

HIGHER ORDER EPISTASIS AND FITNESS PEAKS

KRISTINA CRONA AND MENGMIN LUO

ABSTRACT. We show that higher order epistasis has a substantial impact on evolutionary dynamics by analyzing peaks in the fitness landscapes. There are 193,270,310 fitness graphs for 4-locus systems, i.e., directed acyclic graphs on 4-cubes. The graphs can be partitioned into 511,863 isomorphism classes. We identify all fitness graphs with 6 or more peaks. 81 percent of them imply 4-way epistasis, whereas 9 percent of all 4-locus fitness graphs imply 4-way epistasis. Fitness graphs are useful in that they reflect the entire collection of fitness landscapes, rather than particular models. Our results depend on a characterization of fitness graphs that imply n -way epistasis. The characterization is expressed in terms of a partition property that can be derived from Hall's marriage theorem for bipartite graphs. A similar partition condition holds for any partial order. The result answers an open problem posed at a conference on interactions between algebra and the sciences at the Max Planck institute.

1. INTRODUCTION

Recent empirical studies have detected higher order epistasis in several biological systems (Sailer, 2017; Weinreich et al., 2013). However, the impact of higher order epistasis has not been carefully investigated. The purpose of this study is to relate higher order epistasis and peaks in fitness landscapes. The genotypes for a biallelic 2-locus system are denoted

$$00, 10, 01, 11.$$

We define a fitness landscape as a function $w : \{0, 1\}^n \mapsto \mathbb{R}$, which assigns a fitness value to each genotype. The fitness of the genotype g is denoted w_g .

Epistasis is measured by the expression

$$w_{00} + w_{11} - w_{01} - w_{10}.$$

The genotypes for a biallelic 3-locus system are denoted

$$000, 100, 010, 001, 110, 101, 011, 111,$$

and the (total) 3-way epistasis is measured by

$$w_{111} = w_{000} + w_{011} + w_{101} + w_{110} - w_{001} - w_{010} - w_{100} - w_{111}.$$

We refer to 000, 110, 101, 011 as even genotypes, and 100, 010, 001, 111 as odd genotypes. "Even" and "odd" refer to the number of 1's in the genotype labels. The definition of n -way epistasis is analogous.

Our analysis of higher order epistasis and peaks depends on fitness graphs. The vertices of a fitness graph are labeled by the 2^n genotypes in the n -locus system. For

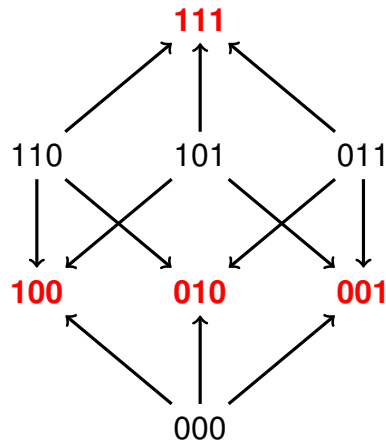


FIGURE 1. The fitness graph has 4 peaks. The graph implies 3-way epistasis.

each pair of mutational neighbors, i.e., genotypes that differ at one locus only, an arrow points toward the genotype of higher fitness (Figure 1). In mathematical terms, a fitness graph is a directed acyclic graph on a hypercube, or a cube orientation. A peak in a fitness landscape is a genotype of higher fitness than all of its mutational neighbors. By abuse of notation, vertices in fitness graphs are referred to as peaks if they correspond to peaks in the fitness landscapes. Fitness graphs were originally used for analyzing empirical data (De Visser et al., 2009; Franke et al., 2011, e.g.), and in more recent time for theoretical results (Crona et al., 2017; Wu et al., 2016; Crona et al., 2013), see also the Discussion section. Fitness graphs are of interest since they capture important aspects of evolutionary dynamics.

The topic of higher order epistasis and peaks was considered in Crona et al. (2017), mainly for 3-locus systems. There are 1862 fitness graphs for 3-locus systems, partitioned into 54 isomorphism classes. 20 out of the 54 graphs imply 3-way epistasis. The relation between higher order epistasis and peaks can be summarized as follows (Crona et al., 2017).

