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Genetic Causation in Complex Regulatory Systems: An Integrative Dynamic Perspective

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The logic of genetic discovery has changed little over time, but the focus of biology is shifting from simple genotype–phenotype relationships to complex metabolic, physiological, developmental, and behavioral traits. In light of this, the traditional reductionist view of individual genes as privileged difference-making causes of phenotypes is re-examined. The scope and nature of genetic effects in complex regulatory systems, in which dynamics are driven by regulatory feedback and hierarchical interactions across levels of organization are considered. This review argues that it is appropriate to treat genes as specific actual difference-makers for the molecular regulation of gene expression. However, they are often neither stable, proportional, nor specific as causes of the overall dynamic behavior of regulatory networks. Dynamical models, properly formulated and validated, provide the tools to probe cause-and-effect relationships in complex biological systems, allowing to go beyond the limitations of genetic reductionism to gain an integrative understanding of the causal processes underlying complex phenotypes.

1. Introduction

Systems biology aims to provide a causal understanding of the complex regulatory processes—metabolic, physiological, and developmental—that generate the phenotypes of living organisms. But what exactly do we mean by "causal understanding," and how can it be achieved? The dominant approach across the life sciences continues to be genetic reductionism, which seeks to explain biological phenomena in terms of the effects of genes. In recent decades, a shift of focus among biologists from simple gene–trait relationships to more complex phenotypes has exposed the limitations of this approach. The causal effects of genes become fragile when they are embedded in a web of multi-level, nonlinear interactions. Although we do possess genetic methods to tackle such complex regulatory processes, they often remain at the level of statistical correlations, which can be predictive but do not usually provide causal understanding.

To better understand the current efforts that systems biology is making to overcome this limitation, it is useful to philosophically reflect on our concepts of causation and their role in causal explanation. A close analysis of different types of causes in regulatory processes allows us to precisely delimit the domain of applicability for genetic reductionism. Outside of this restricted domain, a richer, integrative, dynamic perspective on causation is needed.

As systems biology goes beyond the limitations of genetic reductionism, our thinking about causation faces two main challenges. Both have to do with the fact that genes and their networks of interactions are embedded within contexts of complex nonlinear regulatory dynamics that occur at multiple levels of organization, from molecules to cells, tissues, and organisms. The first challenge is to reach an understanding of causality compatible with the heavily feedback-driven dynamics of regulatory processes. The second is to provide an understanding of gene action in systems with a multi-level structure. The recent short commentary by Bizzari et al.,[1] which provides the background for the papers in this collection, points in the right direction by suggesting that scientists would do well to pay closer attention to the underlying philosophical and conceptual issues. Our contribution to this collection of papers is tasked with connecting the general outlook presented in Bizzari et al.[1] to arguments from the rich historical tradition of philosophical work on the topic. In doing so, we propose a novel processual approach to causes as difference-makers in complex and multi-level regulatory systems. This grounds intuitive notions of dynamic causality that many systems biologists are holding, provides a number of useful concepts to analyze causality in complex regulatory processes, and delimits the role of genetic causation in biological systems.

2. How Should We Understand Causation?

Much of the classic philosophical literature on causation has focused on the basic question of what counts as a cause, that is,

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Box 1. Causal concepts

Stability: A causal relation between variables *X* and *Y* is stable if it holds across a wide range of background conditions (see ref. [16, p. 291]). "Instability" of causal relationships is sometimes called sensitivity or contingency. Highly stable causal relations can be considered laws. They are abundant in physics, but not in biology. But since stability is a matter of degree, this notion can capture the extent to which causal relations in biology approximate law-like regularities.

Proportionality: A causal relation is proportional if it includes enough relevant information, and only relevant information, to describe when the alternative states of the effect variable will be realized [16, p. 298]. Proportionality is closely related to the choice of the appropriate level of explanation. For example, many complex traits are robust to perturbations in the molecular regulatory mechanisms that generate them. In such cases, changes in the molecular details underlying the genetic architecture of the trait are not "proportional" to the phenotypic change. The molecular details contain extra irrelevant information, and an explanation at the systems-level is more proportionate.

Specificity: A causal relation between variables *X* and *Y* is specific if the possible states of *X* map onto the possible states of *Y* in a fine-grained and approximately bijective way. More precisely, specificity means that if there are many possible states of *X* (x_1, ... x_n), many possible states of *Y* (y_1, ... y_n), and a mapping *F* from *X* to *Y* such that states of *X* each have a unique image under *F* in *Y*, not too many states of *X* map onto the same state of *Y*, and most states of *Y* are the image under *F* of some state of *X* (see ref. [16, p. 305]).

what conditions some relationship must satisfy in order to qualify as a cause-and-effect relation. Central concerns include whether causation can be analyzed noncircularly, in terms of notions that do not presuppose causation, or what the relata of causation are.[2] Most of this work is not immediately useful for specific scientific problems such as the ones with which this collection of papers is concerned.

