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**Next steps in integrative biology: Mapping interactive processes across levels of biological organization**

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Perspective

Next steps in integrative biology: Mapping interactive processes across levels of biological organization

Sonia Cavigelli <sup>1\*</sup>, Jeff Leips <sup>2</sup>, Qiu-Yun (Jenny) Xiang <sup>3</sup>, Dawn Lemke <sup>4</sup>, Nicolai Konow <sup>5</sup>

<sup>1</sup> Department of Biobehavioral Health, Pennsylvania State University, University Park PA 16802

<sup>2</sup> Department of Biological Sciences, University of Maryland Baltimore County, Baltimore MD 21250

<sup>3</sup> Department of Plant and Microbial Biology, North Carolina State University, Raleigh NC 27695

<sup>4</sup> Department of Biological and Environmental Sciences, Alabama A&M University, Huntsville AL 35811

<sup>5</sup> Department of Biological Sciences, University of Massachusetts Lowell, Lowell MA 01854

Synopsis

Emergent biological processes result from complex interactions within and across levels of biological organization, ranging from molecular to environmental dynamics. Powerful theories, database tools, and modeling methods have been designed to characterize network connections within levels, such as those among genes, proteins, biochemicals, cells, organisms and species. Here, we propose that developing integrative models of organismal function in complex environments can be facilitated by taking advantage of these methods to identify key nodes of communication *across* levels of organization. Mapping key drivers or connections among levels of organization will provide data and leverage to model potential rule-sets by which organisms respond and adjust to perturbations at any level of biological organization.

\*Corresponding author:

Sonia A. Cavigelli  
219 Biobehavioral Health Building  
Pennsylvania State University  
University Park, PA 16801  
Email: [sac34@psu.edu](mailto:sac34@psu.edu)  
Phone: 814.863.0210  
Fax: 814.863.7525

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Biological systems are organized into and controlled across a size-hierarchy, from molecular to cellular, tissue to organ, organ to organism, and organism to society, ecosystem, and biome. Different entities within each level of organization interact at varying spatial and temporal scales, and their interactions determine static as well as dynamic properties of the system. In addition, interactions within a given level influence and are influenced by interactions at both higher and lower levels of organization, which together determine emergent properties of biological systems. For example, at the organism level, the introduction of rabbits to Australia and sub-Antarctic islands in the 1800s and the removal of wolves from North-American ecosystems had dramatic cascading effects across multiple trophic levels throughout those ecosystems (Ripple and Beschta 2004; Pansu et al. 2015). Across different levels of organization, cellular function is affected by nutrients and molecular processes, which in turn affect function of the whole organism and ecological communities (reviewed in Warne 2014). In the current perspective paper, we identify examples of how cross-level biological analyses can accelerate our understanding of complex and relatively elusive systems. We then provide a general framework in which to develop cross-level modeling without ignoring inherent within-level system complexity, and we propose a specific strategy by which to structure and conduct this work. We review specific methods and current resources that are powerful to include, and identify some limitations to address. We summarize by identifying potential benefits of integrative, cross-level modeling with a brief nod to areas to address which will make this work most efficient.

**Examples of cross-level biological analyses to better understand complex systems**

**Multi-level approaches would undoubtedly lead to a better understanding of most complex biological systems. Here we highlight two examples to illustrate this point.** Vertebrate skeletal muscle has an enigmatic capacity to self-repair after a single bout of activated muscle stretch, for instance when landing from a jump or running down a hill. These insults initially cause injury, but after recovery and repair, the muscle may be stronger and more resilient to re-injury (McHugh et al. 1999). Although this phenomenon has been known for almost a century, mechanisms that govern it remain unclear despite decades of intense research (Lieber 2018). Indeed, muscle stretch injury is a physiological “black box”. Hierarchical composition, coupled with multi-level complexity of muscle structure and function (e.g. Roberts et al. 2019) is one reason that it has been difficult to determine which attributes of stretch are the prime cause(s) of injury (i.e. force, speed, strain, or starting length; Lieber et al. 1991; Proske and Morgan, 2001; Butterfield and Herzog 2006), and which level(s) of biological organization (molecules, proteins, filaments, fibers, fascicles) sustain primary injury. We know that stretch-injury triggers cytokine storms, inflammatory responses, and genetic signaling cascades (Barash et al. 2004) but without dedicated multi-level approaches, it will remain difficult to make genome-to-phenome connections. Major discoveries elude us, such as explanations of how subject behavior, environmental factors, and drug interventions affect the susceptibility of re-injury (Mishra et al. 1995; Toumi and Best 2003), and why re-injury risk is reduced at the affected muscle and also unaffected neighboring muscle (McHugh et al. 1999).

