Koch's Postulates

For Pathogen Discovery Today

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Joint Graduate Seminar

Department of Microbiology

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Agenda Today

01

Causation in Philosophy

- About causation
- Causation Types

02

Hill's Criteria of Causation

03

Koch's Postulates

- Dissecting K's P's
- Limitations

04

Revised K's P's

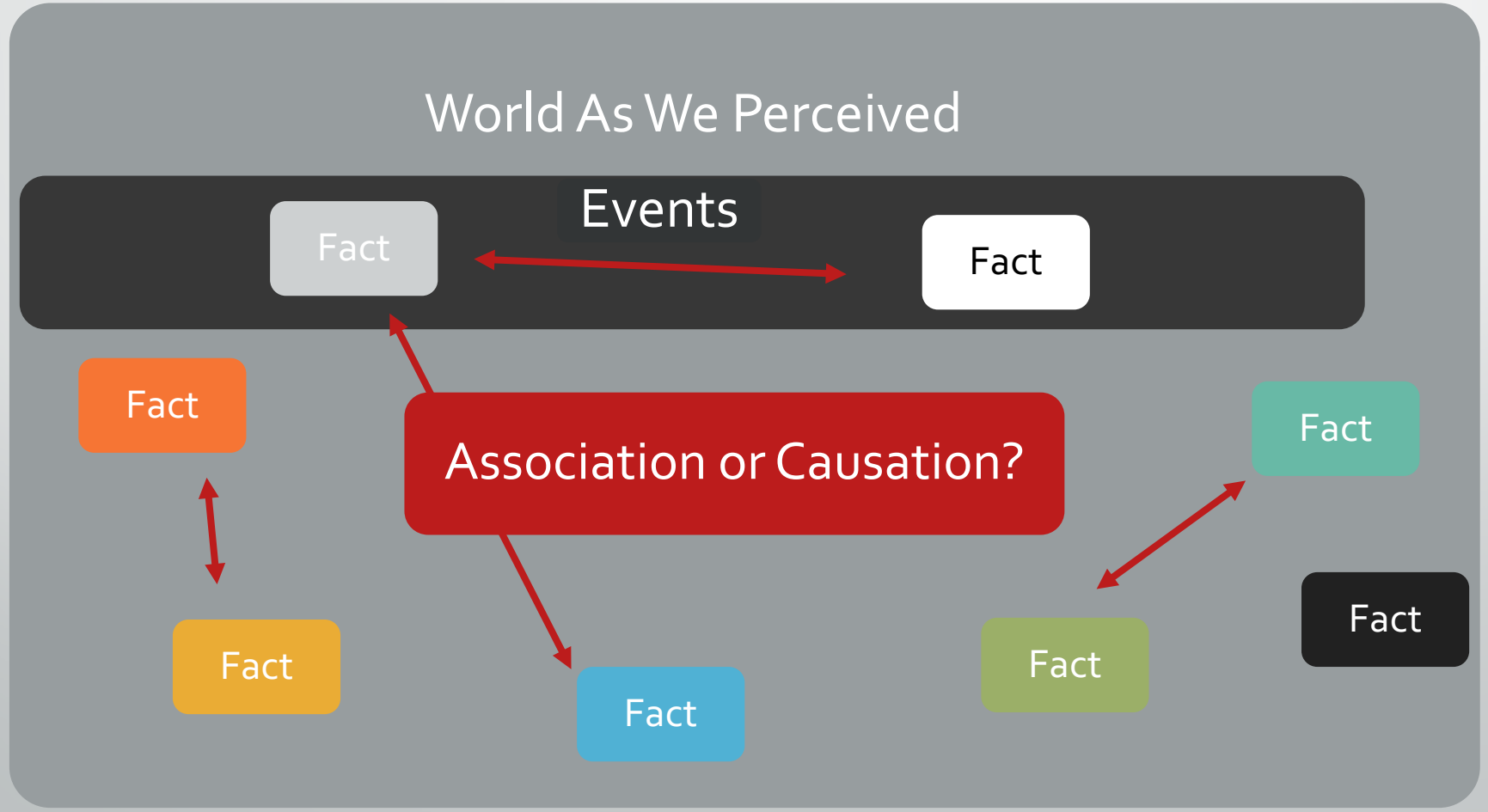
- Postulates on Viral Diseases
- Elements of Immunological Proof of Causation
- Molecular K's P's
- K's P's for 21st Century
- Timeline of revised K's P's

