Distribution of phenotype sizes in sequence-to-structure genotype-phenotype maps

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Abstract

An essential quantity to ensure evolvability of populations is the navigability of the genotype space. Navigability relies on the existence of sufficiently large genotype networks, that is ensembles of sequences with the same phenotype that guarantee an efficient random drift through sequence space. The number of sequences compatible with a given structure (e.g. the number of RNA sequences folding into a particular secondary structure, or the number of DNA sequences coding for the same protein structure) is astronomically large in all functional molecules investigated. However, an exhaustive experimental or computational study of all RNA folds or all protein structures becomes impossible even for moderately long sequences. Here, we analytically derive the distribution of phenotype sizes for a hierarchy of models which successively incorporate features of increasingly
realistic sequence-to-structure genotype-phenotype maps. The main feature of these models relies on the characterization of each phenotype through a prototypical sequence whose sites admit a variable fraction of letters of the alphabet. Our models interpolate between two limit distributions: a power-law distribution, when the ordering of sites in the prototypical sequence is strongly constrained, and a lognormal distribution, as suggested for RNA, when different orderings of the same set of sites yield different phenotypes. Our main result is the qualitative and quantitative identification of those features of the sequence-to-structure map that lead to different distributions of phenotype sizes.

Keywords: genotype-phenotype map, neutrality, RNA, phenotype size, evolution

1 Introduction

How genotypes map into phenotypes counts amongst the most essential questions to understand how evolutionary innovations might come about and how evolutionarily stable strategies are fixed in populations. With some of its features seemingly dependent on the system studied and on the description level considered, the genotype-phenotype (GP) map appears far from trivial. Many studies have addressed the effect of mutations on phenotype: point mutations, genome fragment deletion, duplication or inversions, or the knockout of specific genes —among others— may or may not have an effect at the molecular, metabolic, regulatory, or organismal level. The probability of experiencing different genome mutations and how these mutations modify the current phenotype depends on the structure of genotype spaces and on the properties of the GP map; the latter eventually determine how genome space is explored, and what are the chances that a population survives or innovates in the face of endogenous or exogenous changes.

Despite the plethora of different model systems used to explore the GP map, and the seemingly relevant underlying differences, a number of commonalities at the molecular level have emerged. Exhaustive research on the GP map was pioneered by studies of RNA sequence-to-secondary-structure mappings. Most topological properties identified in RNA spaces are shared by other simple systems, such as the existence of huge genotype networks, the increase in phenotype robustness with size, and a very skewed distribution of phenotype sizes. The set of genotypes that yield the same phenotype typically forms a network, since those genotypes are pairwise connected through mutations. Sufficiently large genotype
networks were postulated as a condition for the navigability of sequence space long ago [1]. Subsequent studies have shown that such large networks do exist and link genotypes as different as two random sequences might be [2, 3, 4, 5]. Phenotype robustness refers to the average effect of point mutations in the genotypes of a specific genotype network. It has been shown to grow logarithmically with the size of the phenotype in RNA [6] and protein quaternary structure [7]. The existence of qualitative and quantitative statistical properties of the GP maps shared by apparently dissimilar systems suggests that they might arise from basic universal features.

The statistical property of GP maps that has awaken most attention is very likely the distribution of phenotype sizes. Due to the astronomically large sizes of genotype spaces, initial estimations of the size of phenotypes were performed through random samplings of that space, and very often the results were represented as frequency-rank plots, with phenotypes ordered according to their sizes. Those samplings invariably yielded some very abundant phenotypes and a bunch of phenotypes represented by a few or just one genotype [8, 9]. Often, frequency-rank plot was fit to a generalized Zipf’s law [10], implying a power-law-like distribution of phenotype sizes. However, subsequent studies demonstrated that the frequency-rank plot of phenotype sizes actually had a more complex functional shape [11, 12, 13, 14], and specific functional fits were avoided. Subsequent studies have exhaustively mapped the complete sequence space to its corresponding phenotypes, among which RNA sequence-to-secondary structure map [12, 15], the hydrophobic-polar (HP) model for protein folding [13, 5], or toyLIFE, which includes a sequence-to-structure-to-function description [14]. As a result, complete phenotype size distributions (for short sequences) are now available. Its fitted shapes range from power-law-like curves [16] to lognormal distributions [15].

It has been argued that, among other generic properties, a skewed distribution of phenotype sizes results from the organization of biological sequences into constrained and unconstrained parts. In [17], the authors introduce a simple, artificial model, called the Fibonacci GP map, where sites in a sequence can be coding or non-conding, and either lead to new phenotypes under mutations (coding sites) or yield the same phenotype (neutral, non-coding sites). The model can be analytically solved and yields a power-law phenotype size distribution, in qualitative agreement with some observations.

