched for functionally linked pairs ($P = 3.5 \times 10^{-9}$). Thus, the Min definition of genetic interaction is not optimal for identifying specific functional relationships. Note that this conclusion holds for the Min neutrality function in combination with any fitness measure that is monotonically related to growth rate.

A major value of synthetic interactions is that they reflect compensatory pathways (2). A qualitative comparison of Study S interactions found interactions defined only under the Min definition to be inconsistent with known biochemical relationships. Specifically, synthetic interactions involving the gene pairs *MMS4-RAD52*, *MMS4-RAD57*, *MUS81-RAD54*, and *MUS81-RAD55* are seemingly at odds with the role of the Mms4–Mus81 complex in resolving DNA intermediates downstream of homologous-recombination repair (28, 29). These interactions would be expected *a priori* to be alleviating, given that the importance of resolving repair intermediates should be diminished in the absence of genes responsible for the production of those intermediates. Whether the Min definition is generally less specific in defining compensatory gene pairs or rather more sensitive than the Product definition remains unresolved. In either case, it is clear that the choice of definition matters.

The Additive and Product definitions produced broadly similar results when applied both to Studies S and J except where one mutant had an extreme fitness defect and the other had at least a moderate defect. In such cases, the Additive definition (while being statistically distinct from the Product definition) was less ideal than the Product definition in that it resulted in a larger positive bias in ε.

Another major difference between the Product and Additive definitions is observed for gene pairs with a sum of single-mutant

fitness values that is <1. For such pairs, the Additive definition predicts the combination of two noninteracting mutations to yield a negative fitness, i.e., to have death rates that are greater than birth rates. For measures of growth rate that do not measure death rates (e.g., growth rates derived directly from observed doubling times of individual cells), the Additive definition will classify any double mutant for which the corresponding single-mutant fitness values sum to <1 as alleviating (positive ε), so long as the double mutant grows. Although neither Studies J nor S in the absence of MMS reported pairs for which the sum of single-mutant fitness values was <1, Study S in the presence of MMS yielded 35 pairs with this property. Every one of these pairs had measurable growth rates (with a mean of 0.197). Although the Min and Product definitions respectively classify 0 and 8 of these pairs as alleviating interactions, the Additive definition classifies all 35 as alleviating (the bias of $\varepsilon_{\text{Additive}}$ for these 35 pairs alone was 0.364). Thus, the Additive definition produces systematically biased ε values for a substantial subset of gene pairs, a characteristic suffered by neither Product nor Min definitions.

Both Log and Additive definitions have been used in population genetics studies that aim to understand the theoretical advantages of sexual reproduction (4–7). In particular, Study J concluded that genetic interaction had a significantly positive bias using a close variant of the Additive definition**. Our analysis of Study J data shows that the Log definition showed a smaller yet still significant positive bias (see *Results*). However, when we excluded pairs involving mutations with extreme fitness defects (because of the potential artifact discussed in *SI Text*), conclusions differed between the Additive and Log definitions: Although the Additive definition showed a significantly positive bias, the Log definition did not (see *Results*). Thus, the choice of definition alters conclusions relevant to the adaptive value of sex and recombination. Fitness measures based on growth rates of isogenic cultures can be mapped to fitness measures based on allele frequencies within a population (22), so that the choice of definition should also be of great concern to population geneticists.

We conclude that the definitions of genetic interactions used in recent large-scale genetic interaction studies diverge both mathematically and practically. Although we cannot yet rule out the Min definition as a viable alternative under some circumstances, multiple lines of evidence favor the Product or Log definitions of genetic interaction for studies seeking to identify functional relationships. Furthermore, differences we have shown between the Additive and Log definitions motivate further study of the most appropriate definition for population genetics studies. Last, we recommend that future studies report quantitative growth measures to allow reclassification of interaction under any definition pending a broader agreement on these fundamental questions.

Methods

Scoring Genetic Interactions. Under each definition, a genetic interaction was assigned to a pair of genes (x, y) if W_{xy}, the fitness phenotype of the double mutant, was significantly different from $E(W_{xy})$, the fitness phenotype of the double mutant that would be expected if x and y were noninteracting. When the relative-growth-rate fitness measure is used, the phenotype $E(W_{xy})$ predicted for a strain mutated in genes x and y under the appropriate neutrality function of each definition is $min(W_x, W_y)$, $(W_x \cdot W_y)$, $\log_2[(2^{Wx} - 1)(2^{Wy} - 1) + 1]$, and $(W_x + W_y - 1)$, for the Min, Product, Log, and Additive definitions, respectively. For each gene pair, the difference between the means of W_{xy} and $E(W_{xy})$ was assessed by using a Z test ($\alpha = 0.01$). Means and estimated measurement errors of W_x, W_y, and W_{xy} were derived from replicate growth experiments as described in ref. 19. Interactions were classified as synergistic if $W_{xy} < E(W_{xy})$ and alleviating if $W_{xy} > E(W_{xy})$.

Defining Shared Function or Functional Links. Functional links among genes were defined by using a general approach applied to a variety of large-scale studies (8, 12, 19). Briefly, a set of specific functions was defined by the set of terms in the Biological Process branch of the Gene Ontology (27) vocabulary assigned to ≤30 genes. We considered two genes to have a specific functional relationship (or ''link'') if any given specific function was held by both genes.

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Note Added in Proof. A positive shift in ε bias similar to that observed here for all definitions for pairs involving extreme defects was previously observed for the Product definition using an independent *E. coli* dataset (30).

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