Another example of the benefits of multi-level integration involves our understanding of how/why individuals develop and maintain different behavioral traits (like aggression/hostility,

boldness/risk-taking, etc.). Phenotypic variance in behavior and social status are often associated with health and fitness, but we have limited understanding as to why (e.g. Friedman 2000; Dingemanse et al. 2004; Sapolsky 2004; Biro and Stamps 2008). Recent integrative research has provided the first steps to identify ‘lower’ and ‘higher’ level processes that are associated with these phenotypes, which provides the first key steps to identify mechanisms by which behavioral traits influence organismal health and fitness (e.g. Robinson et al. 2008; Van Oers & Mueller 2010; Rey et al. 2013; Lea et al 2018; Snyder-Mackler et al. 2016; Ruiz-Ortiz and Tollkuhn 2021). Further integrative work will provide key information to understand how simple behavioral traits evolve, are maintained, and influence individual and group health and fitness in different or changing environmental conditions.

These examples illustrate the complex bidirectional cascading effects that occur across levels of biological organization, and the potential benefits of developing cross-level models to understand these complex phenomena. With the exception of ecological network models (e.g., Fath et al. 2007; Niquil et al. 2020) and recent advances in evo-devo models (e.g., McMillan et al. 2020), most methods have been developed to model within-level interactions (e.g. genetic networks, social networks, etc.). Improved characterization of interactions among levels will facilitate more accurate predictions of wide-scale effects that result from relatively simple perturbations at a single level (e.g. a simple mutation, organ injury, species introduction, shifting environmental conditions). Given our capacity to develop complex models, and a rapid increase in large data sets to understand within-level biological organization, the next step will be to push for targeted integration of these methods and resources to provide more nuanced modeling of cascading interactions across levels of biological organization.

**General framework for conducting complex but manageable cross-level modeling**

Understanding how processes interact across levels of biological organization allows us to predict how biological systems will respond to perturbations at different levels (how perturbations at lower levels affect higher level function, and vice versa). Establishing the strength and direction of linear and non-linear pathways between levels will provide better predictive power for feedforward and feedback models within networks at any given level. Identification of *key nodes of communication* between different levels of organization would make this task manageable. When appropriately identified, key nodes of communication should reduce noise within network connections and strengthen the predictive ability of models within and across levels. The strength of the proposed integrative modeling is its ability to understand impacts across widely-separated levels such as understanding the impacts of climate change down to the cellular level, which may have long-term impacts on organism fitness and survival. Fundamentally, a better understanding of key pathways of communication between hierarchical levels will improve our understanding of the *Rules of Life* (*sensu* the US National Science Foundation).

Technological advances have greatly increased our ability to collect large, robust data sets at a single level of biological organization (e.g. genomics, transcriptomics, proteomics, metabolomics, etc.). Now we can branch out to identify and test connections among levels and

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systems. Network analyses tend to model single levels of organization (e.g. molecular, cellular), leaving details about connections among levels, including their strength, direction and feedback/forward nature, poorly understood. How might this be done? Large-scale within-level data sets can be used to statistically identify possible key nodes within a given level of organization (i.e. nodes that are highly connected to many others, similar to the concept of ‘hub’ genes within a genetic network or ‘centrality’ in a social network; Lehner et al. 2006; Stephenson and Zelen 1989). Statistical techniques similar to weighted gene correlation network models (e.g., Langfelder and Horvath 2008) can be used to identify “nodes” that connect networks across levels of organization using large data sets. Then, the relative significance of these key nodes at one level can be manipulated to test their impact on processes or nodes at levels above or below in the hierarchy. Using classic or modern experimental techniques (e.g. CRISPR/Cas, DREADDS, optogenetics, etc.), we can experimentally test whether key, within-level nodes represent drivers of key processes at higher and lower levels of organization. Identification and testing of key nodes of communication, within and between levels of biological organization, provides a means to reasonably model complex interactions among levels. (See Figure 1 for a visual depiction of the goals for convergence science to map key communication nodes among levels of biological organization.)

