SIGN EPISTASIS AND THE GEOMETRY OF INTERACTIONS

KRISTINA CRONA

ABSTRACT. Approaches to gene interactions based on sign epistasis have been highly influential in recent time. Sign epistasis is useful for relating local and global properties of fitness landscapes, as well as for analyzing evolutionary trajectories and constraints. The geometric theory of gene interactions, on the other hand, provides complete information on interactions in terms of minimal dependence relations.

We propose a new framework that combines aspects of both approaches. In particular, we provide efficient tools for identifying sign epistasis and related order perturbations in large genetic systems, with applications to the malaria-causing parasite *Plasmodium vivax*. We found that order perturbations beyond sign epistasis are prevalent in the drug-free environment, which agrees well with the observation that reversed evolution back to the ancestral type is difficult. As a theoretical application, we investigate how rank orders of genotypes with respect to fitness relates to additivity.

1. INTRODUCTION

Approaches to gene interactions based on sign epistasis, a concept introduced in Weinreich et al. (2005), have been highly influential in recent years. Sign epistasis is useful for relating local and global properties of fitness landscapes (Weinreich et al., 2005; Poelwijk, 2011; Crona et al., 2013), as well as for analyzing evolutionary predictability and other aspects of evolutionary potential and constraints (Weinreich et al., 2006; Franke et al., 2011; De Visser and Krug, 2014). A system has sign epistasis if the sign of the effect of a mutation, whether positive or negative, depends on genetic background. For instance, a biallelic 2-locus system has sign epistasis if

\[ w_{00} > w_{10}, \quad w_{11} > w_{01}, \]

where \( w_g \) denotes fitness of the genotype. Here the mutation \( 0 \rightarrow 1 \) at the first locus decreases fitness if the background is 0, and increases fitness if the background is 1.

The geometric approach to gene interactions developed in Beerwinkel et al. (2007) provides complete information on interactions in terms of a class of linear forms (circuits, as defined in matroid theory, see the Result section for details). For two-locus system, one considers the linear form

\[ u = w_{11} + w_{00} - w_{10} - w_{01}. \]

Notice that the conditions \( w_{00} > w_{10} \) and \( w_{11} > w_{01} \) implies that \( u > 0 \). This simple observation suggests a relation between approaches focused on sign epistasis and the geometric approach.
Here, we investigate how the different approaches relate and propose a framework that combines aspects of both theories. In brief, the sign of the effect of a double mutant, or any higher order mutant, may depend on background. Such order perturbations are similar to sign epistasis, except that we consider the effect of replacing blocks rather than a single locus. In particular, it is of interest to identify block replacements that are universally beneficial. Importantly, we need not assume block independence, but rather that the sign of the effect is independent.

2. RESULTS

We consider biallelic $n$-locus systems. For simplicity, we assume that there exists a total order of the genotypes with respect to fitness, usually referred to as the rank order.

It is straightforward to analyze sign epistasis in the two-locus case. A system has sign epistasis if at least one of the following four conditions are satisfied:

$$w_{00} > w_{10} \text{ and } w_{11} > w_{01},$$
$$w_{00} > w_{01} \text{ and } w_{11} > w_{10},$$
$$w_{00} < w_{10}, \text{ and } w_{11} < w_{01},$$
$$w_{00} < w_{01}, \text{ and } w_{11} < w_{10}.$$

Differently expressed, a system has sign epistasis exactly if at least one of the following expressions is negative.

$$\begin{align*}
(w_{00} - w_{10})(w_{01} - w_{11}) & \quad (1) \\
(w_{00} - w_{01})(w_{10} - w_{11}) & \quad (2)
\end{align*}$$

For the rank order $w_{11} > w_{10} > w_{01} > w_{00}$ both (1) and (2) are positive. On the other hand, for the rank order $w_{11} > w_{00} > w_{10} > w_{01}$ both (1) and (2) are negative. Such a "double flip" is sometimes referred to as reciprocal sign epistasis (Poelwijk, 2011).

For a general analysis of $n$-locus system, one can consider a set of expressions analogous to (1) and (2). It is convenient to introduce circuits for a systematic description. By using linear algebra, we can keep the discussion elementary.