In recent decades, however, several novel frameworks for thinking about causation have been developed that are directly applicable in our context. One key example is the causal modeling approach developed in Spirtes et al.,[3] which provides formal tools for statistically inferring causal models from data. A related approach derives from structural equation modeling,[4] and includes the interventionist framework of Woodward[5] (see also ref. [6]). We will focus on Woodward's approach here.

The interventionist approach is based on the idea that causal relationships, unlike mere statistical correlations, can be exploited for purposes of manipulation and control. For two variables *X* and *Y* to be causally related, there has to exist some intervention on *X* that changes the value of *Y* under a range of background conditions.[5] An intervention on a gene—via overexpression, knock-down, or knock-out perturbations—often results in a corresponding phenotypic change. As a classic example from developmental genetics, if we were to remove one copy of the brachyury gene from the genome of a mouse, it would result in reduced tail length and defects in the sacral vertebrae; if we were to remove both copies, it would lead to embryonic lethality.[7] Such "if-then" statements regarding what would happen under various possible conditions are called counterfactual conditionals, or counterfactuals.

Interventionism is considered a type of "counterfactual theory" of causation,[8–10] because relations between variables that are manipulable in the interventionist sense generate true counterfactual statements. Counterfactual views of causation construe causes as difference-makers.[8] The following counterfactual—if *X* had not had the value x_1, then *Y* would not have had the value y_1—is stating that the value of *X* makes a difference to the value of *Y*. In biology, counterfactual theories have been more productive than earlier accounts of causation based on regularities/laws,[11,12] or the transfer of matter or energy.[13–15] There are few, if any, strict laws in biology, and it is neither practical nor desirable to always trace complex biological processes in terms of flows in underlying physical units.

Three concepts developed within the interventionist approach are particularly useful to frame the problems facing causal explanations in systems biology. These are the notions of causal stability, proportionality, and specificity (see **Box 1**).[16]

The notion of specificity in particular has had a major and enduring influence on thinking about causation in genetics and molecular biology. When it comes to understanding the mechanisms underlying phenotypic traits, genes are generally thought to be more causally specific than nongenetic variables. The unique specificity of genes is often used as a key justification for reductionist genetic and molecular approaches to development and evolution.[17,18]

We do not contest that genes have a high degree of causal specificity for the regulatory processes of transcription and translation. However, this does not entail that individual genes have high specificity in more complex regulatory systems, such as those involved in morphogenesis. Later, we reassess the stability, proportionality, and specificity of individual genes as compared to other biological causes with a range of examples. But first, we examine how the notion of difference-making genes has operated in biological thinking about causation, both classical and contemporary.

3. Genes as Specific Actual Difference-Makers

Our understanding of genetic causation has not changed significantly from early work in classical genetics, despite radical advances in contemporary methods for genetic manipulation and genome engineering. Relying on Woodward's interventionist framework,[5] Waters[19] reconstructs a common pattern of

