

Principles and Illustrations of Real-World Causal Investigations

last revised, July 05, 2026

James B. Grace ^{1,2*}

¹ Affiliation: Department of Biology, Concordia University; Montreal, QC H4B 1R6, Canada

² Current Address: 610 Colonial Drive, Lafayette, Louisiana, USA 70506

* Correspondence: james.grace@concordia.ca; Tel.: +1-337-298-1671

Abstract

Causal investigations represent a long-standing tradition in science that serves to build causal knowledge and understanding of the real-world. In fact, human civilizations have been built on the mechanistic causal knowledge accumulated from this process. While philosophers and scientists have long discussed and advanced this form of inquiry, the subject has been ignored in causal statistics. This unfortunate omission has reached a critical point due to the widespread promotion of counterfactual statistical methods as if it is the only valid route to support causal inferences. A recent, expanded paradigm for causal studies, the Multi-Evidence Paradigm, directs attention to the long-term goal of building causal knowledge through sustained investigation. In this Research Brief, a workflow for the conduct of causal investigations is derived from the classic work *In Search of Mechanisms* (2013) by the philosophers Carl Craver and Lindel Darden. Three classic examples of causal investigations are summarized to illustrate the general guidance given by Craver and Darden. The workflow presented for real-world causal investigations is contrasted with the currently popular “imagined-worlds” causal inference approach from statistics to illustrate the fundamentally contrasting viewpoints that the multi-evidence paradigm seeks to resolve. A brief commentary is presented at the end discussing the barriers to progress in unifying causal methods.

Keywords: causal investigations; causal inference; mechanistic causal investigations.

1. Introduction

The general principles of mechanistic causal investigation are described by Craver and Darden (C&D) in their book, *In Search of Mechanisms* (2013). They set the context for the work they discuss as follows:

“Science is an engine of discovery. From the farthest reaches of space to the most fundamental units of matter, the crowning aim of science is to open the black box of nature and show how it works.”

Consideration of such a broad range of phenomena and the methods used to develop causal understanding necessitates a reliance on examples accompanied by a discussion of common approaches.

In their treatment, C&D describe causal investigations in terms of the steps commonly taken to establish an understanding of real-world causal mechanisms. Note that my use of the term “real-world” is meant to distinguish mechanistic causal investigations from counterfactual statistical inference studies, which deal with “imagined worlds” that ignore mechanistic information and are poorly suited to building causal knowledge.

In this treatment, I summarize some of the most generalizable advice provided by C&D and illustrate the scale of the ambition by summarizing three historical examples pointed to by C&D, the study of the human cardiovascular system, the advance of our understanding from Mendelian genetics to molecular genetics, and the determination of the mechanisms that control the behavior of the carnivorous plant, the Venus Flytrap. By focusing here on these mature examples of causal knowledge, I hope to redirect attention away from the currently popular but incorrect notion that the study of causation is a statistical activity accomplished through data analysis. Assuming that point is made here, a contemporary illustration of mechanistic causal investigation involving mangrove trees and climate change is provided in Grace and Osland (2026).

C&D’s characterization of mechanistic causal investigations is predicated on recent advances in the “new mechanistic philosophy of science” (Machamer, Darden and Craver 2000; Glennan 2017). From that perspective, mechanisms are understood to be made up of structures and processes spatio-temporally organized such that they are productive of behaviors consistent with the mechanism. The goal in mechanism discovery is to describe and characterize the entities and activities, to describe how they are organized, and to understand how that organization produces the phenomenon of interest.

2. A Workflow for Mechanistic Causal Investigations

Figure 1 presents a simplified workflow for real-world causal investigations based on C&D. An investigation is often initiated based on a desire to understand some phenomenon of interest. Grace (2024) suggests an early step is to chronicle existing causal knowledge relevant to the phenomenon. This will typically involve describing (a) the structural components of potential mechanism(s), (b) the processes potentially connecting structural components and the underlying physics, chemistry, and biology (i.e., mechanistic processes) (c) identifying key mechanistic elements that may control the behavior of the phenomenon, and describe the expected behavior of the phenomenon based on pre-existing mechanistic knowledge. C&D suggest consulting the stores of knowledge related to the system and the general type of mechanism. This information may then be organized in some fashion such that it can be critiqued and revised by future studies. Grace (2024) and Grace et al. (2025a and 2025b) illustrate a process referred to as *causal knowledge analysis*, which may be aided by developing a causal knowledge diagram, a reference table of source materials, and possibly a schematic

representation. This information should then lead to hypothesized patterns of expected behavior specific to that information.