For the genotypes 00, 10, 01, 11, one can form vectors in $\mathbb{R}^3$ by adding an extra coordinate $1$

$$(0, 0, 1), (1, 0, 1), (0, 1, 1), (1, 1, 1).$$

The four vectors are linearly dependent,

$$(1, 1, 1) + (0, 0, 1) - (1, 0, 1) - (0, 1, 1) = 0$$

Notice that the dependence relation corresponds exactly to

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

The form $u$ is referred to as a circuit. In general, circuits are defined as minimal dependence relations, in the sense that each proper subset of the vectors [with non-zero coefficients] are linearly independent. (Here each proper subset of the vectors $(0, 0, 1)$, $(1, 0, 1)$, $(0, 1, 1)$ and $(1, 1, 1)$ is linearly independent.) The description works in general,
since one can form vectors in $\mathbb{R}^{n+1}$ from the vertices of the $n$-cube by adding a coordinate 1. The circuits are the minimal dependence relations, exactly as in the two-locus case.

**Remark 2.1.** The relation between circuits and sign epistasis can be summarized as follows for $n = 2$.

(i) A rank order of the genotypes 00, 10, 01 and 11 with respect to fitness implies that $u > 0$ or $u < 0$ exactly if the system has sign epistasis.

(ii) From the information that the rank order implies $u > 0$ (or $u < 0$) alone, one cannot determine if there are one or two order perturbations, i.e., whether or not the system has reciprocal sign epistasis.

(iii) The system has reciprocal sign epistasis if both expressions (1) and (2) are negative, and sign epistasis if at least one of them is negative.

A similar approach works for a general $n$-locus systems, although one needs to consider more general order perturbations. For $n = 3$, the set of circuits includes

$$w_{111} + w_{000} - w_{100} - w_{011}.$$ 

Similar to the study of the two-locus case, it is natural to consider the two related expressions

$$\begin{align*}
(w_{111} - w_{011})(w_{100} - w_{000}) \\
(w_{111} - w_{100})(w_{011} - w_{000}).
\end{align*}$$

(3) (4)

If the rank order implies that (3) is negative, then the system has sign epistasis. If the rank order implies that (4) is negative, then the order perturbation is of a different type. Specifically, the sign of the effect of a double mutation 00 $\rightarrow$ 11 at the second and third loci depends on background.

**Definition 2.2.** A system has a rectangular perturbation if the sign of the effect of replacing a subset of loci, according to the rule 0 $\rightarrow$ 1 and 1 $\rightarrow$ 0, depends on background. The size of the perturbation refers to the number of loci replaced.

In particular, a rectangular perturbation of size one is a case of sign epistasis. However, the next example shows that a system can have rectangular perturbations even if it does not have sign epistasis.

**Example 2.3.** Consider the system:

$w_{000} = 1, w_{100} = 1.1, w_{010} = 1.12, w_{001} = 1.09, w_{110} = 1.2, w_{101} = 1.22, w_{011} = 1.19, w_{111} = 1.3.$

The change 0 $\rightarrow$ 1 at any locus increases fitness regardless of background. Consequently, there is no sign epistasis. However, the sign of the effect of the change 10 $\rightarrow$ 01 at the first pair of loci depends on background since

$$w_{100} < w_{010}, \quad w_{101} > w_{110}.$$
Example 1 is interesting for another reason. The example shows that the rank order

\[ w_{111} > w_{101} > w_{110} > w_{011} > w_{100} > w_{001} > w_{000}, \]

is incompatible with additive fitness, even though the system has no sign epistasis.

For investigating rectangular order perturbations, one can use the class of circuits \( C \) where exactly four variables have non-zero coefficients. For \( n = 3 \), there are 12 such circuits. It follows that one can identify all rectangular perturbations by checking 24 expressions, including (3) and (4) above, (see the appendix for the complete list).

**Proposition 2.4.** Let \( C \) be the class of circuits with non-zero coefficients for exactly four elements.

(i) Each case of sign epistasis corresponds a signed circuit interaction for an element in \( C \). i.e., the rank order of genotypes with respect to fitness implies that the circuit is positive or negative.

(ii) More generally, each rectangular perturbation corresponds to a signed circuit interaction in \( C \).

(iii) Each circuit in \( C \) corresponds to exactly two (potential) rectangular perturbations.

**Theorem 2.5.** The total number of (potential) rectangular perturbations for an \( n \)-locus system is

\[ 2 \left( \frac{6^n}{8} - 4^{n-1} + 2^{n-3} \right). \]

Moreover, the number of rectangular perturbations of size \( k \) (exactly \( k \) loci are replaced) equals

\[ 2^{k-1} \binom{n}{k} \binom{2^{n-k}}{2}. \]

For a proof of this result, see Methods. In particular, it follows from Theorem 2.5 that the number of potential order perturbations for a three-locus system is 24, as mentioned (see the appendix). However, it is not possible that all expressions are negative for one and the same system.

The next result follows immediately from the theorem.