- (i) The mean number of peaks for the graphs implying 3-order epistasis is 1.8;
- (ii) the mean number of peaks for the remaining graphs is 1.5,
- (iii) graphs with the maximal number of peaks imply 3-way epistasis.

However, there are 193,270,310 fitness graphs for 4-locus systems (see the Supplementary Information) and some theory is necessary for determining if a fitness graph implies 4-way epistasis. More generally, it is of interest to determine whether or not a given partial order for an n -locus system implies n -way epistasis. Here we develop the necessary theory, and use the results for a systematic investigation of higher order epistasis and peaks for 4-locus systems.

2. RESULTS

As mentioned in the introduction, 3-way epistasis is measured by

$$u_{111} = w_{000} + w_{011} + w_{101} + w_{110} - w_{001} - w_{010} - w_{100} - w_{111}.$$

Notice that the coefficients for even genotypes are positive, and for odd genotypes negative, where even and odd are as in the introduction. For a given n -locus system it is useful to consider k -locus subsystems. For instance, the genotypes with last coordinate zero

$$000, 100, 010, 110,$$

constitute a subsystem. The conditional epistasis relevant for the 2-locus subsystem is defined by the equation

$$w_{000} + w_{110} - w_{100} - w_{010}.$$

There are in total six 2-locus subsystems (corresponding to the sides of the cube), and each subsystem is defined by fixing one of the coordinates at 0 or 1.

Definition 2.1. An n -locus system has k -way epistasis for $2 \leq k \leq n$ if it has conditional k -way epistasis for at least one k -locus subsystem.

Notice that similar concepts in the literature may disagree with this definition. It is easy to verify that if a system has no k -way epistasis, then it does not have $k + 1$ epistasis. Fitness landscapes fall naturally into three categories:

- (i) additive landscapes;
- (ii) landscapes with pairwise interactions, but not higher order epistasis,
- (iii) landscapes with higher order epistasis.

If one wants to be more precise, one can say that a landscape has k -way epistasis but not higher order interactions.

Some rank orders imply 3-way epistasis, in the sense that every fitness landscape that satisfy the rank order has 3-way epistasis. For instance, it is easily seen that $u_{111} > 0$ for the order

$$w_{000} > w_{011} > w_{101} > w_{110} > w_{001} > w_{010} > w_{100} > w_{111},$$

since the fitness for every single even genotype exceeds the fitness of the odd genotypes. A complete characterization of rank orders that imply n -way epistasis is given in Crona et al. (2017). Here we are interested in understanding when partial orders, in particular fitness graphs, imply n -way epistasis. Fitness graph are of special interest since they capture important aspects of the evolutionary dynamics. We illustrate our method for the fitness graph in Figure 1. The 8 vertices of the graph can be partition into 4 pairs

$$(000, 100), (110, 010), (101, 001), (011, 111)$$

such that the even node in each pair has the lowest fitness. It is easy to verify that $u_{111} < 0$, i.e., the fitness graph implies 3-way epistasis.

More generally, it is straight forward to verify that any fitness graph that satisfies a similar partition condition implies higher order epistasis (Crona et al., 2017). We will

show that the partition property is a necessary condition for the fitness graph to imply epistasis. The proof relies on the following result for bipartite graphs.

Halls Marriage Theorem (Hall, 1935). Given a bipartite graph $G = (V, E)$ with bipartition $A \cup B$ ($V = A \cup B$), the graph G has a matching of size $|A|$ if and only if for every $S \subseteq A$, we have $|N(S)| \geq |S|$, where

$$N(S) = \{b \in B, \text{ such that there exists } a \in S \text{ with } (a, b) \in E\}.$$

Before we give a proof, we demonstrate the main idea of our argument in an example.

Example 2.2. Assume that

$$1111 \succ 1110 \text{ and } 1111 \succ 1101,$$

and that \prec does not constrain 1110 and 1101 in any other way. Then we can assign fitnesses such that

$$w_{1111} - w_{1110} - w_{1101}$$

is an arbitrarily large negative number. By assigning fitnesses to the remaining variables we can define a fitness landscape which respects \prec , such that $u_{1111} < 0$.

A similar argument will be used in the proof.