In this contribution, we attempt an identification of the elements in the organization of sequences that characterize the quantitative properties of the distribution of phenotype sizes. We show in a constructive fashion that the model in [17] is an example of a broad spectrum of sequence-to-structure GP models. Starting
with the simplest case, where sequences are separated into constrained and neutral parts, and adding subsequent elements in the organization of the sequences and versatility levels of the sites, we show how the distribution of phenotype sizes changes from pure power-law (with an exponent dependent on how genotypes are distributed among phenotypes) to lognormal. Our final example corresponds to the RNA sequence-to-secondary structure map, where we demonstrate that the combinatorial properties of the distribution of sites of variable neutrality along sequences causes the distribution of phenotypes to follow a lognormal distribution, with parameters that can be traced to properties of the genotype set. Our main result is that a lognormal distribution of phenotype sizes is the expected result in any GP map where sufficient variation in the number of phenotypes of similar size is present.

2 Definitions

We will study four models that interpolate between the simplest case of sequences divided into neutral and non-neutral sites separated into two groups and a general case (represented by RNA), and calculate for each of them the size of a phenotype given the sequence organization of its corresponding genotypes, the number of phenotypes with the same size, the frequency rank ordering of phenotypes, and eventually the distribution of phenotype sizes.

The following quantities, of relevance for all the cases to be discussed, are used.

- $L$ is the length of the sequence, or genotype;
- $k$ is the size of the alphabet ($k = 2$ for a binary alphabet \{0, 1\}; $k = 4$ for DNA or RNA, \{A, C, G, T\} or \{A, C, G, U\});
- The \textit{versatility} $v_i$ of site $i$ is defined as the average number of different letters of the alphabet that can occupy a given sequence position $i$. In general $k \geq v_i \geq 1$ for all sites $i$. This is a quantity closely related to neutrality. We will study the simplified case where sites can take one out of two different degrees of versatility, $v_1$ and $v_2$, with $k \geq v_1 > v_2 \geq 1$.
- $\ell \in \{0, 1, \ldots, L\}$ is the number of sites in the low-versatility class. Sites are called constrained if $v_2 = 1$.  


Figure 1: Schematic of the quantities involved in the calculation of the abundance $f(r)$ of a phenotype as a function of its rank $r$. Here $\ell$ represents the number of more constrained sites and works as an intermediate variable to simplify calculations. $C(\ell)$ is the number of phenotypes with $\ell$ constrained sites and $S(\ell)$ the corresponding size.

- $L - \ell$ is the number of sites in the high-versatility class. If these sites are fully neutral $v_1 = k$.
- The size $S(\ell)$ of a phenotype is the number of different genotypes compatible with that phenotype.
- The number of phenotypes with the same size is $C(\ell)$. From the former definitions, it is a function of $\ell$ solely.
- The number of genotypes compatible with $\ell$-phenotypes (or the set of $\ell$-genotypes, for short) is $N_c(\ell) \equiv S(\ell)C(\ell)$.
- The rank of a phenotype is $r(\ell)$, and $r(\ell) = \sum_{i=0}^{\ell-1} C(i)$ holds for the first phenotype in size class $C(\ell)$. The total number of phenotypes coincides with the maximum rank, $R \equiv r(L)$.
The probability density that a phenotype has size $S$ can be obtained, up to normalisation, through inversion of $S(\ell)$ as $p(S) \propto C(S(\ell)) \left| S'(S(\ell)) \right|^{-1}$.

Figure 1 illustrates relevant quantities.

A succinct definition of the hierarchy of models introduced in this work is as follows:

- **Model 1**: *Constrained sites occupy fixed positions*. Sequences are formed by $\ell$ fixed sites with $v_2 = 1$ and the remaining $L - \ell$ sites with $v_1 = k$. Two minor variants considered are (i) phenotypes are all viable and (ii) lethal mutations occur independently of the site class.

- **Model 2**: *Constrained sites occupy variable positions*. This is illustrated by means of two examples: (i) constrained sites are splitted into two fragments at the beginning and at the end of the sequence and (ii) constrained sites can occupy arbitrary positions in the sequence.

- **Model 3**: *Versatile sites occupy fixed positions*. Two different types of sites with fixed versatilities $v_1$ and $v_2$ are considered.

- **Model 4**: *Versatile sites occupy variable positions*: RNA. In a first approximation, RNA sequences contain two types of sites that occupy different positions in the sequence subject to secondary structure constraints: those forming pairs in the secondary structure have average versatility $v_2$, and those unpaired have average versatility $v_1$. The model can be generalized to an arbitrary number of site classes.