05

Summary

- (K's P's Limits)
- (Revised K's P's)
- The Road Ahead

Causation in Philosophy
World As We Perceived



Causation in Philosophy: World As We Perceived

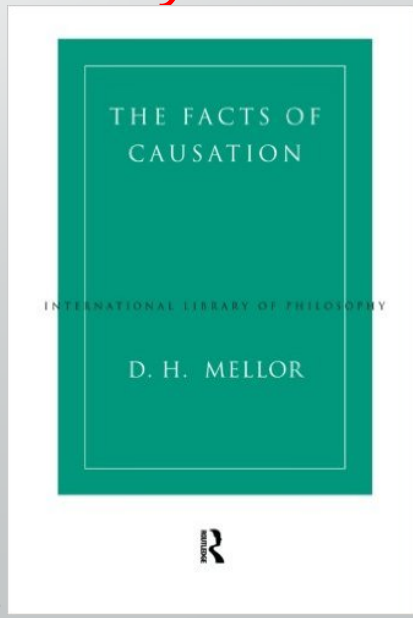
- Both cause and effects are *facts*.
- *Causation is not observable*. The only observable form is the events (Marley & MicMichael, 1991).
- These individual links however, are at most *association*.
- *Concordance of evidences*: prove causation rather than association.

Marley, J. E., & McMichael, A. J. (1991). Principles behind practice. 6. Disease causation. The role of epidemiological evidence. 155:95–101. 69. McCune, J. M.,. *Medical Journal of Australia, 155*, 95-101.

Mellor, D. H. (1999). *The Facts of Causation.* New York: Routledge.

Causation in Philosophy
What Mellor Thinks

- Causation are embodied in facts (Causation links facts).
- df: Causes raise the chance of their effects.
- Causation can be *deterministic* or *indeterministic*, *sufficient* or *necessary*.
- <u>Deterministic</u>
  - For C is sufficient - If C, then E
  - For C is necessary – If not C, then not E
- <u>Indeterministic</u>
  - For C is sufficient – $Ch(E|C) > Ch(E|\perp C)$
  - For C is necessary – $Ch(\perp E|\perp C) > Ch(\perp E|C)$