Theorem 2.3. Let o_i denote the odd genotypes and e_i the even genotypes. A partial order \prec of the genotypes with respect to fitness implies (positive) n -way epistasis if and only if there exist a partition $\{o_j, e_j\}$ of all genotypes such that

$$o_j \prec e_j$$

for all j .

Proof. It is straight forward to verify that the condition is sufficient (Crona et al., 2017). Consider all fitness landscapes that respect a partial order \prec of the genotypes. Let V_o denote the genotypes with odd subindices and V_e the genotypes with even subindices. Consider the graph with vertices $V_e \cup V_o$, where (o, e) is an edge if $o \prec e$.

Let N be defined as above. Suppose that there exists a set of nodes $S_o \subseteq V_o$ such that $|N(S_o)| < |S_o|$. Then we can assign fitnesses to the set of nodes in $S_o \cup N(S_o)$, such that

$$\sum_{e_i \in N(S_o)} w(e_i) - \sum_{o_j \in S_o} w(o_j)$$

is an arbitrarily large negative number. It follows that one can define a fitness landscape which respects \prec and does not have (positive) n -way epistasis. Consequently, if the partial order \prec implies n -way epistasis, then there does not exist any set S_o as described. By Hall's theorem, a matching exists and therefore the desired partition. \square

Theorem 2.3 answers an open problem posed at a conference on interactions between algebra and the sciences at the Max Planck institute in Leipzig, May 2017 (see also the acknowledgement).

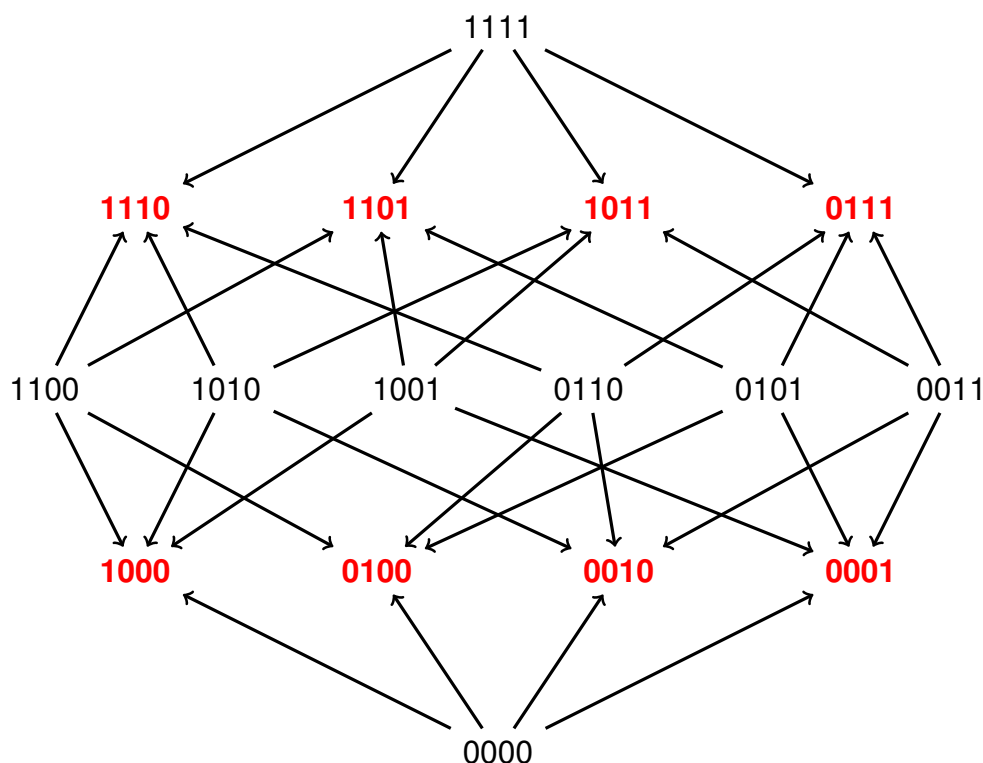


FIGURE 2. The fitness graph has 8 peaks. The graph implies 4-way epistasis.

The 193,270,310 fitness graphs for 4-locus systems can be partitioned into 511,863 isomorphism classes (see the Supplementary Information). In order to analyze the relation between peaks and higher order epistasis, we investigated all graphs with 6 or more peaks. The maximal number of peaks for a 4-locus system is 8 (Haldane, 1931), see Figure 2.