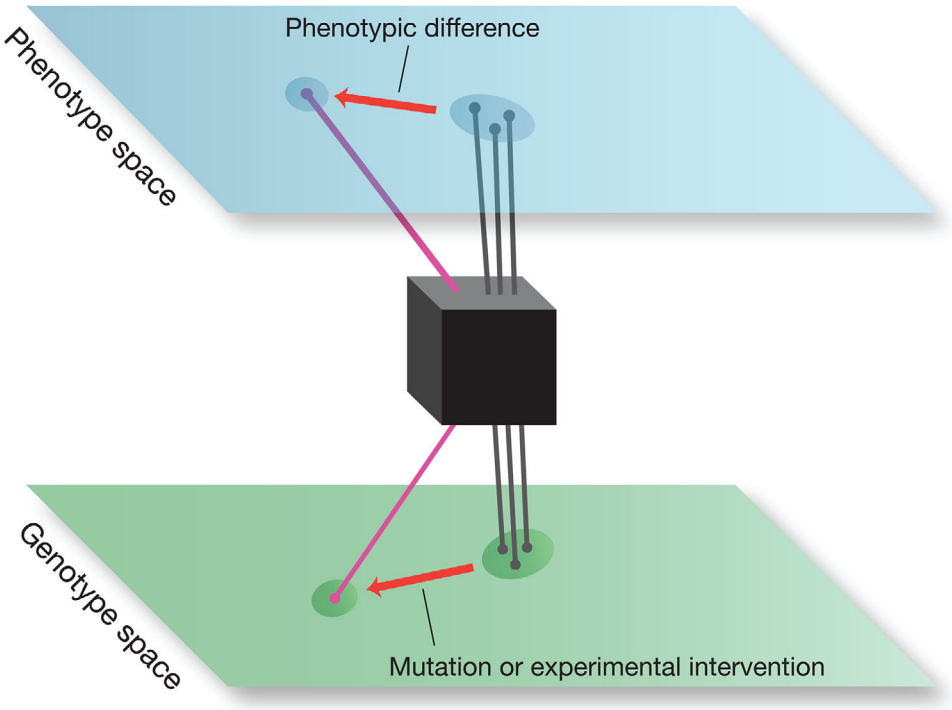

Figure 1. Genes as specific actual difference-makers: for simple genotype–phenotype mappings, particular genotypes (indicated by dark green circles in genotype space) map onto particular phenotypes (dark blue circles in phenotype space). An experimental intervention (or spontaneous mutation) leads to a particular change in phenotype (e.g., from red to pink eye color in *Drosophila,* see text). Other interventions will lead to different phenotypes (not shown). In such straightforward cases, we can treat the structure of the genotype–phenotype mapping as a black box, and the gene which is perturbed/mutated is a specific actual difference maker for the phenotype.[25]

reasoning about genetic causation tracing back to T. H. Morgan.[20,21] It begins with the identification of a natural polymorphism or the generation of a mutant in the lab. As a specific example, Morgan's group studied mutations causing differences in eye color in the vinegar fly *Drosophila melanogaster*. Let us say that red-eyed flies have value y_1, and pink-eyed flies value y_2, of the trait variable *Y*. This phenotypic difference is associated with a genetic difference: allele x_1 versus allele x_2 of gene *X*. If y_1 (red eyes) is present only in individuals with allele x_1, and y_2 (pink eyes) is present only in individuals with allele x_2, and no other genetic differences between them exist, then gene *X* is the actual difference-maker for the observed variation in *Y*.[19] Accordingly, gene *X* was named *pink* (*p*) by Morgan and colleagues.[20] The difference-making relationship can be expressed by the following counterfactual: if the individual had not had allele x_n, it would not have had trait value y_n. The variable *Y* can be manipulated with an intervention on gene *X*.

The difference-making relationship between gene *X* and trait *Y* does not exclude other genetic and nongenetic factors from having a causal influence on trait *Y*. Morgan and colleagues[20] knew of 25 different mutations that affect eye color in *D. melanogaster*, and hypothesized that each genetic variant causes a distinct eye color phenotype. They write: "In this sense we may say that a particular factor (*p*) is the cause of pink, for we use cause here in the sense in which science always uses this expression, namely, to mean that a particular system differs from another system only in one special factor".[20] The *p* gene is the "actual difference-maker" here.[19] In contrast, the other genes affecting eye color are "potential difference makers." If we intervened on them, eye color would change, but they do not actually vary in this particular case.

There are many other causes involved in the development of pink eyes, such as tightly regulated cell divisions and mechanical processes such as the movement of the morphogenetic furrow in the eye imaginal disc of the larva of *D. melanogaster*.[22,23] Moreover, genetic determinants of eye color causally depend on enzymes like RNA polymerase and functional cell metabolism to exert their effect. However, these other causes remain largely constant in populations (or vary in ways not correlated with eye color), and therefore cannot be said to cause the observed phenotypic differences. These factors are nonspecific[17,19,24]: perturbing them will turn off the phenotypic effect by blocking gene expression or cell division. Genetic determinants therefore have a privileged status as causes of phenotypic variation (**Figure 1**): they are specific actual difference-makers.[25] We call this classic reductionist approach the "difference-making gene" paradigm.

Over recent decades, experimental techniques for genetic interventions have been revolutionized. We can now target specific genes for knock-down through RNA interference,[26,27] or delete genes, replace alleles, and make the most subtle changes to coding and regulatory sequences through various techniques for genome engineering.[28,29] Despite all this methodological refinement, the basic logic of genetic discovery has remained the same. Saturation mutagenesis—exemplified by the Nobel-Prize winning work of Nüsslein-Volhard and Wieschaus[30] on segmentation genes in *Drosophila*—and techniques such as linkage mapping for quantitative trait loci (QTL) or genome-wide association

studies (GWAS)[31,32] allow us to identify lists of genes involved in generating complex traits that would have been inaccessible to empirical study at the time of Morgan. And yet, in order to functionally characterize the causal interactions between these genes we still use the same kind of intervention strategies the classical geneticists did.