## 3 Results

### 3.1 Model 1: Constrained sites occupy fixed positions

This is probably the simplest non-trivial model in the class of GP maps, very similar in spirit to that presented in [17]. Phenotypes are characterized by $\ell$ constrained sites in the first part of the sequence. For a fixed $\ell$, mutations in a constrained site change the phenotype, and mutations in fully neutral sites yield genotypes compatible with the phenotype. Therefore,
\[ S(\ell) = k^{L-\ell}w(\ell) \quad (1) \]
\[ C(\ell) = k^\ell \quad (2) \]
\[ r(\ell) = \frac{k^\ell - 1}{k - 1} \quad (3) \]

We have introduced a generic factor \( w(\ell) \) in the size of phenotypes that may take into account additional restrictions in the assignment of genotypes to phenotypes. Note that, if \( w(\ell) = 1 \), the complete genotype space is partitioned among the \( \ell \)-phenotypes for every value of \( \ell \). This implies that, if we consider all possible phenotypes (i.e. all \( \ell \) values), a particular genotype is simultaneously compatible with many different phenotypes. In other words, if \( \Omega = (L + 1)^{-1} \), each genotype is compatible with multiple phenotypes.

In general, size as a function of rank is derived by eliminating \( \ell \) in \( r(\ell) \) and substituting into \( S(\ell) \) to get \( S(r) \). In this case, from Eq. (3),

\[ \ell = \log_k [(k - 1)\ell + 1] \approx \log_k [(k - 1)\ell] \quad (4) \]

and substituting in (1)

\[ S(r) \approx w(r) \frac{k^L}{k - 1}r^{-1} \quad (5) \]

To obtain the probability density \( p(S) \) we first notice that Eq. (1) implies \( k^\ell = k^{L-S^{-1}} \), hence \( C(S) = k^LS^{-1} \). On the other hand \( S'(\ell) = -(\log k)S \), thus

\[ p(S) \propto S^{-2} \quad (6) \]

Hence the probability distribution is a power-law with exponent \( \beta = 2 \).

### 3.1.1 Non-viable genotypes arise from uniformly distributed lethal mutations

In the same scenario as above, let us assume that a fraction \( \delta \) of mutations is lethal, thus leading to a non-viable genotype. In this case, Eqs. (1) to (3) are identical, with \( k \) substituted by \( k(1 - \delta) \). Therefore, \( S(r) \) and \( p(S) \) are as above with the latter
change. This result shows that the existence of a non-viable class to which viable genotypes can mutate does not necessarily imply relevant functional changes in the distribution of phenotypes, which is in either case of the form \( p(S) \sim S^{-\beta} \), with \( \beta = 2 \). The effect of uniformly distributed lethal mutations could be therefore absorbed as a constant into \( w(\ell) \).

### 3.2 Model 2: Constrained sites occupy variable positions

In any realistic model (e.g. the case of RNA) the position of constrained and neutral sites should matter in the definition of a phenotype. While \( S(\ell) \) does not change its functional form as a result, \( C(\ell) \) does (and \( r(\ell) \) as a consequence), causing potentially relevant modifications in \( S(r) \) and \( p(S) \). In general, the number of different phenotypes would take the form \( C(\ell) = k^\ell Q(L, \ell) \), where \( k^\ell \) accounts for changes in the letter of the constrained site (yielding a different phenotype, as assumed) and \( Q(L, \ell) \) is a model-dependent combinatorial number that counts the different ways in which the \( \ell \) sites can be arranged to yield meaningful (and different) phenotypes. In general, the factor \( S^{-2} \) in \( p(S) \) stems from mutations in neutral sites, while the arrangement of constrained and neutral sites along the sequence is weighted by \( Q(L, \ell(S)) \), with effects on the functional form of \( p(S) \) that, in general, depend on the permitted arrangements. As will be shown, \( Q(L, \ell) \) might enormously increase the number of phenotypes and, especially, the relative abundances of \( \ell \)-phenotypes.

#### 3.2.1 Constrained sites are split into two groups at the extremes of the sequence

As a way of example, let us consider one of the simplest situations where the position of the constrained sites matters. Suppose that those sites can be split into two groups with lengths \( \ell_1 \) and \( \ell_2 \) and placed at the beginning and at the end of the sequence (such that \( 0 \leq \ell_1, \ell_2 \leq L \) and \( \ell_1 + \ell_2 = \ell \)). This gives \( Q(L, \ell) = \ell + 1 \) different phenotypes with \( \ell \) constrained sites, and

\[
S(\ell) = k^{L-\ell} \Omega, \quad C(\ell) = (\ell + 1)k^\ell, \quad r(\ell) = \frac{k^\ell(\ell k - \ell - 1) + 1}{(k-1)^2}.
\]