Table 1 summarizes the interactions for 4-locus systems with 6-8 peaks. There are in total 64 graphs in the category, 52 out of them imply 4-way epistasis. The sole graph that does not imply higher order epistasis has 6 peaks.

Table 2 summarizes the relation between 4-way epistasis and peaks. The information for graphs with 6 – 8 peaks is exact. The remaining statistics depend on a sample of 200 randomly chosen graphs among the 511,863 isomorphism classes.

The maximal number of peaks for an n -locus system is 2^{n-1} (Haldane, 1931). However, in the absence of higher order epistasis, the maximal number of peaks is 3 (rather than 4 in general) for 3-locus systems, and 6 (rather than 8 in general) for 4-locus systems. A landscape compatible with the graph in Figure 2 is defined as

$$w_g = 1 + 0.1d - \binom{d}{2} 0.06 \quad \text{where} \quad d = \sum g_i.$$

# Peaks	# Graphs	2-way	3-way	4-way
8	1	0	0	1
7	4	0	1	3
6	59	1	10	48
6–8	64	1	11	52

TABLE 1. The table shows how gene interactions and the number of peaks relate for 4-locus systems. The rightmost column shows the number of graphs that imply 4-way epistasis. The adjacent column shows the number of graphs that imply 3-way epistasis, but not higher order epistasis. The middle columns shows the number of graphs that are compatible with pairwise epistasis only.

# Peaks	4-way interactions
6 – 8	81 %
5	50 %
4	15 %
3	10 %
2	10 %
1	5 %
All graphs	9 %

TABLE 2. The table shows how the proportion of graphs that imply 4-way epistasis depends on the number of peaks.

Notice that the fitness of each genotype is determined by the number of 1's. In particular,

$$w_{0000} = 1, w_{1000} = 1.1, w_{1100} = 1.14, w_{1110} = 1.12, w_{1111} = 1.04.$$

A similar construction works for any number of loci (see the Supplementary Information). One concludes that the maximal number of peaks for landscapes with only pairwise interactions is

$$\geq \binom{n}{\lfloor \frac{n}{2} \rfloor}.$$

The bound is exact for $n = 2, 3, 4$. Whether or not the bound is exact in general is an open problem. Asymptotically,

$$\binom{n}{\lfloor \frac{n}{2} \rfloor} \mapsto \frac{4^n}{\sqrt{\pi n}}$$

(see also the Supplementary Information).

One can ask if the landscapes with extreme peak density are likely to occur in nature, or rather should be considered theoretical constructions. In fact, the unique fitness graph with 6 peaks that is compatible with pairwise interactions is biologically meaningful (Figure 3). The graph is compatible with stabilizing selection. Stabilizing selection

occurs for traits where an intermediate value is preferable as compared to the extremes in the population. Notice that the six genotypes

1100, 1010, 1001, 0110, 0101, 0011,

have the same number of 0's and 1's, and any mutation that makes the distribution more equal is beneficial.

Moreover, the fitness graph with eight peaks (Figure 2) is compatible with egg box models (Bank et al., 2016). Egg box models agree with some empirical data sets, although such examples are probably not common.

3. DISCUSSION

We have studied the relation between higher order epistasis and peaks in fitness landscapes. Fitness graphs (directed acyclic hypercube graphs) reveal all possible constellations of peaks in the landscapes. We provide a characterization of fitness graphs that imply higher order epistasis. A similar result holds for partial orders. Our proof depends on Hall's marriage theorem for bipartite graphs. The theoretical results are of independent interest.

We applied the characterization extensively in a systematic study of fitness graphs for 4-locus biallelic systems, in total 193,270,310 fitness graphs. The graphs can be partitioned into 511, 863 isomorphism classes. We identified all graphs with 6 or more peaks and found that 81 percent of them imply 4-way epistasis. In contrast, 9 percent of all fitness graphs imply 4-way epistasis. Our result agrees well with properties of 3-locus systems (Crona et al., 2017).

Fitness graphs allow for analyzing fitness landscapes in general, rather than focusing on a particular parametric model, such as the NK model or the Mount Fuji model (De Visser and Krug, 2014, e.g.). Results on fitness graphs are in a sense universal, although one should keep in mind that some graphs occur frequently in nature, whereas other graphs are rare.

