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Ecology helps bound causal explanations in microbiology

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Abstract
Experimental manipulations are a key means to establish causal relationships in microbiology. However, challenges remain to establish the applicability of such experiments beyond the precise conditions in which they were conducted. Ecological information can help address these challenges by describing the extent to which an experimentally-determined mechanism can explain the natural phenomenon that it is purported to cause.

Keywords Microbiome · Causality · Function · Koch's postulates · Ecology

There is currently enormous interest in the idea that microbiomes (i.e., the microbes that live on and in most large organisms) can be beneficially manipulated. However, this presents a critical question: how can we know that a microbiome (an individual microbe within it) causes an effect on its host? As Lynch et al. (2019) now excellently explain, such questions are often more complex than they first appear. Formal frameworks to establish causality have a long history in microbiology, dating back to 1890 when Robert Koch and Friedrich Loeffler published what are now known as Koch's postulates (based on earlier work by Jakob Henle; Evans 1976). These state that for a microbe to cause disease: (1) that microbe must occur in all diseased hosts; (2) that microbe must not occur in healthy hosts; (3) cultures of that microbe must cause the original disease when inoculated into a healthy host. Although Koch's postulates have been successfully used for over a century, not all microbes cause disease or are easily cultured. This has caused many researchers to explore the causal underpinnings of Koch's postulates in the hopes of adapting them to a more modern view of microbiology that extends beyond diseases caused by a single pathogen

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(Evans 1976; Fredericks and Relman 1996; Falkow 2004; Zhao 2013; Klassen 2014; Byrd and Segre 2016; Neville et al. 2018; Vonaesch et al. 2018).

Lynch et al. (2019) focus especially on Koch's third postulate as the core interventional approach that microbiologists use to demonstrate causality. This involves infecting a naïve host with a microbe and comparing this infected host to an uninfected control. Other variants of this interventional approach that manipulate genes, metabolites, or entire microbiomes work similarly (Fredericks and Relman 1996; Falkow 2004; Byrd and Segre 2016). The basic logic here is that without a causal agent, no corresponding effect of that agent will be observed. In a parallel body of literature, such experimentally-derived causes are also known as "Causal Role functions" (Cummins 1975; Klassen 2018). Importantly, these experimentally-determined causes remain strictly valid only for the experimental systems in which they are tested. This presents a critical limitation of the manipulative approach for revealing causality because further experiments must be conducted to determine the full context in which each causal explanation remains valid. Any experiment tests only one of many possible conditions, and so further work is required to determine the related systems to which a result can be generalized. Lynch et al. (2019) explore several different dimensions along which causal explanations derived from manipulative experiments can be probed to expand the frame of reference in which they maintain their explanatory power. These include confirming reproducibility in complex (and therefore noisy) biological systems, broadening replicability to related experimental setups, and evaluating the operative level of biological complexity (e.g., molecules, organisms, communities). Together, these criteria set a high bar for causal explanations derived from experimental microbiome research.

Much of the biological complexity described above relates to problems and processes in microbial ecology. This is arguably presaged by Koch's first two postulates stating that a disease caused by a microbe must only occur in the presence of that microbe and not in its absence. To explore this, we must keep in mind the nineteenth century context in which Koch worked. In Koch's time, only a few microbial taxa were known, and these were almost exclusively associated with disease. (Studies of food and beverage fermentation, e.g., by Louis Pasteur, are an exception that proves this rule by their emphasis on spoilage being analogous to disease.) With this context in mind, it is no surprise that Koch's postulates focus on diseases and single microbial species. However, I suggest that it is not a stretch to rephrase Koch's first two postulates to highlight disease as one example of many ecological strategies that a microbe might use. Considered in this way, Koch's first two postulates might more generally state that "a microbe causes some effect when its ecology coincides with when and where that effect occurs". Rephrased slightly, this states that a microbe's realized niche (i.e., where it actually exists in the real world, not some abstract condition that it could ideally occupy in principle) must co-occur with the expressed traits that this microbe uses to establish that niche. For Koch, all microbial niches and traits involved diseases (as the only known effects of microbes). However, this framework readily accepts our newer understanding that beneficial microbes can similarly cause effects: a microbe has to actively express beneficial niche-defining traits for those traits to beneficially affect their host. This also includes the possibility that a trait-encoding microbe occurs in a microbiome but does not express that

trait and therefore does not affect its host, because in this example the non-expressed trait does not help maintain the microbe's host-associated niche. Whether or not a microbe will express such traits depends on if and why these traits have been maintained by evolutionary selection, and these "Selected Effect functions" provide a second and more restrictive approach to defining causality in biological systems (Wright 1973; Klassen 2018). Understanding how evolution has shaped the ecology of a microbe is therefore an important and complementary approach to determining the generality of phenotypic effects that are identified using experimental manipulations.