The persistence of these strategies speaks of their power, versatility, and reliability. However, their reductionistic nature does impose a couple of serious methodological limitations, as mentioned in Bizzari et al.[1] We now know that complex traits are generated by intricate networks of regulatory processes that interact with each other, as well as with their cellular, tissue-level, organismic, and external environment. For such traits, the identification of specific actual difference-makers becomes problematic, if not outright impossible. Causal effects of individual genes in such systems depend on heavily feedback-driven regulatory dynamics across multiple levels of organization. Furthermore, the regulatory processes involved are highly redundant and robust,[33,34] so that interventions on many genes have no phenotypic effect. In what follows, we will take a closer look at causality in the context of such robust, feedback-driven, multi-level dynamic regulatory systems.

4. Understanding Causation in Complex Regulatory Systems Requires Dynamical Modeling

4.1. Genetic Causation Depends on Dynamical Regimes in Multistable Systems

The effects of individual genes on complex traits may be specific at the molecular level, but become unstable and nonproportional (see Box 1) when considered at the level of network dynamics. Nonlinear regulatory networks often exhibit multistability, generating a range of qualitatively different dynamic behaviors under different background conditions (see, e.g., ref. [35]). These different behaviors are called dynamic regimes.[36] In multistable networks, the effect of a genetic intervention becomes dependent on regulatory context: the same gene has different effects in different dynamic regimes. This means that the roles of individual genes are causally unstable as explanations for the behavior of the system.[19] The power of the reductionist approach is limited when the effect of a component becomes dependent on the behavior of the system as a whole.[33]

For instance, sub-circuits of the gap gene system in the embryo of *D. melanogaster* fall into a switch-like or oscillatory patterning regime depending on their network context and initial regulatory inputs.[37,38] Since each gap gene participates in more than one sub-circuit across different embryonic regions, its effect becomes sensitive to spatial and regulatory background conditions. Multistable networks are often also multifunctional in terms of their phenotypic effects: an example of this is provided by a recent study of pattern formation driven by the Notch signaling pathway.[39] Depending on a tissue-specific signaling input, the same network can account for either lateral inhibition or lateral activation, two pattern-forming modes of Notch that are based on completely opposite dynamic behaviors.[40] This example illustrates that the structure of a network does not uniquely determine its function.[41] It also highlights that the same genetic components can mediate completely opposite dynamic behaviors, depending on tissue-level context.

4.2. Robustness and Redundancy Complicate Causal Analysis

Cross-talk between regulatory processes further complicates the situation, since it leads to effects that depend on multiple chains of causation. These effects are often counterintuitive—sometimes synergistic, sometimes antagonistic, sometimes redundant—and therefore not easily classifiable into simple combinations of "necessary" and "sufficient" causes as is often done in traditional and contemporary genetic studies.[1] Antagonistic and redundant interactions are particularly problematic, since interventions on them often have no observable effect on system-level behavior due to regulatory compensation. Redundancy and compensatory regulation are two of the main mechanisms underlying the robustness of biological systems.[34] Interventions on factors involved in such robust regulation still have measurable effects on molecular interactions, and there are quantitative methods to assess causal specificity of interacting causal chains.[25,42] However, an intervention without effect is no longer directly relevant, and hence proportional, for explanations of systems-level dynamics.

4.3. Feedback Regulation Makes Genetic Causation Time Dependent

Another problem arises from feedback regulation, which means that effects "feed back" to modulate their causes. This can occur directly, through auto-regulation, or indirectly through intermediary factors. It can be localized (modular) or distributed (affecting the global behavior of the system). The most important consequence of feedback regulation is that causal effects of individual components become dependent not only on regulatory context, but also on the history of the system. An intuitive example is provided by bistable behavior due to positive feedback in the genetic toggle switch (see ref. [35], and the references therein). Toggle switches are small networks with two regulatory factors that mutually repress each other. Small adjustments in the initial concentrations of the regulators can lock the system into either one of the two alternative states, a situation in which one factor is active to the exclusion of the other. Once the switch is triggered, and a stable state is established, a much larger intervention is required to revert the system to its alternative state. Such history-dependent sensitivity modulation is called hysteresis. It implies that the timing of an intervention makes a crucial difference for its effect. In feedback-driven systems, causality is time dependent, so that it cannot be expressed in terms of simple atemporal counterfactuals.

Although time plays a key role in dynamical representations of causal processes, difference-making representations of causation tend to abstract from time.[43] It is tempting to conceptually identify difference-makers with things whose properties vary so as to cause changes in the properties of other things. In fact, this tendency fits with a tradition in early molecular biology, where biomolecular interactions were characterized as highly specific, due either to chemical conformation and "lock-and-key" fitting