**Corollary 2.6.** A complete investigation of sign epistasis for an \( n \)-locus system, requires that one checks the sign of

\[ n \binom{2^{n-1}}{2}, \]

expressions.

We applied rectangular perturbations to a study of the malaria-causing parasite *Plasmodium vivax* (Ogbunugafor and Hartl, 2016). The original study concerns a 4-locus system exposed to different concentrations of the anti-malarial drug pyrimethamine (PYR). The quadruple mutant denoted 1111 has the highest degree of drug resistance, whereas the genotype 0000 has the highest fitness among all genotypes in the drug-free environment.
TABLE 1. Rectangular perturbations for drug exposed and drug free malaria fitness landscapes. The prevalence of sign epistasis (size 1 perturbations) is similar. However, rectangular perturbation of size 2 and 3 differ substantially between the landscapes.

| Perturbation size | 1 | 2 | 3 | size (1-3) |
|-------------------|---|---|---|-----------|
| Drug-exposed      | 54 | 39 | 9 | 102 |
| Drug-free         | 55 | 21 | 5 | 81 |

We compared the highest concentration of the drug and the drug-free environment. Sign epistasis were prevalent in both fitness landscapes; the landscapes had 54 and 55 rectangular perturbations of size one, respectively. However, the latter landscape had approximately twice as many perturbations of size two and three. This finding agrees well with the authors’ observation that resistance development is a relatively straightforward process, whereas reversed evolution from the four-tuple mutant 1111 back to the ancestral type is difficult.

One can also apply rectangular perturbations for an analysis of rank orders and additivity. For a three-locus system, there are $8! = 40,320$ rank orders. As remarked in Crona et al. (2017), exactly 384 orders are compatible with absence of sign epistasis. By using the list in the appendix, one can verify that 288 orders imply rectangular order perturbations. Moreover, one can verify that the remaining 96 orders are compatible with additive fitness. In summary, 0.24 percent of all rank orders for three-locus systems are compatible with additive fitness. In fact, after relabeling only the following two orders are compatible with additive fitness.

\[ w_{111} > w_{110} > w_{101} > w_{011} > w_{100} > w_{010} > w_{001} > w_{000} \]
\[ w_{111} > w_{110} > w_{101} > w_{100} > w_{011} > w_{010} > w_{001} > w_{000} \]

3. METHODS

For counting rectangular perturbations, one needs to find all rectangles with vertices in an $n$-cube. We provide a proof for the reader’s convenience. The proof depends on Stirling numbers of the second kind. We refer to Grimaldi (2006) for concepts used in this section.

**Lemma 3.1.** For Stirling numbers of the second kind $S(n, k)$ the following identities hold.

\[ S(n, 2) = 2^{n-1} - 1 \]
\[ S(n, 3) = \frac{1}{6}(3^n - 3 \cdot 2^n + 3) \]

**Proof.** The first formula holds since there are $2^n - 2$ non-empty proper subsets of $n$ elements, and each partition corresponds to exactly two subsets. Similarly, the second formula can be derived from the observation that one can construct three labeled subsets of $n$ elements in $3^n$ ways. After reducing for all cases with empty sets, the number
of alternatives is
\[ 3^n - 3(2^n - 2) - 3 = 3^n - 3 \cdot 2^n + 3. \]

Each partition corresponds to 6 alternatives, which completes the argument. □

Lemma 3.2. There are
\[ \frac{6^n}{8} - 4^{n-1} + 2^{n-3} \]
rectangles with vertices in an \( n \)-cube.

Proof. For each vertex \( s_1 \ldots s_n \) in an \( n \)-cube, one can construct a rectangle with vertices on the \( n \)-cube as follows. Distribute the set of \( n \) loci into three subsets \( S_1, S_2 \) and \( S_3 \), where the intersection of each pair of sets is empty, and where \( S_1 \) and \( S_2 \) are non-empty.

From the vertex \( s_1 \ldots s_n \), one constructs the remaining vertices by replacing sets of loci, according to the rule \( 0 \mapsto 1 \), and \( 1 \mapsto 0 \). Specifically, one vertex is obtained by replacing all elements in \( S_1 \), one by replacing all elements in \( S_2 \), and the last by replacing all elements in \( S_1 \cup S_2 \). In case \( S_3 \) is empty, one can construct \( S(n, 2) \) rectangles. If all three sets are non-empty, then one can choose \( S_3 \) in three ways, and consequently construct \( 3 \cdot S(n, 3) \) rectangles. In total one obtains \( 3 \cdot S(n, 3) + S(n, 2) \) rectangles starting from a particular vertex. There are \( 2^n \) vertices in the \( n \)-cube and each rectangle has four vertices. By the previous lemma, the number of rectangles is
\[ \frac{2^n}{4} \cdot (3 \cdot S(n, 3) + S(n, 2)) = \frac{6^n}{8} - 4^{n-1} + 2^{n-3}, \]
which completes the proof. □

We can now prove the main result.