Fitness graphs have been applied for relating global properties, such as the number of peaks and mutational trajectories, and local properties of fitness landscapes Crona et al. (2013). The capacity of fitness graphs to detect higher order epistasis was demonstrated in Wu et al. (2016). The authors observed that one can deduce 3-way epistasis from particular graphs. A complete description of fitness graphs that imply 3-way epistasis was provided in Crona et al. (2017). For related results, see also (Poelwijk, 2011; Weinreich et al., 2005). The graphs have potential for other aspects of higher order epistasis as well, including the relation between higher order epistasis and (accessible) mutational trajectories.

One would expect that higher order epistasis implies that adaptation is more difficult according to almost any measure. In any case, more systematic studies would be valuable. An obvious limitation of fitness graphs is that they encode pairwise fitness comparisons only, and not for instance the curvature of fitness landscapes. Other methods are necessary as well for analyzing higher order epistasis.

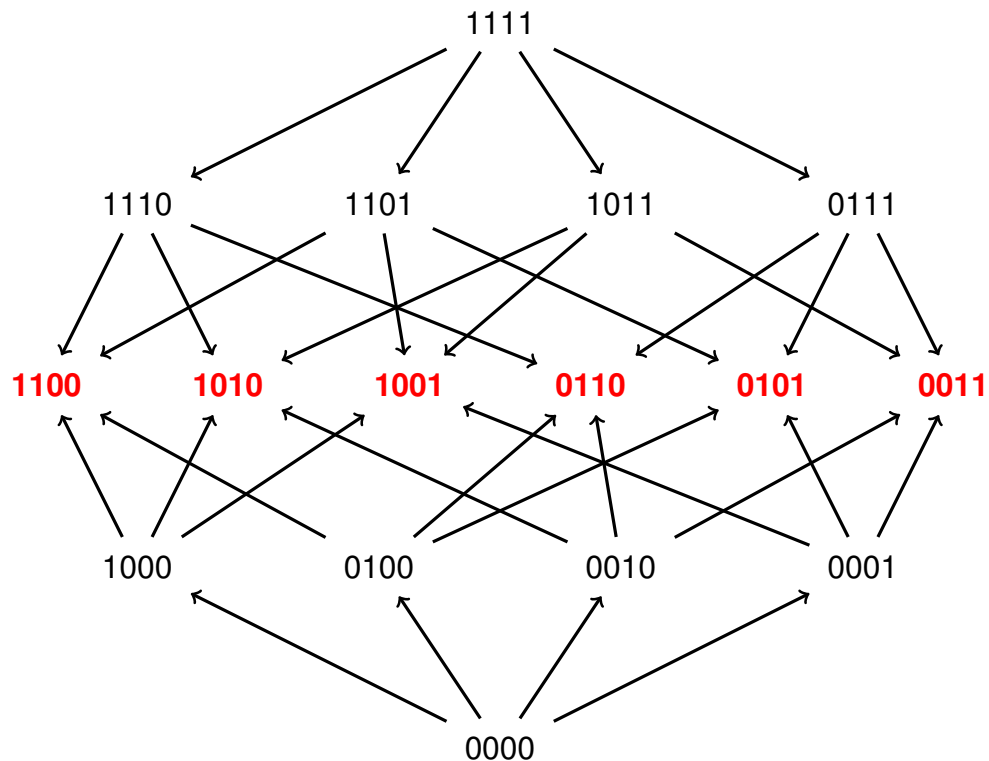


FIGURE 3. The fitness graph has 6 peaks. The graph is compatible with pairwise interactions only.

The utility of fitness landscapes have been discussed in recent years (e.g. Hartl, 2014) and sometimes questioned altogether (e.g. Kaplan, 2008). Fitness graphs and other efficient representations of fitness landscapes may ameliorate some of the issues addressed. In any case, fitness graphs are intuitive and at the same time well suited for developing theory.

Acknowledgements. Devin Greene produced all code for the project. The code made it possible to identify isomorphism classes of graphs, compute the number of peaks and automatically produce pictures.