### How do we begin to understand key interactions across levels of biological organization?

Tackling questions about the *Rules of Life* requires collaborative and convergence research. Convergence research, or transdisciplinary research, involves deep integration among scientific disciplines to address specific problems (<https://www.nsf.gov/od/oia/convergence/index.jsp>). Such integration requires expertise of biologists broadly trained with multidisciplinary skills and collaboration with mathematicians, physicists, engineers, and/or social scientists for modeling hierarchical processes (e.g., Iglesia and Ingalls 2010; reviewed in Padilla et al. 2014). Such an approach can mitigate the common problem in single-discipline science that strong patterns in data are mechanistically inexplicable; an outcome caused by changes at organizational level(s) that are not measurable with the tools of a single discipline. For example, muscle-stretch injury causes contractile functional changes that are difficult to explain without information on genetic regulation and protein expression (Barash et al. 2004). Rapidly-developing techniques in genomics (e.g., genome sequencing, single-cell and single-nuclei RNA-seq, in-vitro cell system, genome editing, genome imaging, and artificial intelligence; Nguyen et al. 2021; Hettige et al. 2020) and proteomics (Torson et al. 2020) can be combined with classic methods such as the *in-vitro* motility assay (Warshaw 1996) and *in vivo* muscle ergometry (Tijs et al. 2021) to measure contraction force/speed/power of single muscles, as well as their individual cells (myocytes), and contractile protein ensembles (actin, myosin, troponin, tropomyosin). Such multi-method integration facilitates the initial steps needed to identify functional connections across levels and determine cascades of genetic-molecular signaling and post-translational protein changes that tune muscle function after injury. Similarly, bridging across multiple levels of organization, altered expression patterns of key regulators of inflorescence affect its ontogeny and structure, which affect species reproduction, ecology, and evolution, which cannot be understood without careful studies of morphology, development, reproductive biology,



phenology, demography, molecular genetics, and phylogeny of related species (Harder and Prusinkiewicz 2013; Kirchoff and Claßen-Bockhoff 2013). **Specific outcomes at each level provides information on key nodes to enable cross-level process integration through appropriate modeling.** An additional example in plants comes from the phenomenon of polyploidy and how it can generate novel phenotypes (Levin 1983). Doyle and Coate (2019) reviewed the many mechanisms, at different levels of organization (genetic, transcriptomic, proteomic, nucleic, cellular, and metabolic), in the complex pathway by which polyploidy contributes to novelty. Only by modeling complex mechanisms at each level, and understanding how these levels interact, can we understand how polyploidy has evolved and how it affects plant morphology and success. Last, changes in the biodiversity of species that play key ecological roles in communities can have cascading effects across an ecosystem. Nowhere is this better exemplified than in the case of the global loss of pollinator biodiversity and the potential for this loss to produce not only extreme ecological impacts but also on human society (Christmann 2019). Such cascade changes cannot be explained without integrative, multilevel information on taxonomy, phylogenetics, physiology, biogeography, and ecology of individual species and their communities. Convergence research approaches will need to be implemented to model interactions across levels in order to capture otherwise unquantifiable relationships between form, function, and mechanics, within and across systems, and enable prediction of their responses to perturbations across biological levels of organization.

How do we begin to tackle this problem? **First**, we propose research on relatively simple systems, with manageable complexity of interactions across few levels of organization (e.g. protein interactions within and across cells). **If the rules of biological interactions across different scales are consistent from scale to the next, this initial work can help train network analysis and Artificial Intelligence (AI) algorithms to scale across levels.** **Second**, we propose using classic “model” organisms (e.g. *Caenorhabditis elegans*, *Danio rerio*) to capitalize on data sets that include documentation of complex networks at single levels, plus data on across-level interactions to further train algorithms. **Third**, we propose application of suites of bench-marked algorithms on classically challenging systems, like muscle processes, complex structure changes, and behavior. **Fourth**, moving on from steady-state systems, we propose developing predictive analysis approaches to tackle truly complex systems, such as environmental change (in a cell, or across the planet), where innumerable feed-back loops currently compound our ability to draw conclusions that could inform policy-making, governance, and emergency preparedness. **More accurate/effective monitoring of environment, food, and health allow for prediction of population susceptibility and responses to environmental challenges, injury/illness, and aging.** This broad and hierarchical methodological game plan capitalizes on currently-available data and methods, but also requires wide-scale collaboration to integrate expertise and techniques across disciplines.