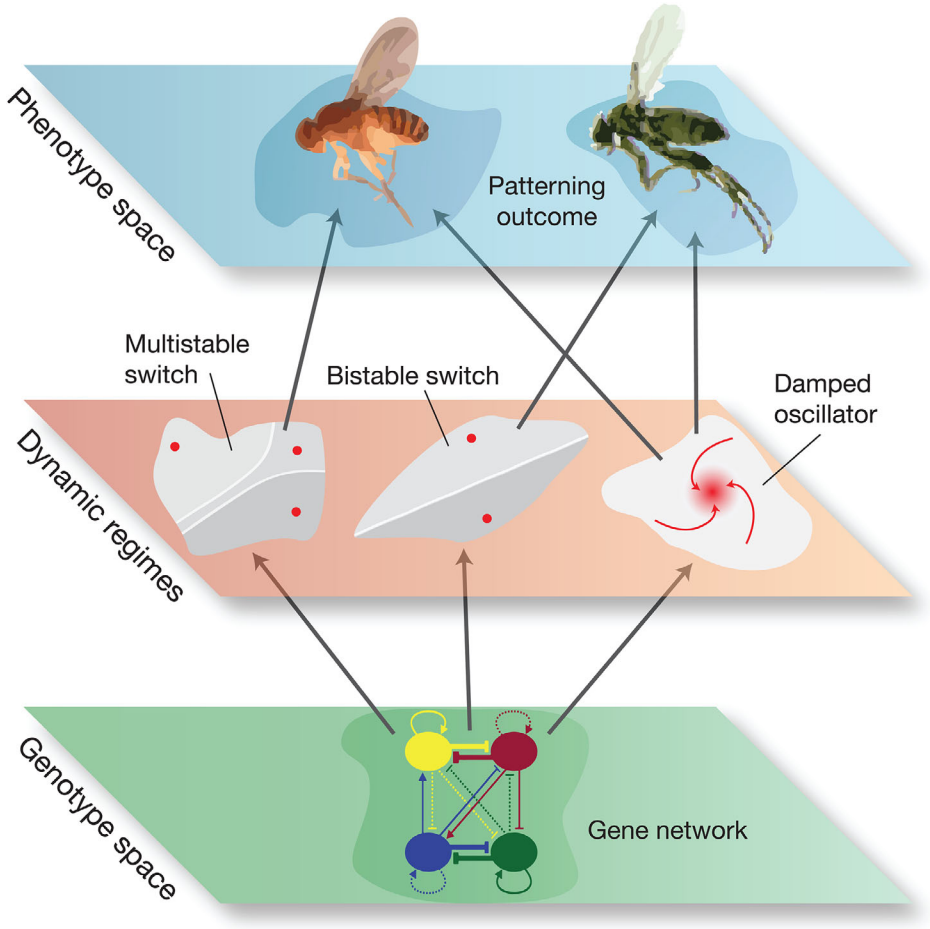

Figure 2. Genetic causation situated in the context of dynamics with robust nonlinear feedback. The same regulatory network can exhibit qualitatively different dynamic behaviors depending on the strengths, timings, and rates of interactions, its regulatory context, as well as the history of the system. Genotype and phenotype spaces are shown as in Figure 1. The dynamical regulatory processes that mediate between genotype and phenotype are no longer black boxed as in the reductionist approach shown in Figure 1, but are shown explicitly as their own level of explanation (in red). In the example shown, the actual difference-makers for the process of segmentation determination in *Drosophila melanogaster* compared to *Megaselia abdita* (a distantly related species of the fly) are not individual genes. Instead, they are the strengths, timings, and rates of interaction in the gap gene network, mediated by its dynamic regimes (multistability, bistability, damped oscillations).[37,38]

between structures[44–46] or "information" contained in the sequence of nucleic acid base pairs.[24,45,47,48] In either case, when causal specificity is based on stable structural properties, the causal relation is invariant to differences in the timing and rate of interaction, so these dynamical features can be safely ignored. For such time-invariant cases, statistical inference using structural equations is the method of choice for inferring the causal architecture of a system.[3,4]

However, this simplification is no longer valid in dynamic systems with feedback regulation, where the difference-makers are not just things with structural properties, but also rates, timings, and interaction strengths—parameters that affect the probability of the system being in a certain dynamic regime. They have largely been neglected throughout the history of genetics, because it is technically challenging to design specific interventions on such parameters, rather than disrupting the function of particular genes. Practically, this is now becoming feasible through the refinement of genomic engineering methods.[28] For instance, deleting all the auto-regulatory binding sites of the transcription factor encoded by the gap gene giant (gt) from its own regulatory region blocks the dynamic transition between early and late phases of this gene's expression.[49] This means that we need conceptual representations of causality that take dynamics at the network level into account. The same applies for networks with redundant and antagonistic interactions. In both cases—feedback and compensatory regulation—individual genes may be specific difference-makers at the molecular level, but they are unstable—in terms of their radical sensitivity to both context and history—at the level of the overall dynamics of the system (**Figure 2**).