Now,
\[
\ell = \frac{W \left( \left[ (k-1)^2 r - 1 \right] c_k e^{-c_k} \right)}{\log k} + \frac{1}{k-1}, \quad c_k \equiv \log k \frac{1}{k-1},
\]
with \( W(x) \) Lambert’s product-logarithm function \cite{18, Def. 4.13.1}. Since \( W(x) \sim \log x + O(\log \log x) \) as \( x \to \infty \) \cite{18, Prop. 4.13.10}, we can approximate

\[
\ell \approx \log_k \left( c_k \left[ (k-1)^2 r - 1 \right] \right) \approx \log_k \left( c_k (k-1)^2 r \right),
\]
from which

\[
S(r) \approx \frac{k^L \Omega}{c_k (k-1)^2 \gamma r^{-1}}.
\]

On the other hand, eliminating \( \ell = L + \log_k (\Omega/S) \) we obtain

\[
p(S) \propto \left[ L + \log_k (\Omega/S) \right] \frac{\Omega}{S^2}.
\]

Therefore, even in this simple case with quite a limited number of possible organization of constrained sites, \( S(r) \) and \( p(S) \) are no longer pure power-laws, though the dominant term of the phenotype size distribution (size still dominated by mutations in neutral sites) is characterized by an exponent \( \beta = 2 \). The total number of genotypes compatible with \( \ell \)-phenotypes is also modified, \( N_c(\ell) = (\ell + 1)^k \), and is seen to increase with \( \ell \).

3.2.2 Constrained sites can occupy any position in the sequence

We now assume that the constrained and unconstrained sites can occupy any site of the chain. In that case

\[
S(\ell) = k^L - \ell \Omega, \quad C(\ell) = \binom{L}{\ell} k^\ell,
\]

whereas there is no simple expression for \( r(\ell) \). Let us focus, however, on the size distribution \( p(S) \), and consider the case where \( L \gg 1 \). Asymptotically for \( L \to \infty \)

\[
\binom{L}{\ell} \sim 2^L \sqrt{\frac{2}{\pi L}} \exp \left\{ -\frac{2}{L} \left( \ell - \frac{L}{2} \right)^2 \right\}.
\]
Changing $\ell$ to $S$ through $\ell = L + \log_k \Omega - \log_k S$

$$P(S) \propto \frac{1}{S^2} \exp \left\{ -\frac{2}{L} \left( \log_k S - \frac{L}{2} - \log_k \Omega \right)^2 \right\},$$  \hspace{1cm} (17)

and writing $S^{-1} = \exp(-\log k \log k S)$, we finally obtain

$$P(S) \sim \frac{1}{S \log k} \sqrt{\frac{2}{\pi L}} \exp \left\{ -\frac{2}{L} \left[ \log_k S - \frac{L}{2} \left( 1 - \frac{\log k}{2} \right) - \log_k \Omega \right]^2 \right\},$$ \hspace{1cm} (18)

a log-normal distribution with mean $\mu_L \sim \frac{\log k}{2} \left( 1 - \frac{\log k}{2} \right) L + \log \Omega$ and variance $\sigma^2_L \sim \left( \frac{\log k}{2} \right)^2 L$, very different from the $p(S) \sim S^{-2}$ distribution of the previous cases.

The number of compatible genotypes as a function of $S$ is also affected. Since $N_c(S) = SC(S) = 2^L SP(S)$,

$$N_c(S) \sim \frac{2L}{S \log k} \sqrt{\frac{2}{\pi L}} \exp \left\{ -\frac{2}{L} \left[ \log_k S - \frac{L}{2} - \log_k \Omega \right]^2 \right\},$$ \hspace{1cm} (19)

proportional to a log-normal distribution with mean $\bar{\mu}_L \sim \frac{L}{2} \log k + \log \Omega$ —shifted up with respect to $\mu_L$— and the same variance $\sigma^2_L$.

### 3.3 Model 3: Versatile sites occupy fixed positions

The models analysed above demonstrate that when sites are either constrained or fully neutral, the exponent associated to the power-law part of $p(S)$ is $\beta = 2$. As we show next, this exponent is modified when the sites in the sequence show intermediate degrees of versatility and the number of $\ell$-genotypes depends on $\ell$. In this section, we consider two possible fixed versatilities for sites in the sequence and analyse two variants of this model. In the first one, the number of different phenotypes is defined as an extension of model 1. In the second one, the number of different phenotypes is defined so as to maintain $N_c(\ell)$ independent of $\ell$. Equivalently, we may think of the second definition as a case of $w(\ell)$ variable such that the number of $\ell$-genotypes is constant.
3.3.1 Variable number of \( \ell \)-genotypes

Let us now consider the case where the \( L - \ell \) sites are just less constrained than the \( \ell \) sites, such that the former admit an average of \( v_1 \) different letters of the alphabet and the latter admit \( v_2 \), with \( k \geq v_1 > v_2 \geq 1 \). Relevant functions read

\[
S(\ell) = v_1^\ell \left( \frac{v_2}{v_1} \right)^\ell \Omega, \tag{20}
\]

\[
C(\ell) = (k - v_1 + 1)^L \kappa^\ell, \tag{21}
\]

\[
r(\ell) = (k - v_1 + 1)^L \left( \frac{\kappa - 1}{\kappa - 1} \right), \tag{22}
\]

with \( \kappa \equiv (k - v_2 + 1)/(k - v_1 + 1) \).