The goal is to inform advances needed to progress to a stage in biological research where we are capable of generating predictive network models of the relationships existing across different levels of biological organization. This will enable us to explore how these relationships vary (or remain invariant) across systems with different levels of complexity and the extent to which these relationships are altered by changes in environmental conditions. These goals will

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require substantial (re)integration of knowledge from across disparate STEM fields, not limited to biology. (Sample research framework and methods are provided below and in Figure 2.)

Necessary contributions will include collaborators that collect comprehensive basic datasets at different organization levels, from suitable candidate systems (i.e., relatively low-complexity systems, model-organism systems where data exist at multiple levels, and high-complexity systems). To explore relatively simple interactions, models can be developed to predict organismal phenotypes from gene regulation networks, and then with multiple possible species phenotypes, predict species biotic and abiotic interaction networks, and then model these networks in different environmental conditions. One useful approach to integrate information across levels and identify drivers of a biological property is through comparative analyses of attributes from different levels on a phylogenetic tree or phylogenetic network (e.g., character mapping or reconstructing ancestral character state; Joy et al. 2016). This allows identification of changes that co-occur at a specific phylogenetic branch or node. Cross-level correlated changes can illuminate the key communication links across levels of biological organization. As an example of one node in the molecular network, a mutation in a regulatory gene in the anthocyanin pathway may be associated with lower gene expression, which may be associated with a change in flower pigment production, and further associated with a shift in pollinator, an increase of seed production, a niche shift to high elevation, and range expansion. If networks at each level are well understood, then further cross-level modeling can be pursued. Repeating such analyses for all key nodes at different levels, information can then be linked and connected to build predictive models. To conduct such work, existing or newly developed *in-silico* modeling approaches can be used to build within-level networks (for example: biomechanics (OpenSIM), ecology (MaxEnt), physics (Simulink), genetics (GeneticNetworks, CaiNet), physiology (Physiome projects' JSim), followed by phylogenetic mapping analyses, with appropriate evolutionary models for the specific attributes under consideration, using phylogenetic tools such as Phytools (<http://blog.phytools.org/2011/12/citing-phytools.html>; Revell 2012). Computational scientists must be recruited to generate network analysis approaches that are capable of dynamically relating correlated attributes uncovered by the mapping analyses from different determinant-level "sheets" (formed by the above-mentioned modeling approaches) to one another; this could be helpful for initially establishing system interconnections across levels. The Physiome Projects provides an example of a comprehensive cross-level integration program (Bassingthwaite 2010). Using artificial intelligence approaches, it may then be possible to establish general patterns of feed-forward/feed-back in response to system state-changes that either result through predicted processes (environmental seasonality, maturation, aging) or episodic state-changes (injury, environmental stressors, climate change). This cross-level work will require skill sets, databases, and insight from different scientists, mathematicians, and engineers.

### Current resources and methods and challenges for cross-level modeling

Diverse, open-source "big-data", such as "-omics" data (genomics, proteomics, metabolomics), ecological data (soil, climate, organism movements), and biodiversity inventories, are increasingly available. In addition, efforts have been made to standardize ecological data collection terminology and metrics to support global integration of what are often quite



heterogeneous data sets (*Plant Phenology Ontology*, Stucky et al. 2018). Large-scale and standardized databases permit more comprehensive and effective modeling of some biological systems at multiple levels of biological organization. Some new methods are also available to integrate big data at the micro-level (e.g., to build gene regulatory networks of biological pathways; Gligorijević and Pržulj 2015), mapping genes/transcripts to traits through genome-wide association mapping and weighted gene co-expression network analysis (GWAS, Visscher et al. 2017; WGCNA, Zhang and Horvath 2005), and at the macro-level (e.g., comprehensive ecological niche modeling (Regos et al. 2019) to predict shifts in species distribution range when responding to climatic changes). Phylogenetic tools for comparative analyses are widely available ([https://en.wikipedia.org/wiki/Ancestral\\_reconstruction](https://en.wikipedia.org/wiki/Ancestral_reconstruction)) and can be improved. New methods for mapping and visualization of associated data have been developed (Revell 2013; Yu et al. 2018). **However, in most organisms, networks within certain levels (such as at the molecular and genetic levels and above organismal levels) are still not well understood**, and tools to integrate data across levels (beyond the phylogenetic mapping step) are lacking. Both of these limitations truncate our ability to make connections across higher levels of organization.