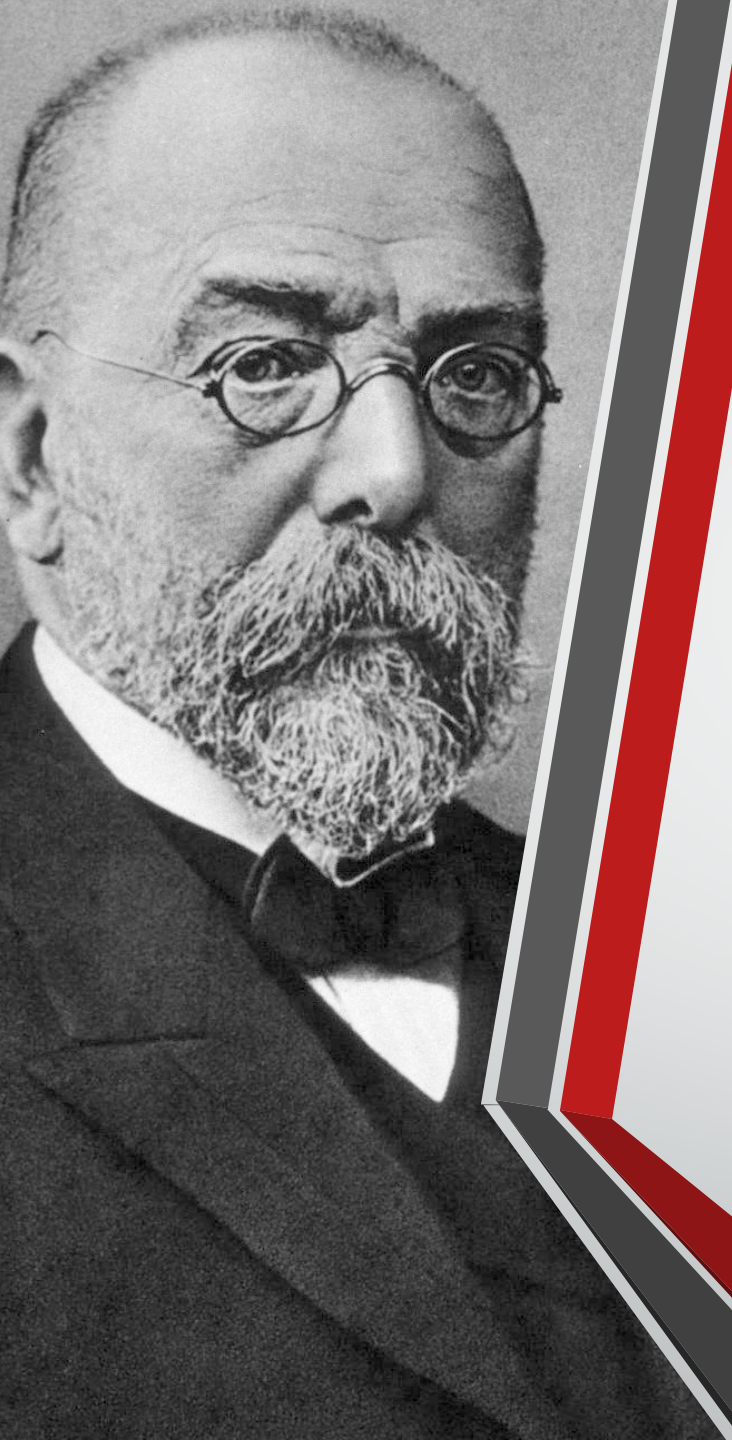
Mellor, D. H. (1999). *The Facts of Causation.* New York: Routledge.

Hills Criteria of Causation

Nine criteria to consider for assigning causality:

- Temporal relationship
- Strength of association
- Dose-response effect
- Replication of observations
- Biological plausibility
- Consideration of alternatives
- Cessation of exposure
- Consistency with other knowledge
- Specificity of association

Hill, A. B. (1965, May). The Environment and Disease: Association or Causation? *Proceedings of the Royal Society of Medicine, 58*(5), 295-300.



Koch's Postulates

- Rules to govern physicians / microbiologists to *sort out cause* (causative agent) *and effect* (diseases)
- "Gold Standard"
- Holds true for human, animal and plant diseases

Pic source: http://alchetron.com/Robert-Koch-1175442-W

Gradmann, C. (2008). «Common sense, proper training and sound reasoning» – Koch's postulates and 20th century medicine. *Michael, 3*, 217-228.

Lipkin, W. l. (2013). The changing face of pathogen discovery and surveillance. *Nature Microbiology, 11*, 133-141.

Woodford, N., & Johnson, A. P. (2004). *Genomics, Proteomics, and Clinical Bacteriology* (Vol. 266). London: Humana Press.

Koch's Postulates – What Evidence They Seek

a. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
b. The microorganism must be isolated from a diseased organism and grown in pure culture.
c. The cultured microorganism should cause disease when introduced into a healthy organism.
d. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Koch, R. (1876). Untersuchungen über Bakterien: V. Die Ätiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des Bacillus anthracis. *Cohns Beitrage zur Biologie der Pflanzen, 2*(2), 277-310.

Koch's Postulates: The Caveat (1)

- a. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

- *Some asymptomatic carriers due to various host factors (immunological status, physiological status, genetic variability). e.g. Cholera.*

Fredrick, D. N., & Relman, D. A. (1996, Jan). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews*, *9*(1), 18-33.
Lipkin, W. I. (2009). Microbe hunting in the 21st century. *PNAS*, *106*(1), 6-7.
Lipkin, W. I. (2013). The changing face of pathogen discovery and surveillance. *Nature Microbiology*, *11*, 133-141.

Koch's Postulates: The Caveat (2)

- b. The microorganism must be isolated from a diseased organism and grown in pure culture.