In mechanistic investigations, validation is based on empirical studies that seek to determine whether real-world evidence is consistent with mechanistic understanding. These further studies initiate an adaptive process shaped by the specifics of the situation. C&D use the slogan – “the product guides the process.” The thesis is that characterizing a mechanism (the product) provides feedback to guide the reasoning in the discovery process (Craver and Darden 2013). Thus, causal investigations are distinguished by a few major features, but without a fixed roadmap.

Several points should be kept in mind in mechanistic investigations. First, mechanisms are often best understood through direct observation and measurement. Note it is expected that there will almost always be relevant pre-existing knowledge regarding the system and potential mechanisms to be considered. Causal investigations, by definition, attempt to build causal knowledge and understanding through a series of studies. Characterizations of regulatory systems are typically progressive, focusing initially on structures and processes that are expected to be most reliably influential (e.g., limiting effects of constitutive physiological requirements or physically determined). The notions of complete knowledge and certainty when dealing with mechanisms are understood to be replaced by concepts such as useful approximations, sufficiency, and refinement of understanding (aka deepening of knowledge). It should be recognized that mechanistic relationships do not correspond to the idealized behavior of statistical relationships. Consideration of appropriate interpretations for results should be described (see Grace et al. 2025a, 2025b). First-order focus is on qualitative correspondence, as an indication of external validation. Variance explanation is not typically a sole basis for conclusions, though it can be selectively useful as evidence as to whether alternative model forms conform to real-world manifestations as expected. Sufficiency is typically based on consistency between expectations and external observations as judged by scientific experts. Most of the time, useful progress is celebrated, particularly when accompanied by improved forecasts of expected outcomes.