As it can be readily seen by substitution, these expressions reduce to Model 1 for \( v_1 = k \) and \( v_2 = 1 \). Now,

\[
\ell = \log_\kappa \left( 1 + \frac{\kappa - 1}{(k - v_1 + 1)^L r} \right), \tag{23}
\]

yielding

\[
S(r) = v_1^\ell \left( \frac{v_1}{v_2} \right)^{-\log_\kappa \left( 1 + \frac{\kappa - 1}{(k - v_1 + 1)^L r} \right)} = v_1^\ell \left( 1 + \frac{\kappa - 1}{(k - v_1 + 1)^L r} \right)^{-\log_\kappa (v_1/v_2)}. \tag{24}
\]

For large \( r \) this scales as \( S(r) \sim cr^{-\alpha} \), where \( \alpha \) depends on \( v_1 \) and \( v_2 \) as

\[
\alpha = \log_\kappa \left( \frac{v_1}{v_2} \right). \tag{25}
\]

Furthermore

\[
\ell = -\frac{1}{\alpha} \log_\kappa \left( \frac{S}{v_1^\ell \Omega} \right), \tag{26}
\]

hence

\[
p(S) \propto \kappa^{-\frac{1}{\alpha} \log_\kappa \left( \frac{S}{v_1^\ell} \right)} S^{-1} \propto S^{-1-\alpha^{-1}}. \tag{27}
\]

Again \( p(S) \) maintains its power-law shape but its exponent depends on \( v_1 \) and \( v_2 \).
The number of $\ell$-genotypes now becomes

$$N_c(\ell) = \Omega v_1^L (k - v_1 - 1)^L \left( \frac{v_2}{v_1} \kappa \right)^\ell.$$  \hfill (28)

This number can either increase or decrease with $\ell$ depending on whether $v_2/v_1\kappa$ is larger or smaller than 1. Both situations are possible under the constraint $v_1 > v_2$.

### Fixed number of $\ell$-genotypes

We assume now that the set of different phenotypes for fixed $\ell$ is defined in such a way that $N_c(\ell) = k^L$, i.e.,

$$S(\ell) = v_1^{L-\ell} v_2^\ell = v_1^L \kappa^{-\ell}, \quad \kappa \equiv \frac{v_1}{v_2},$$  \hfill (29)

$$C(\ell) = \left( \frac{k}{v_1} \right)^{L-\ell} \left( \frac{k}{v_2} \right)^\ell = \left( \frac{k}{v_1} \right)^L \kappa^\ell,$$  \hfill (30)

$$r(\ell) = \left( \frac{k}{v_1} \right)^L \frac{\kappa^\ell - 1}{\kappa - 1}.$$  \hfill (31)

Thus

$$\ell = \log_\kappa \left( \frac{v_1}{k} \right)^L (\kappa - 1) r + 1 \approx \log_\kappa \left( \frac{v_1}{k} \right)^L (\kappa - 1) r,$$  \hfill (32)

for large $r$, and therefore

$$S(r) \approx \frac{k^L}{\kappa - 1} r^{-1}.$$  \hfill (33)

On the other hand $\ell = \log_\kappa(v_1^L/S)$ and therefore $p(S) \propto S^{-2}$.

### Model 4: Versatile sites occupy fixed positions. RNA

In a first approximation (which has been shown to yield acceptable fits to data [6]), RNA sequences can be divided in two classes of sites: those in stacks (bound) and those in loops (unbound). They have different degrees of neutrality (see e.g. [19] and Fig. 4 in [20]). Their position in the sequence also matters to define the phenotype. In the following, the abundances or phenotypes are ruled by the average
values \(v_2\) and \(v_1\) of the number of letters that can be changed in stacks or loops, respectively, without affecting the phenotype. We will call \(\ell\) the number of paired nucleotides \((\ell = 2, 4, 6, \ldots, L - j)\), with \(j = 3\) if \(L\) is odd and \(j = 4\) if \(L\) is even), and \(L - \ell\) will be the number of nucleotides in loops \((L - \ell \geq 3\), which is the size of the minimal —hairpin— loop).

The distribution of secondary structure sizes (i.e. number of sequences compatible with a given secondary structure) with a fixed number of stacks or loops has been obtained in [21] for the general case of structures with pseudoknots, in [22] and [23], and in [24] in a form that will be used here.