- *Most bacteria are actually uncultivable; viruses cannot be grown in pure culture.*

Fredrick, D. N., & Relman, D. A. (1996, Jan). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews, 9*(1), 18-33.
Lipkin, W. I. (2009). Microbe hunting in the 21st century. *PNAS, 106*(1), 6-7.
Lipkin, W. I. (2013). The changing face of pathogen discovery and surveillance. *Nature Microbiology, 11*, 133-141.

Koch's Postulates: The Caveat (3)

- c. The cultured microorganism should cause disease when introduced into a healthy organism.

- *Ethical issues when dealing with human and animal pathogens. E.g. HIV.*

Lipkin, W. I. (2009). Microbe hunting in the 21st century. *PNAS*, *106*(1), 6-7.
Lipkin, W. I. (2013). The changing face of pathogen discovery and surveillance. *Nature Microbiology*, *11*, 133-141.

Koch's Postulates: The Caveat (4)

- d. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

- *If prior postulates cannot be fulfilled, this one can't too.*

Lipkin, W. I. (2009). Microbe hunting in the 21st century. *PNAS*, *106*(1), 6-7.
Lipkin, W. I. (2013). The changing face of pathogen discovery and surveillance. *Nature Microbiology*, *11*, 133-141.

Revising Koch's Postulates

Additional rules / a reworked version of K's P's may be needed

Thomas River - Postulates On Viral Diseases

- He believes that blindly following K's P's had became a hindrance.
- River argued that K's P's not to be fulfilled in viral disease.
  - Most viral infections become asymptomatic
  - Viruses can't be cultured on lifeless medium.

Pic source: http://centennial.rucares.org/index.php?page=Modern_Virology

River, T. M. (1937). Viruses and Koch's Postulates. *Journal of Bacteriology*, *33*(1), 1-12.

Postulates On Viral Diseases

a) A specific virus *must be found associated* with a disease with a degree of regularity.

b) The virus *must be shown to occur* in the sick individual not as an incidental or accidental finding but as the cause of the disease under investigation.

River, T. M. (1937). Viruses and Koch's Postulates. *Journal of Bacteriology*, *33*(1), 1-12.

Alfred Evans - Elements of Immunological Proof of Causation

- Evans noticed that the original *K's P's poorly encompass viral diseases*.
- The postulates were derived based on Evans' work on EBV and infectious mononucleosis.
- Assign causality based on *seroreactivity* to the suspect pathogen's antigen
- Does not require the causative agent be isolated and grow in pure culture

Evans, A. (2012). *Causation and Disease: A Chronological Journey.* New York and London: Springer Science & Business Media.Ravindran, B. (2016). New pathogen discovery. *Current Science, 110*(4), 549-551.

Elements of Immunological Proof of Causation

a) Antibody to the agent is *regularly absent* prior to the disease and exposure to the agent.

b) Antibody to the agent *regularly appears* during illness and includes both immunoglobulin G and M classes.

c) The presence of antibody to the agent *predicts immunity* to the clinical disease associated with primary infection by the agent.

d) The absence of antibody to the agent *predicts susceptibility* to both infection and the disease produced by the agent.

e) Antibody to no other agent should be similarly associated with the disease unless it is a cofactor in its production.

Evans, A. (2012). *Causation and Disease: A Chronological Journey.* New York and London: Springer Science & Business Media.Ravindran, B. (2016). New pathogen discovery. *Current Science, 110*(4), 549-551.

Stanley Falkow - Molecular Koch's Postulates

- Falkow believes that pathogenicity is caused by genes carried in pathogenicity island.
- Explained some asymptomatic status?
- Causality based on molecular evidences.

Pic source: http://med.stanford.edu/news/all-news/one-to-one/2008/stanley-falkow-on-his-lifes-work.html

Falkow, S. (2004). Molecular Koch's postulates applied to bacterial pathogenicity — a personal recollection 15 years later. *Nature Microbiology*, *2*, 67-72.