4.4. Systems-Level Interventions Require Dynamical Modeling

An understanding of causation in feedback-driven, redundant regulatory systems requires a quantitative, integrative, and dynamical approach. Genetic reductionism—with its focus on individual genes as specific actual difference-makers—must be supplemented with interventions on network-level dynamics. Such

interventions can be set up with the aid of data-driven dynamical models.[35,36] If formulated properly, dynamical models of regulatory networks allow us to keep explicit track of local interactions between factors in highly complex, feedback-driven systems (this point is elaborated in more detail in the introduction of ref. [50]). Such models must be rigorously validated against experimental evidence. The type and strength of each relevant regulatory interaction should be captured as accurately as possible. In cases where experimental evidence is not available to determine the type and strength of every network connection, we can fit models to quantitative data in order to reverse-engineer the structure of the network.[37,51] Interventions can be carried out by altering parameters that represent specific regulatory interactions, or through "mutation" of a factor by changing its rate of production or decay. Both local and global effects of such interventions can then be traced by numerical simulation or, more systematically, by carrying out a global sensitivity analysis (see, e.g., ref. [52]).

In this way, we can learn how the dynamic regimes of the system mediate the effects of the actual difference-makers (strengths, timings, and rates of interactions). For instance, it is the extent to which switch-like and oscillatory behaviors predominate in gap gene-mediated patterning that varies between different species of flies (Figure 2). These different regimes depend directly on changes in the strength of a set of regulatory interactions in the underlying network.[37,38] This systems-level approach, combined with evidence from genetic interventions, yields an integrative dynamic explanation of causal flow through the network with all its redundancy and regulatory feedback.[41]

5. Causation Across Levels of Organization Involves an Interplay between Processes and Constraints

5.1. Causation Differs within and between Levels of Organization

To understand the causal structure underlying complex phenotypes, we not only need to model nonlinear dynamics of lower-level entities such as genes and gene products. We also need a conceptual framework for understanding how causal influence is channeled between lower and higher levels of organization, from molecules to cells, tissues, organisms, and so on.[33,53,54] In the "difference-making gene" paradigm, these levels are collapsed down to two abstract levels—genotype and phenotype (see Figure 1). The identification of difference-making genes then amounts to finding correlations between genotypic differences and phenotypic outcomes. However, the causal role of genes is typically more complex than this. Moving from individual difference-making genes to causal mechanism requires a more integrative understanding of causation across multiple levels of organization.

In approaching this issue, it is instructive to confront two apparently contradictory ideas about the connection between causation and levels. On the one hand, causal interactions segregate across levels of organization, so that molecules interact primarily with other molecules, cells with other cells, and organisms with other organisms.[33,55–59] On the other hand, entities at lower and higher levels clearly do interact. Mutations can have large phenotypic effects, and higher-level phenomena such as tissue-level interactions or environmental triggers can influence gene expression—leading to nonlinear hierarchical feedback. The tension between these ideas is attenuated by recognizing that same-level and inter-level interactions tend to have a different character. Same-level interactions usually take place between entities of similar spatial, temporal, and force scales, are more frequent and regular relative to those time scales, and often involve direct transfers of matter and energy. With inter-level interactions, in contrast, interactions between scale-separated entities are more indirect, infrequent (or constant) relative to the time scales of each level, and are often mediated by constraints. Although constraints are not always involved in inter-level interactions, constraint-mediated causation between levels is particularly significant in biological systems because constraints are typically specialized structures that can be targets of natural selection.

5.2. Constraints Can Mediate Inter-Level Causation

A constraint is a material structure that acts on some dynamic process by reducing its degrees of freedom, but without itself being altered at the spatial or temporal scale of the process.[60] A paradigmatic example is provided by the enzymes that enable metabolism by altering the rates of the underlying chemical reactions without being altered by those metabolic reactions themselves. Another example is the cell membrane, which spatially constrains the diffusion of biochemical components without being affected directly by the diffusing molecules.

Constraints are generally represented as being at a higher level than the entities whose dynamics they constrain. From a dynamical perspective, lower levels determine the initial conditions as well as the possible states of a biological process, whereas higher levels determine the context within which the process operates.[61,62] Constraints can therefore be viewed as one important class of boundary conditions. They do not alter the range of possible behaviors of the lower-level process, but change the probabilities of specific dynamic regimes. Enzymes do not change the kind of chemical reactions that are possible, or whether they are exergonic or endergonic. They only change the rates of the reactions.