A better understanding of how processes at one level affect mechanisms at another level requires diverse data and integration of data across different scales and from diverse organismal/ecological systems. New omics tools and long-term ecological monitoring systems allow for data collection that fills in gaps in knowledge for a more complete picture of processes **at different hierarchical levels and across a diversity of taxa and systems**. New tools for modeling gene regulation networks, protein networks, network driver nodes, iterative in-silico modeling frameworks including Artificial Intelligence, and mathematical modeling tools for extracting biological information/knowledge from molecular or higher hierarchical level networks continue to emerge (e.g. Liu et al. 2011; Cowan et al. 2012). Multi-scale modeling is now an active area of applied mathematics with potential approaches that have already been demonstrated to be directly applicable to hierarchical aspects of biological systems (see: MultiScale Modeling Consortium supported by NIH (<https://www.nibib.nih.gov/research-funding/interagency-modeling-and-analysis-group-imag>)). New technologies that are emerging for real-time imaging at micro and macro scales include estimates of functional connectivity, improved remote sensing with improved spectra-spatial-temporal resolution, state-of-the art microscopy, and subtractive modeling approaches provide tools to develop novel integration across levels of biological organization. Computational tools to integrate large biological datasets are available (Lapatas et al. 2015). Across-level modeling is particularly important to determine the deep effects of environmental change on diverse biological systems. Understanding how processes communicate and affect one another across levels allows for prediction of changes of the living environments of populations affected by climate change.

Challenges to the proposed advances exist at multiple biological levels. These challenges present key areas for focused inquiry and funding support. Specific challenges *within* levels of biological organization include identifying key functional elements in and across each network at a certain level (e.g. protein networks in different cell types and key communication points among different cell protein networks). This requires: (1) efficient **and standardized** measures of functional elements within levels to provide ample data (genes, proteins, cellular processes,

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organ and organismal-level processes, ecosystem processes, etc.), and (2) network analyses to characterize how functional elements interact within levels. This will allow identification of the key/hub elements within each system at each level of organization. Improving the design of large ‘-omics’ databases and information depositories will make these databases usable across multiple disciplines through explicit metadata and extraction tools. Additional development of AI tools to search databases and extract relevant data to address a particular research problem is necessary. Further, identifying and extracting common metrics within each level of organization allows us (and AI technology) to more easily identify and characterize connections across levels. Information within each level of analysis provides the basic building blocks to experimentally test cascading pathways among levels (ecosystem to organismal to cellular and molecular processes and back). A challenge to modeling communication across levels of networks will be the identification and quantification of key pathways, feedback mechanisms, and measurement at appropriate time scales. However, identification of some core pathways across levels will allow us to test the benefits of cross-level communication to predict multi-level organismal and ecosystem responses to environmental challenges. Methods to facilitate this cross-level modeling include an initial focus on systems and methods that are already well-characterized to test the potential benefits of cross-level modeling (e.g. metabolic networks, Nijhout and Reed 2014; fruit flies or mouse systems; hierarchical Bayesian models). One potential benefit is that we identify universal principles that exist within and across levels of organization.

Based on the current state of technology and the focus on networks across levels, there is great potential for new technology to be integrated and developed beyond their current resolution. In addition, targeted funding to support basic, standardized, and large dataset development in diverse species and systems will provide the data pool necessary for complex modeling. Finally, emerging technologies can allow for visualization of complex processes across levels for real-time systemic analyses including design sensors amenable to multiscale measurements.

**Conclusion and broader impacts**

Knowledge of the nature and types of major feed-forward/back interactions that occur across levels of biological organization stands to enhance our ability to predict organismal function in complex and changing environments. Our ability to understand phenomena at one level of organization will likely be enhanced by incorporating information on processes that occur at other levels of organization and that directly influence the phenomenon of interest. For example, when systems such as muscle are perturbed, at what level(s) should interventions be directed to restore function? In this case the most effective treatment may be to target a key deeper determinant level (e.g. genes, proteins) rather than treating the manifestation itself (i.e. the organ). At even higher levels, cross-level models will also enable us to design solutions to remediate global problems, whilst detecting and minimizing unanticipated adverse risks that result from remediation efforts. Challenges to overcome include data availability, data standardization, species diversity, and data integration. First, network data from different levels for both model and non-model systems provide the necessary basis to identify key network nodes (this has become much more manageable with large ‘-omic’ data collection methods).

