Defining Higher-Order Interactions in Synthetic Ecology: Lessons from Physics and Quantitative Genetics

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A new paper from Mickalide and Kuehn studies a well-controlled microbial trophic chain and identifies a high-order interaction between its species.

Interactions between the components of biological systems are critical for biological function at all levels of organization. Quantitatively understanding these interactions and mapping out their systems-level consequences have been major aspirations in biology (Tekin et al., 2018). Historically, the study of interactions has been reductionist and largely focused on pairwise effects. An underlying assumption was that once understood, these pairwise interactions could then be integrated to predict the behavior of systems composed of many components.

To what extent is this assumption correct? The existence of interactions that exist only when three or more components are present (higher-than-pairwise, or high-order interactions [HOIs]) has been recognized for a long time in fields that deal with higher levels of organization such as ecology (Billick and Case, 1994) or genetics (Weinreich et al., 2013), but their study also has a long tradition in areas of biology that deal with the molecular scale (Estrada et al., 2016). The diversity of fields that deal with HOIs has led to some confusion about what does and does not count as one; and proposing a single definition that is universally valid and useful across fields has been challenging.

Perhaps the closest such "universal" definition is an operational one, as a deviation from the prediction of a null model where only up to pairwise interactions are included. This definition is the one adopted in quantitative genetics (Weinreich et al., 2013), and it has also been recently used to characterize the function of systems of neurons, the activity of drug cocktails, the folding of proteins or the effect of microbiome composition on host life-history traits (see (Tekin et al., 2018) and references therein). Defined in this way, interactions emerge from the failure of a null model that does not include them.

The crux of the problem is therefore not the identification of putative HOIs, but the validity of the null model itself. Interactions may be found simply because the null model fails to capture the basic biology with sufficient realism. In other fields of science, more notably in physics and chemistry, it is often possible to formulate quantitative models that both accurately describe experimental systems and are also derived from first principles and laws of nature. The failure of such first-principles pairwise models can be interpreted as evidence of fundamental high-order interactions, because it demonstrates that pairwise interactions alone are inadequate to explain observations (Büchler et al., 2007; Weinberg, 1992). However, first-principles theory is very difficult to formulate at higher levels of organization in biology, where models often consist of a coarse-grained, simplified representation of our current understanding of complex phenomena (Gunawardena, 2014).

In the particular case of ecology, at least three different definitions of HOIs have been historically proposed (Billick and Case, 1994). One is mechanistic, as the modification of a pairwise interaction when a third species is present. A second one is typically invoked in theoretical models, as a non-additive effect of two species on the per-capita growth of another. The third definition aligns with the generic "operational" definition presented above, as the failure of a null pairwise model. Mathematically, these pairwise models do not contain terms (neither explicitly nor implicitly) that are multiplicative on the densities of three or more species (x1*x2*x3) (Billick and Case, 1994).

In this issue of *Cell Systems*, Mickalide and Kuehn (2019) present a study of a synthetic "ABC" ecosystem, where they identify the presence of an HOI that solidly complies with the first of these definitions. A mechanistic "interaction modification" is observed, where a single-celled algae (*Chlamydomonas reinhardtii*; species "A") modifies an interaction between a predatory ciliate (*Tetrahymena thermophila*; species "C") and the bacterium *E. coli* (species "B"). This interaction is mechanistically caused by a phenotypic change of *E. coli* in the presence of *C. reinhardtii*: the algae inhibits aggregation of *E. coli* cells, making them more vulnerable to predation by the ciliate. A main strength of this work is the unambiguous demonstration of an interaction modification in a well-controlled microbial trophic chain, which allows a direct quantitative comparison between models and experiments.

A question could be raised of whether a well-controlled experimental system, such as the one studied by Mickalide and Kuehn (2019), may help us bridge the three definitions of HOIs outlined above, bringing it closer to the kind of analysis one can do in the physical sciences. Unlike in physics, ecological models describe systems at higher levels of organization and are often phenomenological. Additivity is thus an assumption that does not arise from first principles and is often violated, even in the simplest of scenarios. Indeed, non-additive effects arise quite easily in a wide range of mechanistic ecological models (e.g., in consumer-resource dynamics under most conditions [Letten and Stouffer 2019]), as a result of coarse-graining mechanistic interactions into effective interaction coefficients. In the paper by Mickalide and Kuehn, the authors provide an example of this, by formulating a minimal mechanistic model of interactions in their system, which they parameterized empirically. The authors show that this mechanistic model does not explicitly contain any terms that are that are multiplicative on the densities of three or more species (x1*x2*x3), yet these multiplicative terms arise when they coarse-grain their mechanistic model into density-dependent interaction parameters. Thus, a theoretical model that reflects the mechanism of interaction proposed by the authors exhibits non-additive effects on per-capita growth, consistent with the second definition of HOIs.

Although this mathematical model predicts invasion dynamics that are in qualitative agreement with experiment, the lack of a quantitative agreement (which is exceedingly rare in ecological systems) makes it challenging to unambiguously and definitively prove the second definition. This brings us to the third "operational" definition of HOIs. In a recent piece, Letten and Stouffer (2019) have thoughtfully argued that HOIs defined in this manner are "emergent phenomenological representations of underlying non-additive processes," and a property of the models themselves that are used to study competition. One might thus question the value of such an "operational" definition of interactions in general and of HOIs in particular.

Beyond their potential utility to improve prediction in ecology (which is often [Letten and Stouffer, 2019] though not always granted, e.g., [Sanchez-Gorostiaga et al., 2018]), an example of the validity and usefulness of such an operational definition of interactions at high-levels of organization may be found in another field that deals with higher-levels of organization: quantitative genetics (Mackay, 2014; Weinreich et al., 2013). Here, an interaction between two mutations (i.e., epistasis) is defined as the deviation between the quantitative phenotype of a double mutant and the prediction from a (typically additive) null model. In turn, HOIs (high-order epistasis) can be defined as deviations from adding up all of the pairwise interactions (Weinreich et al., 2013). This latter situation reflects that interactions between two mutations can be modulated by the genetic background.

All the problems of coarse-graining mechanistic interactions that were discussed above in the context of ecological systems exist as well in the definition of epistasis. However, this has not subtracted from its usefulness as a quantitative concept. Despite the many mechanistic reasons why additivity may fail to describe the combined effect of mutations, it often represents a very strong approximation to quantitative traits in fitness landscapes (e.g., Mackay, 2014). In ecology, we have recently shown that additivity may counterintuitively emerge even in consortia that are dominated by strong competitive interactions (Sanchez-Gorostiaga et al., 2018). These results reinforce the idea that "operationally defined" interactions—and HOIs in particular—may be useful even when we are far from the situation one finds in physics, where they reflect fundamental microscopic phenomena.

Given these considerations, definitively identifying HOIs, in ecology and elsewhere, remains a challenge worth pursuing. More broadly, understanding when and why they should improve predictive power, and when they will instead make prediction impossible remains a critical question. Integrating the various definitions of what an HOI is may not be possible, as they may refer to subtly different phenomenon at different levels of organization and across fields. While this may inevitably lead to some ambiguity, it may be a price worth paying for the benefits we get.

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