The partition result, here Theorem 2.3, was proved independently by another group, and will also appear in work by Caitlin Lienkaemper and co-authors.

REFERENCES

- Bank, C., Matuszewski, S., Hietpas, R. T. and Jensen, J. D. (2016). On the (un) predictability of a large intragenic fitness landscape. *Proceedings of the National Academy of Sciences*, 201612676.
- Beerenwinkel, N., Pachter, L. and Sturmfels, B. (2007). Epistasis and shapes of fitness landscapes. *Statistica Sinica*, 17:1317–1342.

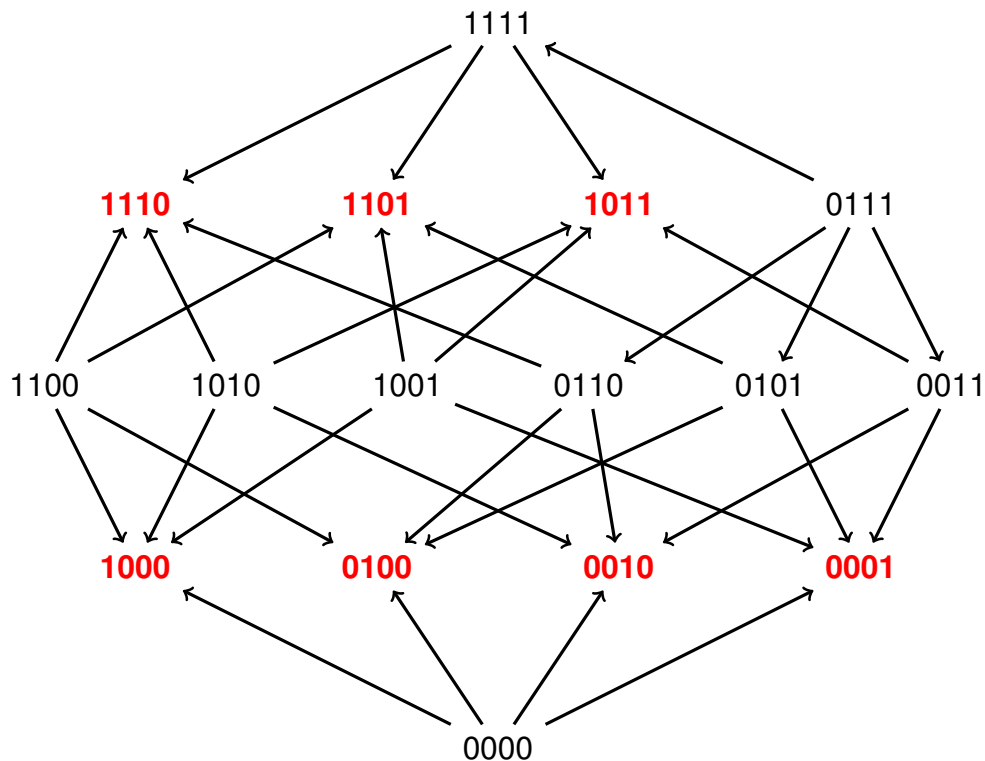


FIGURE 4. The fitness graph has 7 peaks. The graph does not imply 4-way epistasis. However, it implies 3-way epistasis.

- Beerenwinkel, N., Pachter, L., Sturmfels, B., Elena, S. F., and Lenski, R. E. (2007). Analysis of epistatic interactions and fitness landscapes using a new geometric approach. *BMC Evolutionary Biology*, 7:60.
- Crona, K., Gavryushkin, A., Greene, D., and Beerenwinkel, N. (2017). Inferring Genetic Interactions From Comparative Fitness Data. bioRxiv, 137372.
- Crona, K., Greene, D. and Barlow, M. (2013). The peaks and geometry of fitness landscapes. *Journal of theoretical biology*, 317, 1-10.
- De Visser, J. A. G., and Krug, J. (2014). Empirical fitness landscapes and the predictability of evolution. *Nature reviews. Genetics*, 15(7), 480.
- De Visser, J. A. G. M., Park, S.C. and Krug, J. (2009). Exploring the effect of sex on empirical fitness landscapes. *The American Naturalist*, 174(S1), S15-S30.
- Franke, J., Klzer, A., de Visser, J. A. G., and Krug, J. (2011). Evolutionary accessibility of mutational pathways. *PLoS computational biology*, 7(8), e1002134.
- Hall, P. (1935). On representatives of subsets. *Journal of the London Mathematical Society*, 1(1), 26-30.
- Haldane, J. B. S. (1931). A mathematical theory of natural selection. Part VIII. Metastable populations. In *Mathematical Proceedings of the Cambridge Philosophical Society* (Vol. 27, No. 1, pp. 137-142). Cambridge University Press.