### 3.4.1 Number of secondary structures with fixed number of pairs in RNA

Let us call \(p_{L,\ell}\) the probability distribution for secondary structures with \(\ell\) paired nucleotides (sites with \(v_2\)), for sequences of length \(L\) (in the limit \(L, \ell \to \infty\)). It has been shown [21] [22] [24] that this distribution behaves as a normal distribution in \(\ell\) with mean \(\mu_L = \mu L + \mu_0 + O(L^{-1})\) and standard deviation \(\sigma_L = \sigma L^{1/2} + \sigma_0 L^{-1/2} + O(L^{-3/2})\). In the case that structures with stems with less than two base pairs or loops with less than three unpaired bases are forbidden —for energetic constraints— we obtain \(\mu \approx 0.28647\ldots]\), \(\mu_0 \approx -1.36502\ldots\), \(\sigma \approx 0.25510\ldots\), and \(\sigma_0 \approx -0.00713\ldots\). Note that different constraints will lead to different values of these quantities, but otherwise will not change the fact that \(p_{L,\ell}\) is a normal distribution. Finally, the number \(M_{L,\ell}\) of different phenotypes of a sequence of length \(L\) with \(\ell\) paired bases is given, in the limit \(L, \ell \to \infty\), by

\[
M_{L,\ell} \sim \frac{1}{\sqrt{2\pi \sigma_L}} e^{-((L-\mu_L)^2/2\sigma_L^2)} M_L,
\]

with \(M_L \sim 1.48L^{-3/2}(1.85)^L\) (see [21] [22] [23] [24]).

### 3.4.2 Size distribution

In the case that the unpaired sites admit an average of \(v_1\) different letters and the paired sites an average of \(v_2\) letters \((1 \leq v_2 < v_1 \leq k)\), the size of a phenotype is given by \(S(\ell) = v_1^{L-2\ell} v_2^\ell\), with \(\ell = 2, 4, \ldots, L - j\). For the moment, we will consider that a phenotype is formed by all sequences compatible with that phenotype, thus setting \(\Omega = 1\). (A discussion on deviations from this assumption is included in Section [4].) We have
\[ \ell = \frac{L \log v_1 - \log S}{2 \log \left( \frac{v_1}{v_2} \right)}. \] (35)

Denoting
\[ \mu_S = L \log v_1 - \mu_L, \quad \sigma_S = 2 \log \left( \frac{v_1}{v_2} \right) \sigma_L, \] (36)
the size distribution becomes the log-normal distribution
\[ p_L(S) \sim \frac{1}{\sqrt{2\pi \sigma_S S}} e^{-\left(\log S - \mu_S\right)^2/2 \sigma_S^2}. \] (37)

### 3.4.3 Rank distribution

In the same two-sites approximation
\[ C(\ell) \sim (k - v_2 + 1)^{2\ell} (k - v_1 + 1)^{L - 2\ell} M_{L,\ell}. \] (38)

The functional form of the rank \( r(\ell) \) is derived in the appendix. After some algebra we arrive at
\[ S(r) \sim v_1^{L(1 - 2a)} v_2^{2aL} \exp \left\{ \kappa L \sqrt{1 - \frac{\log r}{cL}} \right\}, \quad \kappa \equiv \sigma \sqrt{8c \log \left( \frac{v_1}{v_2} \right)}. \] (39)

### 4 Discussion

The functional shape of the distribution of phenotype sizes is strongly dependent on the sequence organization within phenotypes. In a first approximation that discards the heterogeneity among genotypes in the same phenotype, one may describe that ensemble of sequences through a prototypic sequence whose sites admit a phenotype-dependent, variable number of letters of the alphabet, a quantity that we have dubbed versatility. The substitution of each sequence in a phenotype by the average over the phenotype seems a strong approximation. However, there is evidence that deviations from the average within a phenotype are small: the number of neutral neighbours of genotypes within a phenotype are tightly clustered around an average value characteristic of that phenotype size [6]. With this
proviso, two main elements determine the corresponding distribution of phenotype sizes. The first one, generic for all systems, is the relationship between the size of a phenotype and the versatility $v_i$ of each site $i$. In the framework used in this work, the size of a phenotype can be written in general as

$$S\{\{v_i\}\} = \prod_i v_i. \quad (40)$$

This product yields an intrinsic allometric relation between the size of a phenotype and the length of the sequence. The second element, specific of each sequence-to-structure map, is the number of phenotypes with similar size. This quantity takes the overall form

$$C\{\{v_i\}\} = Q(L, \{v_i\}) \prod_i (k - v_i + 1), \quad (41)$$

with the combinatorial factor accounting for the number of ways in which an ensemble of $L$ sites with $v_i$ values can be arranged into meaningful phenotypes, and the product accounting for the number of neutral sequences within the phenotype. If the values of the combinatorial factor are constrained enough such that the asymptotic behavior of $Q(L, \{v_i\})$ with $L$ is subdominant with respect to that of the product —as in models 1 and 3— the distribution of phenotype sizes is a power-law. If, on the contrary, the dominant term is the combinatorial factor —in particular when the distribution of structural motifs converges to a Gaussian— the distribution of phenotype sizes becomes a lognormal.