Molecular Koch's Postulates

a) The phenotype or property under investigation should be *associated* with pathogenic members of a genus or *pathogenic strains of a species*.

b) Specific *inactivation of the gene(s)* associated with the suspected virulence trait should lead to a *measurable loss in pathogenicity* or virulence, or the gene(s) associated with the supposed virulence trait should be isolated by molecular methods. Specific inactivation or deletion of the gene(s) should lead to loss of function in the clone.

c) *Reversion or allelic replacement* of the mutated gene should lead *to restoration of pathogenicity*, or the replacement of the modified gene(s) for its allelic counterpart in the strain of origin should lead to loss of function and loss of pathogenicity or virulence. Restoration of pathogenicity should accompany the reintroduction of the wild-type gene(s).

Falkow, S. (2004). Molecular Koch's postulates applied to bacterial pathogenicity — a personal recollection 15 years later. *Nature Microbiology*, *2*, 67-72.

Fredricks and Relman - Koch's Postulates for 21st Century

- Fredricks and Relman believe that:
  - advancement in nucleic acid-based microbial detection method
  - uncultivable microorganisms

  made traditional K's P's obsolete.
- Aim to provide a paradigm shift from clinical-based evidence to sequence-based evidence to prove causality.
- Causality by culture-independent molecular method.

Pic source: http://www.antimicrobe.org/authors/david_fredricks.asp
http://fsi.stanford.edu/people/david_relman

Fredrick, D. N., & Relman, D. A. (1996, Jan). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews*, *9*(1), 18-33.

Koch's Postulates for 21st Century (1)

a) *A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease*. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased (i.e., with anatomic, histologic, chemical, or clinical evidence of pathology)and not in those organs that lack pathology.

b) *Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences* should occur in hosts or tissues *without disease*.

c) *With resolution of disease* (for example, with clinically effective treatment), the *copy number of pathogen-associated nucleic acid sequences should decrease* or become undetectable. With clinical relapse, the opposite should occur.

d) When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.

Fredrick, D. N., & Relman, D. A. (1996, Jan). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews*, *9*(1), 18-33.

Koch's Postulates for 21st Century (2)

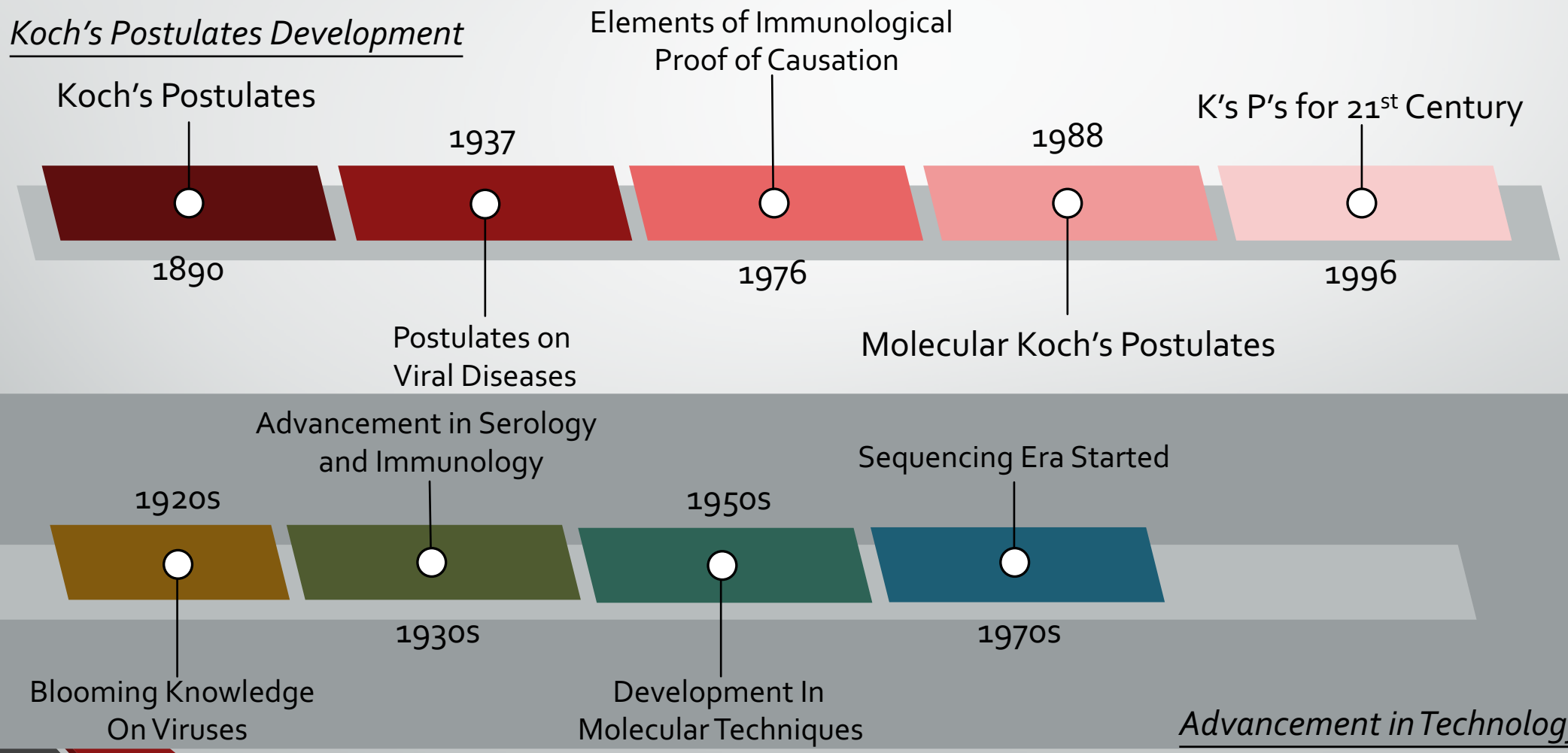
e) The nature of the microorganism inferred from the available sequence should be *consistent with the known biological characteristics of that group of organisms*. When phenotypes(e.g., pathology, microbial morphology, and clinical features)are predicted by sequence-based phylogenetic relationships, the meaningfulness of the sequence is enhanced.