The notion of constraints, especially as used in evolutionary biology, usually carries the negative meaning of preventing something from happening.[63,64] By preventing certain dynamic regimes, however, constraints can promote other more biologically useful ones. Constraints can harness the inherent stochasticity of molecular processes so as to select or bias toward events that would otherwise be improbable.[65–67] An example is provided by the "Brownian ratchet" model of intracellular transport, which explains how motor proteins move along the cytoskeleton in certain directions.[46,68,69] In this model, alternating changes in protein conformation, mediated by ATP hydrolysis, result in an alternating potential energy landscape of the protein–cytoskeleton system. The consequence is that it is energetically easier for the protein to move in one direction than the other. Intrinsically, the protein's movement is determined by random thermal fluctuations that are equally likely to push it in either direction along the cytoskeletal track, but since one direction is effectively blocked by an added boundary condition, we see a systematic bias in the movement of the protein toward the "right"

or functional direction—an improbable outcome under unconstrained Brownian diffusion. Altering the intrinsic behavior or a process by adding boundary conditions is a general mechanism for "downward causation."[62,70]

Another example of constraint-mediated causation between levels occurs when a fluctuating molecular process is converted into a robust on/off signal, as in intercellular signaling or action potential propagation. In the latter case, the voltage-gated ion channels located in neuronal membranes constitute constraints that mediate between changes in a continuous variable (concentrations of potassium, sodium, and chloride ions) and a discontinuous variable (action potential spike or not). As in other signaling processes, activation thresholds sum the underlying molecular variation into a single output (a process called "black-boxing").[67] Combined with other neuron-level inputs, we see a quasi-"logical" branching dynamics of neuronal networks with a degree of autonomy from the biochemical details.

Action potential propagation is an example of a hierarchical nonlinear feedback process. Ion channel proteins influence the electrical potential of the cell by constraining the flow of ions across the membrane. In turn, the gating of these channels is itself influenced by the cell potential. Thus, a cell-level parameter and protein-level parameter are controlling each other in a cyclical fashion.[62] Although many genes are involved in the cycle—for example, in the production of membrane proteins—they do not determine its dynamical behavior. Understanding the dynamics of action potential propagation requires taking into account the multi-level causes that give spatiotemporal organization to the process, such as proteins controlling ion flows and cell potentials feeding back on the voltage-sensitive proteins.

5.3. Constraints Are Inter-Level Difference-Makers

How do these concepts fit into our earlier discussion of causation and genes? Constraints are difference-makers, and their relationship to dynamic processes can be explicated in terms of counterfactual dependence. For example, "if enzyme *E* had not been present in such and such concentration, reaction *R* would have proceeded at such and such a rate." Quantitative counterfactual dependencies like these are much more economically expressed using differential equations. There is an important contrast with the counterfactuals we have seen with individual difference-making genes, however. Those earlier counterfactuals relate presence/absence of genes to phenotypic properties, whereas here the counterfactuals relate constraints to inherently dynamic entities—that is, processes or activities—that have their own causal trajectories without the constraint. Embedded in the causal representation of constraints, then, is some representation of what a system would be doing without the constraint: its intrinsic dynamics or default behavior.

5.4. Constraints Alter the Default Behavior of Underlying Processes

Shifting to representations of dynamics or behaviors gives us a much richer causal vocabulary than the classical one focused narrowly on static entities and properties that are turned on and off by the presence/absence of these difference-making things. For one thing, it facilitates the application of dynamical modeling, as explored in Section 4. The idea of default behaviors also lays the conceptual groundwork for models of "null" cell and tissue dynamics that could contribute to a possible theory of development.[71–74] Default behaviors, such as uniform motion under inertia in classical mechanics, carry counterfactual information about process—how the system would proceed from initial conditions to end states under various perturbations and constraints—rather than merely correlating initial differences with end-state differences. Soto et al.[73] identify the most basic default behavior of biological tissues as cell proliferation with variation and motility (see also ref. [72]). This is supported by evidence showing that cell cycle components are highly conserved throughout evolution. Variation arises naturally through unequal distribution of molecules and cellular components upon each individual division, and from the inherently stochastic nature of gene expression. This default behavior is systematically altered by biasing events, such as environmental triggers, inductive signaling, growth factors, geometric constraints, limited nutrients, time constraints, and so on.[73] Embedded within this multi-level dynamic picture, difference-making genes are recast as components in a wider system, causes that have their effects primarily by contributing to more inclusive causal mechanisms—that is, gene regulatory networks, as well as physical processes at the cell and tissue level.

Bizzari et al.[1] have already highlighted the central issue of integrating gene regulatory networks and cellular–physical mechanisms (see also refs. [43,75–78]). Areas of biology such as developmental genetics are dominated by an explanatory mode in which mechanism is identified with gene regulatory networks.[41,79] While such networks can account for the different transcriptional states that underlie cell fate determination, they do not explain how populations of cells form tissues, change their spatial locations and shapes, articulate into complex morphologies, and shape organismic behaviors.[80] The multi-level dynamic framework of causation we have outlined here may provide the beginnings of a powerful conceptual foundation for examining these complex inter-level influences.