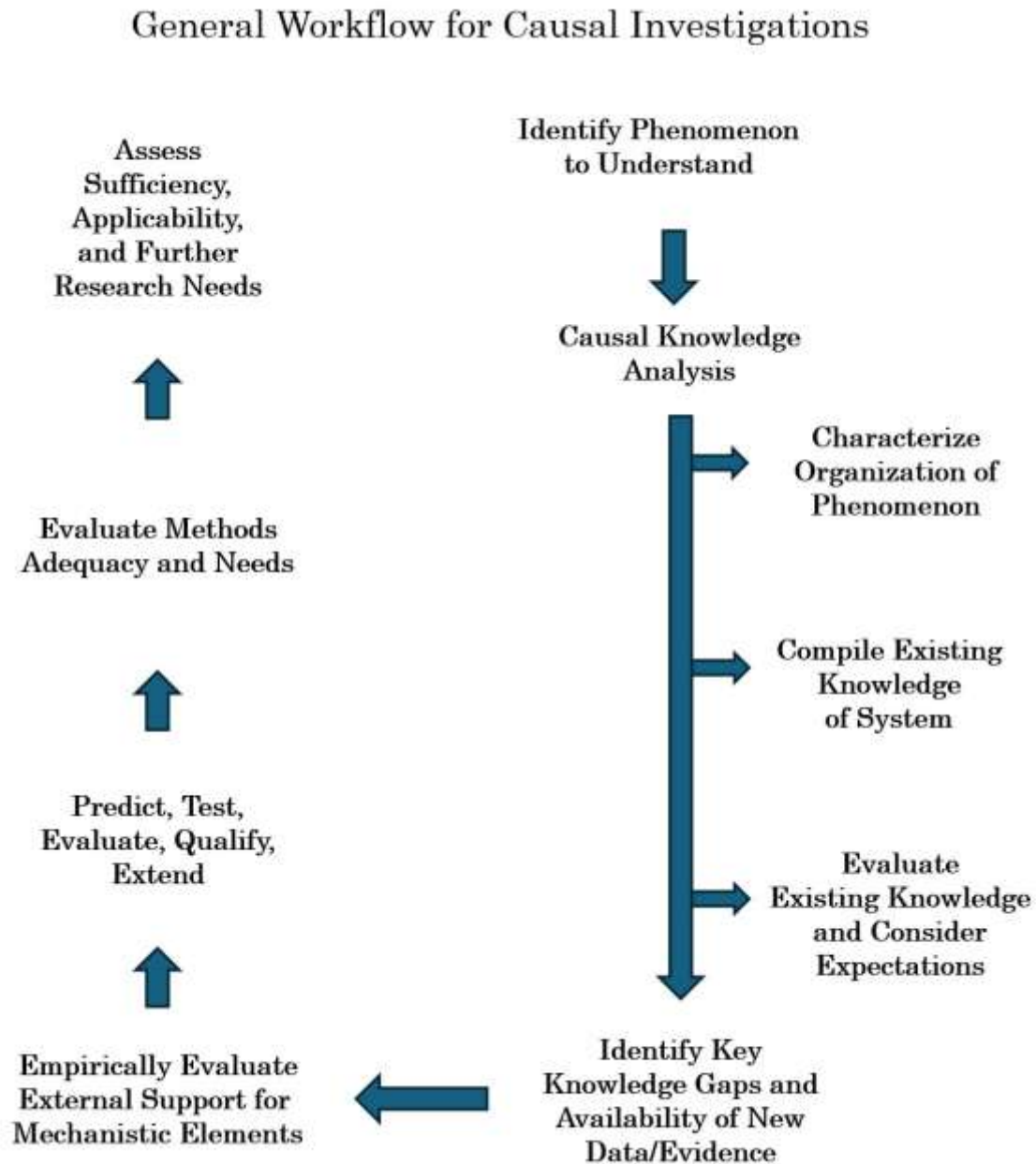


Figure 1. Example workflow following suggestions by Craver and Darden (2013) and Grace (2024).

3. Historical Example I – The Causes of Blood Flow

The development of a mechanistic causal understanding of blood flow in the human body provides an exemplary illustration of a causal investigation (Craver and Darden 2013). The essential features of our current understanding was developed by Willaim Harvey after a very long period where contrasting ideas about the complex interplay of structures and processes determining blood flow were considered and evaluated (Aird 2011). Harvey’s investigations

included the use of manipulations and calculations, along with anatomical examinations to establish that it is a closed, force-pumped system. Harvey's work revolutionized cardiovascular science, replacing non-mechanistic explanations with a mechanical model that allowed for the study of velocity, pressure, and resistance. Figure 2 shows a progression of schemas transitioning from the long-held idea that ingested food was transformed in the liver into blood and then distributed throughout the body passively.