- Hartl, D. (2014) What can we learn from fitness landscapes? *Current Opinion in Microbiology*, 21 (2014): 51-57.
- Kaplan, J. (2008). The end of the adaptive landscape metaphor? *Biology and Philosophy*, 23(5), 625-638.
- Poelwijk, F. J., Tanase-Nicola, S., Kiviet, D. J. and Tans, S. J. (2011). Reciprocal sign epistasis is a necessary condition for multi-peaked fitness landscapes. *Journal of theoretical biology*, 272(1), 141-144.
- Sailer, Z. R. and Harms, M. J. (2017). Detecting High-Order Epistasis in Nonlinear Genotype-Phenotype Maps. *Genetics*, 205(3), 1079-1088.
- Stanley, R. P. (2006). Acyclic orientations of graphs. *Discrete Mathematics*, 306(10), 905-909.
- Weinreich, D.M., Lan, Y., Wily. C.S, and Heckendorn, R.B. (2013) . Should evolutionary geneticists worry about higher-order epistasis? *Curr Opin Gen Dev*, 23: 700-7.
- Weinreich, D. M., Watson R. A. and Chao, L. (2005). Sign epistasis and genetic constraint on evolutionary trajectories. *Evolution*, 59, 1165–1174.
- Wu, N., Dai, L., Olson, C. A., Lloyd-Smith, J. O. and Sun, R. (2016). Adaptation in protein fitness landscapes is facilitated by indirect paths. *Elife*, 5, e16965.

4. SUPPLEMENTARY INFORMATION

4-locus fitness graphs. There are 193,270,310 fitness graphs for 4-locus systems. This can be verified by considering the chromatic polynomial for 4-cube graphs. The chromatic polynomial is

$$(x - 1)x(x^{14} - 31x^{13} + 465x^{12} - 4471x^{11} + 30793x^{10} - 160807x^9 + 657229x^8 - 2137667x^7 + 5564285x^6 - 11536667x^5 + 18740317x^4 - 23081607x^3 + 20308039x^2 - 11372201x + 3040575).$$

Evaluation of the polynomial in -1 gives 193,270,310. By Corollary 1.3 in Stanley (1973), there are 193,270,310 fitness graphs for 4-locus systems. The graphs can be partitioned into 511,863 isomorphism classes.

The argument for finding the isomorphism classes is similar to the 3-locus case (Crona et al., 2017). We generated a list of 511,863 fitness graphs, one for each isomorphism class, and identified all graphs with 6 or more peaks. We sampled graphs with fewer peaks from the list (summarized in Table 2).

Fitness landscapes with no higher order epistasis. Consider fitness landscapes of the following type:

$$w_g = 1 + ka - \binom{k}{2}e \quad \text{where} \quad k = \sum g_i$$

where a and e are positive numbers. Moreover, assume that a and e are chosen so that $w_g > 0$ for all g . For $n = 4$ we obtain the fitness landscapes discussed in the main text if $a = 0.1$ and $e = 0.06$. All genotypes with $\sum g_i = 2$ are peaks.

The same construction works for any n . It is straight forward to verify that a and e can be chosen such that w_g is maximal if $k = \lfloor \frac{n}{2} \rfloor$. Such graphs have $\binom{n}{\lfloor \frac{n}{2} \rfloor}$ peaks. For the category of fitness landscapes with no higher order epistasis, one concludes that the maximal number of peaks is

$$\geq \binom{n}{\lfloor \frac{n}{2} \rfloor}.$$

The bound is exact for $n \leq 4$, as is clear from Table 1 for $n = 4$, and from Crona et al. (2017) for $n = 3$. For asymptotic behavior of this expression as described in the main text, see also <http://oeis.org/A000984> and references therein.