6. Understanding Genetic Causation in Complex Biological Systems Requires an Integrative Dynamic Perspective

Constraints, default behaviors, feedback-driven processes, network dynamics, and physical mechanisms—these notions all contribute to a richer causal framework than a reductionist approach narrowly centered on individual difference-making genes. But how does this relate to claims that individual genes have a privileged status with regard to causal specificity and actual difference-making? Let us re-evaluate each of the aspects of causation introduced earlier in turn—stability, proportionality, specificity, and actual difference-making (see Box 1).

A causal relationship is stable, if it holds under a variety of different background conditions. The stability of genetic causes varies greatly, being maximal for simple Mendelian traits, but

minimal for complex traits where the contribution of any specific gene depends strongly on nonlinear and potentially redundant interactions with other genes and the cellular/external environment. For complex traits in general, the most stable causes are not individual genes, but rather gene networks or regulatory processes. A proportional (i.e., relevant) explanation should therefore center on causes at that higher level.

A causal relationship is specific to the extent that it approximates a bijective mapping between states of the cause and effect variables.[16,25] It is widely presumed that genes have more causal specificity than other cellular components.[17,19,24,25] While this seems to be generally true for the process of gene expression, it is not clear that it can be meaningfully extrapolated beyond the micro-scales of molecular biology. When it comes to complex traits, networks of interacting genes have more specificity than the component genes (see also ref. [81]). In other cases, for example, highly polygenic quantitative traits such as height, genetic contributions are nonspecific compared to higher-level factors such as the rate of cell division and growth. Overall, it is far from clear that individual genes should have a privileged status as causes in biology due to their purportedly unique degree of specificity (see ref. [82] for an earlier argument of this kind).

Lastly, a cause is an "actual difference-maker" if it is the sole factor that co-varies with a target effect in a population.[19,20,24] It is widely assumed that individual genes are the actual difference-makers for most phenotypic traits. Again, this is plausible for simple Mendelian polymorphisms, but not necessarily for complex traits like body parts. The actual difference-makers underlying the distinct processes of segment determination in the vinegar fly *D. melanogaster* and in the scuttle fly *Megaselia abdita* are not individual genes, nor even network structures, but dynamic properties of the regulatory networks (see Figure 2). Just like the genetic determinants themselves, these properties can vary within a population and be inherited across generations.[35]

When assessing the causal role of genes, there are two important considerations to keep in mind. First, we should not underestimate the extent to which claims about the special causal importance of individual genes depend on our techniques for genetic interventions. Compared to biophysical constraints and nonlinear dynamics, individual genes are easy targets for experimental manipulation.[83] This fact tells us more about our technical capabilities (and limitations) than about the causal structure of biological systems. Second, genetic systems are subject to evolutionary change. The stable and specific genotype–phenotype maps familiar from model organisms like *D. melanogaster* represent highly derived and specialized evolutionary conditions.[84–86] In such cases, it may be that part of the causal stability and specificity that genes have acquired is actually explained by the evolution of constraints on the genetic system. The constraints that make body plans more modular, for example, increase the stability of gene–trait relationships against changes in genetic background, which in turn enables the genes within a module to have specific, targeted effects on the corresponding modular trait.

7. Conclusion and Outlook

Current experimental and statistical methods are highly effective for identifying causally relevant genetic components, but less effective for disentangling their regulatory interactions, especially when these interactions are nonlinear and multi-level. One way to address this challenge is through the integration of experimental intervention and dynamical modeling, while working with an expanded conceptual repertoire for kinds of causes like the one we have introduced in this paper, including constraint, default behavior, and physical mechanism. Our dynamical approach is not opposed to difference-making accounts of causation, nor to the successful intervention methods that have historically been tied to the search for individual difference-making genes. Instead, it includes identification of difference-making causes and components, but takes extra steps to put them together and integrate them across time.[87] The goal is to understand and explain how a complex system actually goes from some initial state to some end state, and how it would proceed under different constraints and perturbations.[88]

When the causal structure at stake is nonlinear and multi-level, our models of causal processes will need to include dynamical properties like rate, timing, and strength of interaction (i.e., causes that are not structural properties of things), as well as cell- and tissue-level physical properties. This is certainly more demanding than measuring genetic variables at the initial state and correlating them to phenotypic variables at the end state in order to construct a two-level genotype–phenotype map. It is also more demanding than compiling ever-longer "lists" of components using "-omics" technologies and hoping that computational methods will put it all together.[83] But the demands fit the nature of the current problem: that is, going beyond the identification of genes that are "involved" in a process to a fuller understanding of the causal process itself.

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Conflict of Interest

The authors declare no conflict of interest.

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dynamical systems modeling, genetic reductionism, levels of organization, philosophy of causation, regulatory networks, robustness, systems biology

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