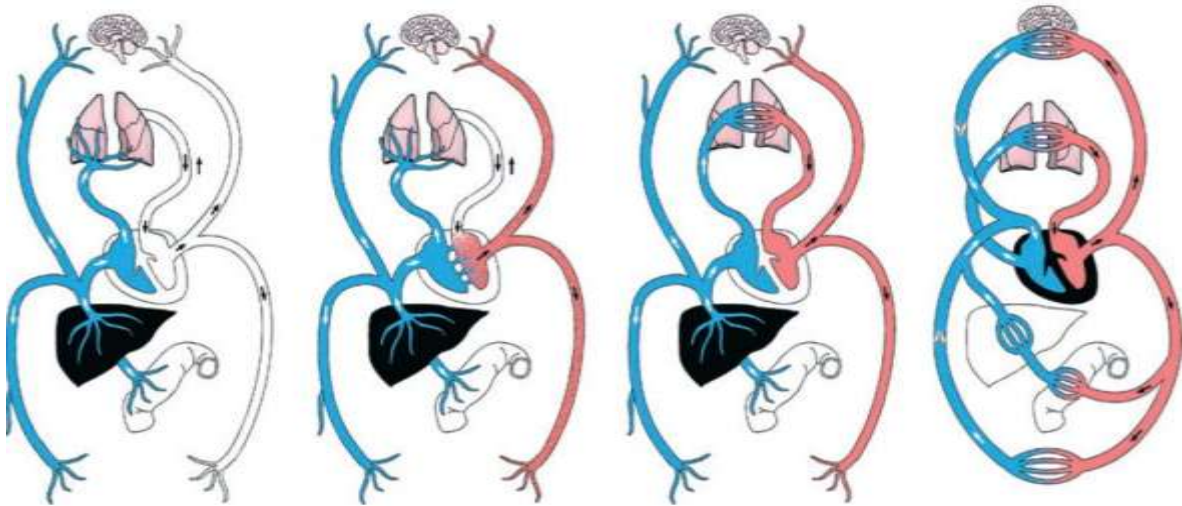


Fig. 2. Schematic of our understanding of the cardiovascular system over time (from left to right). From Aird (2011).

The progressive efforts to develop and deepen a mechanistic causal understanding of blood flow illustrate the general features of causal investigations, including:

1. Characterization of the dynamic behavior of the phenomenon.
2. Investigate and describe system structures and their properties.
3. Consider and evaluate mechanisms that can explain dynamic behavior, including underlying physical, chemical, and biological processes.
4. Develop and refine a summary schema/representation and compile accompanying documentation (aka causal knowledge analysis).
5. Create new empirical methods and approaches in response to the unique features of the mechanism so as to refine and verify accumulated understanding.
6. Project expected behavior and build confidence through repeated evaluations of external consistency with mechanistic understanding.
7. Extend understanding to as wide a variety of related phenomenon as possible.
8. Use mechanistic understanding for knowledge applications (e.g., heart medicines, surgical procedures) and to motivate refined understanding.

4. Historical Example II – Progressing from Mendelian to Molecular Genetics

A classic example of causal investigation involves the discoveries contributing to a mechanistic basis for genetic inheritance. Mersha (2024) provides a concise summary of the

full span of time from Gregor Mendel's phenomenological characterization of Mendelian Inheritance to today's applications of the accumulated knowledge (Fig. 3). Here I will focus solely on the steps leading to the initial discovery of how the structure of DNA encodes the genes indirectly observed by Mendel.