In the case $Q(L, \{v_i\}) \simeq 1$ we should expect a power-law-like distribution of phenotype sizes characterized by an exponent $\beta$. The actual value of $\beta$ stems from a combination of the number of genotypes compatible with a given phenotype and the total number of phenotypes with the same (or similar) size. In a general scenario, let us assume that phenotype sizes can be ordered according to a certain variable $\hat{\ell}$, and let us define the total number of genotypes compatible with $\hat{\ell}$-phenotypes as $N_c(\hat{\ell}) \equiv S(\hat{\ell})C(\hat{\ell})$, formally generalizing the quantity calculated in the specific models tackled in this work. The behaviour of $N_c(\hat{\ell})$ with $\hat{\ell}$ determines the value of the exponent $\hat{\beta}$: If $N_c(\hat{\ell})$ is constant, then $\hat{\beta} = 2$. However, if $N_c(\hat{\ell})$ is exponentially enriched (depleted) in genotypes as $\hat{\ell}$ grows, the value of $\hat{\beta}$ becomes larger (smaller) than 2. In the case of Model 3, for example $N_c(\ell) = AB^\ell$, with $B = (v_2/v_1)(k - v_2 + 1)/(k - v_1 + 1)$ and $\hat{\beta} = 1 + 1/\alpha$. Two examples of enrichment or depletion in the number of genotypes compatible with $\ell$-phenotypes are $\{v_1, v_2\} = \{4, 2.5\}$, with $B = 1.56$ and $\hat{\beta} = 2.95$, and $\{v_1, v_2\} = \{3, 1.5\}$, with $B = 0.875$ and $\hat{\beta} = 1.81$. 

15
Figure 2: Summary of constrained models yielding power-law-like distributions of phenotype sizes and main analytical quantities. Lower case letters in the table represent constants. (a) Model 1: Constrained sites occupy fixed positions; (b) Model 2(i): Constrained sites are split into two groups at the extremes of the sequence; (c) Model 3: Versatile sites occupy fixed positions; (d) Fibonacci GP map [17]; (e) Model 1 with a uniform distribution of lethal mutations; (f) Model 3 with a fixed number of $\ell−$genotypes.

An example in the class of power-law-like $p(S)$ with non-trivial $\beta$ is the model in [17]. Besides the division of sequences into neutral and constrained sites, the authors introduce a stop codon which causes an $\ell−$dependent transition rate to alternative phenotypes, that being the eventual reason for a non-trivial value of $\beta$. In that case, $N_c(\ell) = 2^{L−\ell} \phi^{\ell−1}/\sqrt{5}$, which corresponds to a value of $B = 1.06$ and $\beta = 2.08$ as expected. Figure 2 summarizes the sequence organization of different models with a power-law distribution of phenotype sizes, several relevant quantities, and the corresponding $\beta$ value.

The situation where the combinatorial factor converges to a Gaussian distribu-
tion is expected to be very general for sequence-to-structure GP maps [22], implying that a lognormal distribution of phenotype sizes might be a generic property of such maps. Up to now, there are few quantitative results supporting this statement, very likely due to the impossibility to exhaustively fold genome spaces for large $L$. A remarkable exception is [15], where the lognormal distribution has been suggested as the best fit to computational distributions of RNA secondary structure sizes for lengths up to $L = 126$. It is interesting to highlight that our results depend on some a priori strong assumptions on how genotypes are assigned to phenotypes. In particular, we have taken a uniform assignment of genotypes (represented through our variable $\Omega$) to avoid the many-to-many relationship implicit in our models. However, natural relationships might be more complex. In the case of RNA, for example, the assignment of genotypes to phenotypes is not uniform (phenotypes are not arbitrarily chosen among all those compatible with a sequence) but it is based on thermodynamical rules: the structure corresponding to a sequence is typically that with the minimum free energy fold. It cannot be discarded that genotype-to-phenotype assignment rules based on quantities not considered here might skew the distribution or eventually yield different functional forms. Though this is a possibility that has to be kept in mind, the results in [15] reveal that, at least in the case of RNA, deviations from lognormality cannot be numerically detected.