Of course, defining a microbe's niche and evolutionary history are difficult undertakings (Klassen 2018). Lynch et al. (2019) describe in detail how all types of causal explanations involving microbes are challenged by the ubiquitous presence of complex microbiomes in which the nature of the causal actor is especially confused. This means that all of the relevant contexts need to be identified in which a focal trait operates, including the ecology of each member of a dynamic microbiome, how they modify trait expression, and how the expression of that trait affects the host. One might therefore wonder about our ability to definitively assign general causal explanations at all, considering the diverse interactions that occur between microbes and their microbiomes, microbes and their hosts, the varying environmental conditions that are experienced by the host and all members of its microbiome (with varying consequences), and all of the higher-order interactions between these factors. For example, a pathogen may cause a disease only in the presence (or absence) of certain microbes—themselves subject to the historical contingencies of community assembly—and only in the presence of certain environmental conditions that favor the pathogenesis of that disease-causing microbe and that disfavor other community members that might prevent such pathogenesis. Such a high degree of complexity that includes all temporal, biological, and environmental variables likely renders every microbe an individual that exists in a unique environment at a unique moment in time. If every microbe is an individual, their exact ecology at any particular moment in time cannot be precisely known and no experiment can ever entirely replicate an observed effect once it has occurred. This means that any effect of a microbe on a host will be only incompletely explained because some situation will always exist for which the causal mechanism that governs that interaction is unknown. Causal understanding of microbiomes in the real world must therefore always remain incomplete.

Are we therefore to admit defeat? Certainly not! Yes, ecological explanations will always be imprecise. However, we can still make progress by combining both of the experimental and ecological approaches described above to robustly identify causal mechanisms and their significance. The first of these, represented by Koch's third postulate and emphasized by Lynch et al. (2019), uses manipulative experiments that identify mechanisms where microbes (or microbiomes, metabolites, etc.) produce a specific effect on a host. The second, represented by Koch's first two postulates (interpreted with historical contextualization), determines the conditions under which these mechanisms are likely to operate. Lynch et al. (2019) describe experimental approaches to generalize the mechanisms identified using manipulative experiments to different experimental conditions, which I extend using ecological

considerations to identify which conditions are the most relevant for explaining general phenomena. This paired explanatory framework has strong parallels to the practice of statistical hypothesis testing, where P values describe the likelihood of an observed effect being due to chance and effect sizes describe the strength of the observed relationship (Sullivan and Feinn 2012). Taking care to consider both of these parameters is critical to avoid experimental results that are robust (i.e., have low P values) but that have low explanatory power (e.g., low R^2 values). Applying this analogy to microbiology, considering both experimental manipulations and ecological principles will help us identify causal mechanisms that both have well-defined mechanisms and that occur in widespread environmental contexts, even if those contexts cannot be defined with 100% precision.

As a final thought, it is interesting to consider how microbiologists would view causality if the discipline of microbiology had stronger historical roots in the natural history disciplines (zoology, botany, etc., from which ecology is derived) instead of physics and chemistry (from which molecular biology and biochemistry are derived). Both physics and chemistry have strong experimental traditions that have successfully uncovered general rules that can be expressed mathematically and that are strongly predictive. In contrast, ecology and evolution are more strongly challenged by historical contingency and natural variability, and struggle to create models that are broadly predictive. However, despite leaving substantial variation unexplained and often weakly generalizing to diverse systems, predictive models are considered to provide causal understanding in ecological research (Houlahan et al. 2017). Microbiome research seems to sit squarely at the interface between these scientific traditions, and so further work to define and contrast their varied approaches to defining causality might offer deep insights and paths forward to establish causality in microbiology.

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