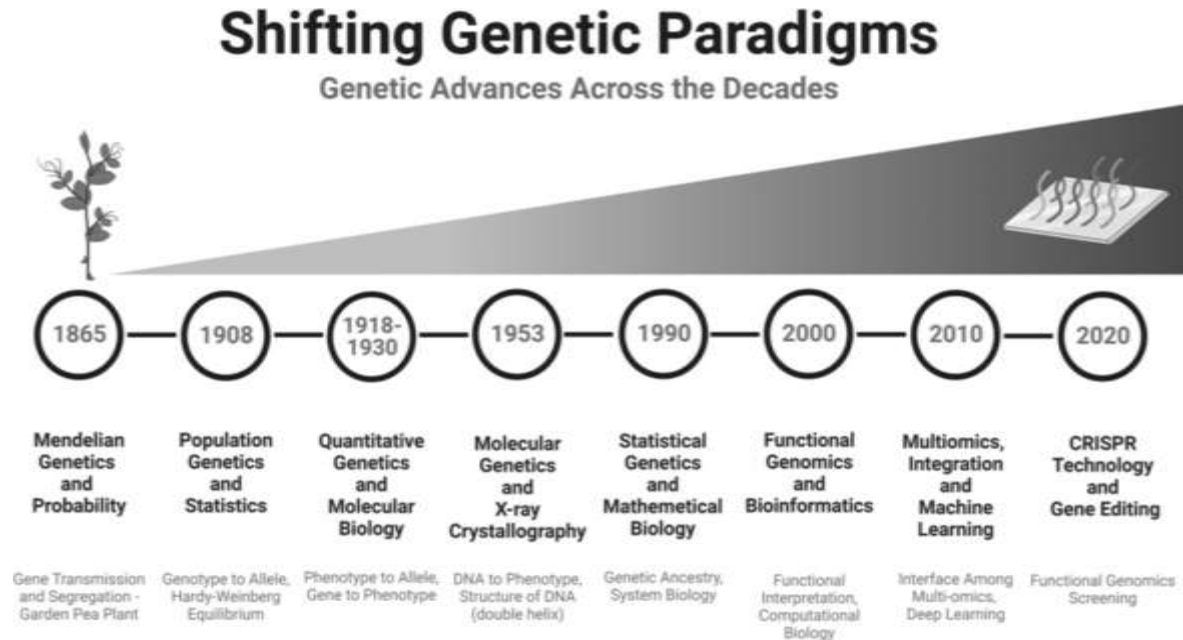


Fig. 3. Key events in the history of genetics research. In this presentation the focus is on how the observable phenomenon of Mendelian inheritance came to be understood in terms of the structure and function of DNA (aka molecular genetics). From Mersha (2024).

Gregor Mendel, an amateur scientist and monk living in what is now the Czech Republic, conducted breeding experiments using pea plants from 1857-1865, keeping meticulous notes focused on the patterns of inheritance for select traits. His own and later work described rules for the inheritance of discrete traits controlled by clear patterns of dominance and recessiveness. This work led to an era of quantitative genetics in which rules of inheritance were studied further without knowledge of the underlying mechanistic basis. The discovery of the mechanistic basis for heredity was a multi-generational journey from the identification of nucleic acid in 1869 by the Swiss chemist Friedrich Miesche, to the demonstration that nucleic acid is the genetic material in 1944, and finally a sequence of discoveries revealing DNA's double-helix structure in 1953. The practical power of this line of research is now to the point that Mersha states, "Genetics is rapidly becoming the backbone of all biomedical research."

5. Historical Example III – The Mechanisms that Control the Behavior of the Carnivorous Plant, Venus Flytrap

The Venus flytrap is a carnivorous plant that Charles Darwin described as one of the most wonderful plants in the world. Its behavior is remarkable among plants (Fig. 4).



Fig. 4. Venus flytrap in action (from <https://makeagif.com/gif/venus-flytrap-jaws-of-doom-2014-compilation-GDe1i4>).

The mechanistic basis for its behavior has fascinated scientists for over 100 years. Darwin was able to determine that it closes its trap when trigger hairs are touched, leading to a change in the shape of the leaf, which is under tension at rest. John Burdon-Sanderson established that the trapping action is accompanied by electrical signals back in the late 1800s. A few decades later it was determined that triggering usually requires contact with two hairs within a time period of 30 seconds to initiate closure and further studies determined that a “receptor potential” state is initiated by the first contact allowing the plant to sum subthreshold stimuli, thereby remembering the first touch. Further work has elucidated the role of ion channels in transforming physical signals into electrical signals (Sachse et al. 2020; Suda et al. 2025).

5. Summary

Causal investigations are mechanistic studies supplemented with knowledge obtained as to the potential behavior of the system. This combined information provides documentation for anticipating the real-world repeatability of controlling mechanisms. Assessments of mechanistic understanding adheres to a minimum requirement of external consistency with mechanistic expectations and a maximum aspiration of a full understanding (rarely achieved). For further discussion, see Grace (2024).

6. Barriers to Progress – The “Imagined Worlds” of Causal Statistics

The literature on causal methods is currently dominated by discussions of statistical approaches based on simplistic “imagined worlds” (in contrast to the real world). It is highly problematic that that literature studiously ignores mentioning the possibility of mechanistic causal inference and the accumulation of causal knowledge. Other problematic elements of statistical/counterfactual approaches are often omitted from discussion as well, which creates tremendous confusion. It is my current assessment that practitioners of counterfactual /

statistical causal inference will have to be externally motivated by the science community to become more forthcoming. More discussions of causal investigations and causal knowledge are vital for that to be accomplished.

The challenge faced by statistical causal inference has been clearly articulated at times. Rubin was first to describe an imagined world for causal studies based on hypothetical and unmeasurable “potential outcomes” where mechanistic knowledge from the real world was consciously and explicitly dismissed from consideration by the following logic,

“Scientists are usually concerned with understanding causal mechanisms. Purely statistical discussions of causality are substantially more limited in scope, because the unique contribution of statistics is to measuring causal effects and not to the understanding of causal mechanisms” (Holland and Rubin 1987).