Our calculations make it explicit that variations in the precise values of versatility, in the number of different classes of sites, or in particular constraints in structures (as, e.g. whether one, two or more base pairs are required as a minimum condition to form a stack) have a quantitative effect on the parameters of the lognormal, but do not affect the shape of the distribution. Computational analyses in the light of the analytical results here presented should reveal the extent to which non-uniform sequence-to-structure assignments or deviations from a prototypic genotype characterizing a phenotype are relevant. As a result, it should be clarified whether the distribution of phenotype sizes is significantly model-dependent or if, on the contrary, a lognormal distribution is a generic property of realistic sequence-to-structure maps. The rationale behind our models and preliminary results with RNA strongly support the latter.

**Acknowledgements**

This work has been supported by the Spanish Ministerio de Economía y Competitividad and FEDER funds of the EU through grants ViralESS (FIS2014-57686-P)
and VARIANCE (FIS2015-64349-P).

Author contributions

SM and JAC designed the study, carried out the calculations, interpreted the results and wrote the manuscript. Both authors read and approved the final text.

Appendix

The rank function for the case of RNA sequences whose sites may take two values of neutrality $v_1$ and $v_2$, a number $M_{L,\ell}$ of secondary structures of length $L$ with $\ell$ sites with neutrality $v_1$ and a total number of $M_L$ different secondary structures of length $L$ is

$$r(\ell) \sim M_L(k-v_1+1)^L \int_{-\infty}^{(\ell-\mu_L)/\sigma_L} \frac{1}{\sqrt{2\pi}} \left( \frac{k-v_2+1}{k-v_1+1} \right)^{2\sigma_L x + 2\mu_L} e^{-x^2/2} dx$$

$$= M_L(k-v_1+1)^L \exp \left\{ \mu_L \xi + \frac{\xi^2}{2} \sigma_L^2 \right\} \int_{-\infty}^{(\ell-\mu_L-\xi \sigma_L^2)/\sigma_L} \frac{1}{\sqrt{2\pi}} e^{-x^2/2} dx,$$

where

$$\xi \equiv 2 \log \left( \frac{k-v_2+1}{k-v_1+1} \right).$$

(42)

Now, since $\ell - \mu_L - \xi \sigma_L^2$ will be negative for all $\mu_L - \sigma_L \leq \ell \leq \mu_L + \sigma_L$, we can use the asymptotic expansion of the complementary error function

$$\text{erfc} x \equiv \frac{2}{\sqrt{\pi}} \int_x^{\infty} e^{-t^2} dt = \frac{2}{\sqrt{\pi}} \int_{-\infty}^{-x} e^{-t^2} dt \sim \frac{e^{-x^2}}{x \sqrt{\pi}}$$

to write

$$r(\ell) \sim \frac{M_L \sigma_L (k-v_1+1)^L}{\sqrt{2\pi} (\mu_L + \xi \sigma_L^2 - \ell)} \exp \left\{ \mu_L \xi + \frac{\xi^2}{2} \sigma_L^2 - \frac{(\mu_L + \xi \sigma_L^2 - \ell)^2}{2 \sigma_L^2} \right\}. \quad (44)$$

In order to find how the size of a phenotype depends on its rank value $r(\ell)$ it is convenient to introduce new parameters. Let us denote $\mu \equiv \mu_L / L$ and $\sigma \equiv \sigma_L / \sqrt{L}$, and
\[ a \equiv \mu + \xi \sigma^2, \quad c \equiv \xi \mu + \frac{\xi^2 \sigma^2}{2} + \log(k - v_1 + 1) + \log \rho \]  

(45)

with \( \rho \approx 1.85 \). The size of a phenotype is given by \( S(\ell) = v_1^{L - 2\ell} v_2^{2\ell} \), therefore

\[
\frac{1}{L} \log S = \log v_1 - 2 \frac{\ell}{L} \log \left( \frac{v_1}{v_2} \right).
\]  

(46)

Now, taking logarithms in (44) and neglecting subdominant terms in \( L \),

\[
\frac{1}{L} \log r \sim c - \frac{1}{2\sigma^2} \left( a - \frac{\ell}{L} \right)^2.
\]  

(47)

Hence

\[
\frac{L}{\ell} \sim a - \sigma \sqrt{2c} \sqrt{1 - \frac{\log r}{cL}}
\]  

(48)

and therefore

\[
\frac{1}{L} \log S \sim \log v_1 - 2a \log \left( \frac{v_1}{v_2} \right) + \sigma \sqrt{8c} \log \left( \frac{v_1}{v_2} \right) \sqrt{1 - \frac{\log r}{cL}}
\]  

(49)

which implies

\[
S \sim v_1^{L(1 - 2a)} v_2^{2aL} \exp \left\{ \kappa L \sqrt{1 - \frac{\log r}{cL}} \right\}, \quad \kappa \equiv \sigma \sqrt{8c} \log \left( \frac{v_1}{v_2} \right).
\]  

(50)

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