Proof of Theorem 2.5. The total number of (potential) rectangular perturbations for an \( n \)-locus system is
\[ 2 \left( \frac{6^n}{8} - 4^{n-1} + 2^{n-3} \right). \]
A rectangle with vertices in the \( n \)-cube corresponds to exactly two rectangular order perturbations, one for each pair of parallel edges. Consequently, the result follows from Lemma 3.1.

The second part of the theorem states that the number of rectangular perturbations where exactly \( k \) loci are replaced equals
\[ 2^{k-1} \binom{n}{k} \binom{2^{n-k}}{2}. \]

The positions of the \( k \) loci that change can be chosen in \( \binom{n}{k} \) different ways. There are \( 2^k \) words of length \( k \). One can choose the word and its replacement in \( 2^{k-1} \) different ways (for instance the replacement of 110 is 001). Finally, that are \( 2^{n-k} \) different backgrounds, so that a pair of backgrounds can be chosen in \( \binom{2^{n-k}}{2} \) ways.
REFERENCES

Beerenwinkel, N., Pachter, L. and Sturmfels, B. (2007). Epistasis and shapes of fitness landscapes. *Statistica Sinica*, 17:1317–1342.

Crona, K., Gavryushkin, A., Greene, D. and Beerenwinkel, N. (2017). Inferring genetic interactions from comparative fitness data. *eLife*, 6.

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.

Franke, J., Klözer, A., de Visser, J. A. G. and Krug, J. (2011). Evolutionary accessibility of mutational pathways. *PLoS computational biology*, 7(8), e1002134.

Grimaldi, Ralph P (2006). *Discrete and Combinatorial Mathematics*, 5/e. Pearson Education India.

Ogbunugafor, C. B. and Hartl, D. (2016). A pivot mutation impedes reverse evolution across an adaptive landscape for drug resistance in Plasmodium vivax. *Malaria journal*, 15(1), 40.

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.

Weinreich, D. M., Delaney, N. F., DePristo, M. A. and Hartl, D. L. (2006). Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science*, 312(5770), 111-114.

Weinreich, D. M., Watson R. A. and Chao, L. (2005). Sign epistasis and genetic constraint on evolutionary trajectories. *Evolution*, 59, 1165–1174.
4. APPENDIX

The complete list of expressions for identifying rectangular perturbations for $n = 3$. The first 18 expressions can identify sign epistasis and the last 6 expressions rectangular perturbations where two loci are replaced.

$$(w_{000} - w_{100})(w_{010} - w_{110})$$
$$(w_{000} - w_{100})(w_{001} - w_{101})$$
$$(w_{000} - w_{100})(w_{011} - w_{111})$$
$$(w_{010} - w_{110})(w_{001} - w_{101})$$
$$(w_{010} - w_{110})(w_{011} - w_{111})$$
$$(w_{001} - w_{101})(w_{011} - w_{111})$$
$$(w_{000} - w_{010})(w_{100} - w_{110})$$
$$(w_{000} - w_{010})(w_{001} - w_{011})$$
$$(w_{000} - w_{010})(w_{101} - w_{111})$$
$$(w_{100} - w_{110})(w_{001} - w_{011})$$
$$(w_{100} - w_{110})(w_{101} - w_{111})$$
$$(w_{001} - w_{011})(w_{101} - w_{111})$$
$$(w_{000} - w_{001})(w_{100} - w_{101})$$
$$(w_{000} - w_{001})(w_{010} - w_{011})$$
$$(w_{000} - w_{001})(w_{110} - w_{111})$$
$$(w_{100} - w_{101})(w_{010} - w_{011})$$
$$(w_{100} - w_{101})(w_{110} - w_{111})$$
$$(w_{010} - w_{011})(w_{110} - w_{111})$$
$$(w_{000} - w_{101})(w_{010} - w_{111})$$
$$(w_{000} - w_{101})(w_{100} - w_{111})$$
$$(w_{010} - w_{001})(w_{110} - w_{011})$$
$$(w_{010} - w_{001})(w_{101} - w_{011})$$
$$(w_{100} - w_{001})(w_{110} - w_{011})$$
$$(w_{010} - w_{001})(w_{101} - w_{011})$$
$$(w_{000} - w_{001})(w_{110} - w_{101})$$

E-mail address: kcrona@american.edu