In this imagined world, the only available *admissible evidence* for conclusions comes from data associations and therefore the only causal quantity that can be estimated is *counterfactual causal effects*. These effects attempt to estimate group differences akin to the differences between treated and untreated individuals subjected to some event using quasi-experimental techniques. By definition, a counterfactual causal effect estimate requires that all sources of possible bias have been controlled for. Thus, *the evidential standard/requirement has been referred to as the perfection standard/requirement* (Grace 2024). It is routinely admitted that there are numerous untestable assumptions that must be defended to even provide a plausible estimate of a counterfactual causal effect. Since any remaining sources of bias are of unknown magnitude, one can never be sure that the estimate obtained has any correspondence to the counterfactual value. *The evidential process* is therefore based on statistical conditioning/control (something familiar to statisticians dating back to the early 1900s). A critical but undiscussed feature of this system is that the estimated causal effects are properties of the individual dataset and not of some property that exists in the real-world. This is why there is so much emphasis on controlling bias because it is not possible to validate results using external consistency, the benchmark for real-world causal investigations. In turn, causal knowledge known to represent features of the real world is not characteristically generated from imagined-world studies.

Pearl (2009), a theoretician, went on to describe an alternative imagined world based on non-parametric continuous structural relations rather than potential outcomes. He also developed graphical modeling rules as an aid to reasoning about approaches to statistical conditioning. The style of his presentations is less often focused on restrictions, more focused on theoretical possibilities, and as a result more optimistic in tone. It is sometimes claimed that graphical models (Directed Acyclic Graphs, DAGs) represent mechanistic knowledge. The earlier portions of this presentation should make it clear that is a false interpretation. Analyses have shown that Pearl’s system is just as vulnerable to confounding bias as is the potential outcomes approach, but the opportunity for misrepresentation of causation is greater.

An example workflow for estimating counterfactual causal effects from an imagined-world study is presented in Figure 5 for discussion. We should keep in mind that the requirement for counterfactual causal effects is control of all forms of bias. The motivating logic is that if there

might be omitted confounding, there is no way to know its potential magnitude. Therefore, there is, by extension, no way to know whether estimated effects correspond even qualitatively to the true effect estimates. The focus in Figure 5 follows the emphasis in Pearl's presentations, which focus on control for known and unknown confounders. A perfection requirement directs one to ask how measurement error, spatial autocorrelation, simultaneous feedbacks, and heterogeneity (non-exchangeability) can be addressed. Procedural questions, such as how can DAG-Data consistency (Step 2) be evaluated with confidence if the control of confounding (Step 3) has not yet been achieved? Finally, external validation is not a part of counterfactual causal inference because causal effects are summary properties of the data sets and not repeatable components of the real world that exist independently from the data. Efforts to replicate counterfactual causal effects have thus far been unsuccessful (Cinelli et al. 2025), which is not surprising given the imagined-world aspect of causal statistics.

Example Workflow for an Imagined-World Causal Investigation under the Structural Causal Model

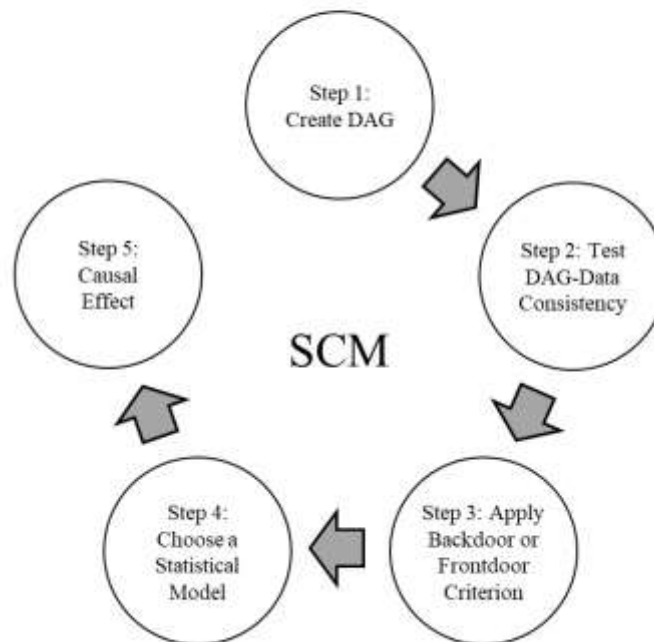


Figure 5. A workflow for causal inference under the Structural Causal Model (redrawn from Arif and MacNeil 2023).