Second, models to integrate information across levels to identify potential relationships among key or other nodes at different levels, and to simulate information to build 3D cross-level networks (as shown in Fig. 1) will eventually allow us to forecast large-scale changes even when nodes within one level/network are unaccounted for or rapidly altered. Lack of diverse yet standardized multi-level data and analytical techniques remain the most important and pressing challenges for convergence science. However, the proposed integrative work is the key to addressing large-scale scientific and societal issues with enormous potential such as reversing environmental degradation, enhancing food production efficiency, improving global health by addressing health disparities, addressing complications associated with aging, and identifying individual susceptibility to disease and injury to alter the way that we treat medical conditions. A better understanding of the network structures that connect different levels of organization will enhance our ability to tackle complex systems, like modeling and predicting impacts of climate change across multiple levels of biological organization to inform public policy decisions, governance, and emergency preparedness. Indeed, such knowledge is necessary to understand the depth of environmental effects on organisms and populations, and vice versa.

**Glossary**

- Levels of biological organization** (or determinant levels, organizational levels, biological hierarchy).
- Levels** (or sheets, layers, planes, scales)
- Signaling** (reserved for molecular information flow)
- Pathways** (information flow within and between levels)
- Interactions** (information flow within levels)
- Entities** (as opposed to components, players)
- Processes** (interactions)

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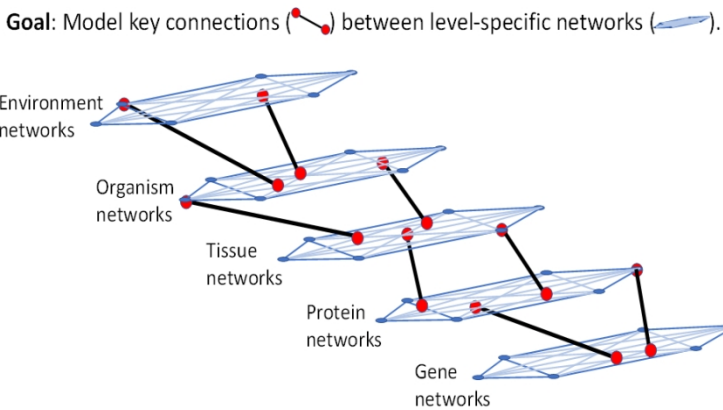
## Interactive processes across levels

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**Figure 1.** Theoretical diagram of key connections among within-level networks to help advance integrative biology. ⬡ = Networks of feedforward/feedback communication among elements within a single level of organization (e.g. protein networks consisting of different signals and receptors within a single tissue). ●—● = Key communication processes among within-level networks (i.e. processes within one level that have a particularly strong influence on processes at a level above or below).

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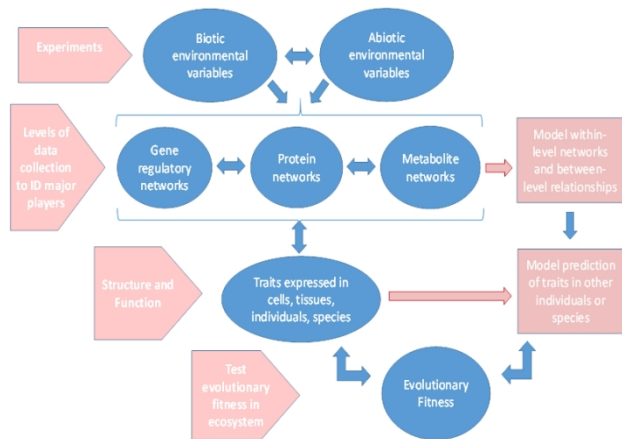


Figure 2. An example of integrating information from different scales to understand complex interactions at different levels of biological organization. This can be done for different species on a phylogenetic framework to understand the evolutionary trends of these networks and interactions for prediction of influences of biotic and abiotic factors on the future evolutionary fates of living organisms. By mapping changes of key nodes of the networks at each level on the phylogeny, correlated changes from different level networks illuminate relationships of processes across levels.

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