A final, larger point to be made about the counterfactual causal inference approach is that scientists have played no role in establishing the evidential system, including the form of admissible evidence, evidential standards/ requirements, or evidential procedures, all of which derived from the imaginings of Rubin and Pearl, neither of which are scientists. The multi-evidence paradigm for causal investigations (Grace 2024), in contrast, incorporates the

centuries-old experiences of mechanistic scientists, who must play a prominent role in judging suitable inferences based on all relevant evidence and knowledge. Importantly, this same conclusion was just reached in 2022 by the National Academies of Science, Engineering, and Medicine in a Consensus Report on Causal Methods (NASEM 2022). The National Academies Report further concluded that workflows such as those shown in Figure 5 are to be deemed incomplete and insufficient for causal determination. Recent efforts to formalize the documentation and characterization of mechanistic evidence (Grace 2024; Grace et al. 2025a, 2025b) aid the operationalization of the National Academies' recommendation.

References

- Aird, W.C., 2011. Discovery of the cardiovascular system: from Galen to William Harvey. *Journal of thrombosis and haemostasis*, 9, 118-129. (Freely available via the Journal of Thrombosis and Haemostasis).
- Arif, S. and MacNeil, M.A., 2023. Applying the structural causal model framework for observational causal inference in ecology. *Ecological Monographs*, 93(1), p.e1554.
- Cinelli, C., Feller, A., Imbens, G., Kennedy, E., Magliacane, S. and Zubizarreta, J., 2025. Challenges in statistics: A dozen challenges in causality and causal inference. *arXiv preprint arXiv:2508.17099*.
- Craver C.F. and Darden, L., 2013. *In Search of Mechanisms*. Univ. Chicago Press.
- Glennan, S., 2017. *The New Mechanical Philosophy*. Oxford University Press.
- Grace, J.B., 2024. An integrative paradigm for building causal knowledge. *Ecological Monographs*, 94, e1628.
- Grace, J.B., 2026. Synoptic Summary of National Academies Assessment of Causal Frameworks. Research_Brief. G2026.3
- Grace, J.B. et al., 2025a. Causal effects vs causal mechanisms: Two traditions with different requirements and outcomes. *Ecology Letters* 28, e70029.
- Grace, J.B. et al., 2025b. Causal interpretations in ecology can be based on mechanistic knowledge. *Journal of Ecology*, 113, 3084–3098.
- Holland, P.W. & Rubin, D.B., 1987. Causal inference in retrospective studies. *ETS Research Report Series*, 1987, 203-231.
- Mersha, T.B., 2024. From Mendel to multi-omics: shifting paradigms. *European Journal of Human Genetics*, 32(2), pp.139-142.
- NASEM 2022. National Academies of Sciences, Engineering, and Medicine, 2022. *Advancing the Framework for Assessing Causality of Health and Welfare Effects to Inform National Ambient Air Quality Standard Reviews* The National Academies Press. <https://doi.org/10.17226/26612>.
- Pearl, J., 2000. *Causality*, Cambridge University Press
- Rubin, D. B. Estimating causal effects of treatments in experimental and observational studies. *ETS Research Bulletin Series* 2, i-31 (1972).
- Sachse, R., Westermeier, A., Mylo, M., Nadasdi, J., Bischoff, M., Speck, T. and Poppinga, S., 2020. Snapping mechanics of the Venus flytrap (*Dionaea muscipula*). *Proceedings of the National Academy of Sciences*, 117(27), pp.16035-16042.
- Suda, H., Asakawa, H., Hagihara, T., Ohi, S., Segami, S., Hasebe, M. and Toyota, M., 2025. MSL10 is a high-sensitivity mechanosensor in the tactile sense of the Venus flytrap. *Nature Communications*, 16(1), p.8280.