f) Tissue-sequence correlates should be sought at the cellular level: efforts should be made to *demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology* and to visible microorganisms or to areas where microorganisms are presumed to be located.

g) These sequence-based forms of evidence for *microbial causation should be reproducible*.

Fredrick, D. N., & Relman, D. A. (1996, Jan). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews*, *9*(1), 18-33.

Timeline of Pathogen Discovery Milestones



The Road Ahead

- Despite how robust the revised K's P's are:

A. Consensus regarding the df of pathogen (e.g. prions).
B. A set of acceptable criteria that applies to all disease-causing agent (along with development of technology).

End Word

- "*The power of Koch's postulates comes not from their rigid application, but from the spirit of scientific rigour that they foster.*" - Fredericks and Relman

- There is no fault in K's P's or any of its revised version, they only serve as a guideline to help us assign causality.
- Know K's P's and their revised form, but don't rely on them.

Evans, A. (2012). *Causation and Disease: A Chronological Journey.* New York and London: Springer Science & Business Media.Ravindran, B. (2016). New pathogen discovery. *Current Science, 110*(4), 549-551.

Acknowledgement

- This powerpoint's logic flow is inspired by Professor Neil Mcroberts from University of California, Davis on his class GDB 101 – Epidemiology (Week 1, causation).

Thank You! That's All I have!

Summary (Koch's Postulates' Limitations)

Koch's Postulates (Not in exact wording)	Problems Associated
The microorganism must be found in diseased organism. The opposite holds for healthy individual	Asymptomatic carriers; false diagnosis
The microorganism must be isolated from a diseased organism and grown in pure culture	Non-cultivable microorganisms
The cultured microorganism should cause disease when introduced into a healthy organism	Ethical Issues in human and animal Inoculation problem in plants
The original specific microorganism must be identified by isolation from the inoculated, diseased experimental host.	Prior postulates must be fulfilled

Summary (Revised K's P's)

Proof of Causation	Mode of Proof	Limitations
Koch's Postulates	Clinical-based evidences	See last slide
Postulates on Viral Diseases	Association and other concordance of evidences	N/A to asymptomatic carriers
Elements of Immunological Proof of Causation	Serological and Immunological reactions	N/A to neurological infectious diseases
Molecular Koch's Postulates	Based on virulence genes	Provide little help to original K's P's
Koch's Postulates for 21st Century	Based on sequence-based molecular method	N/A to prions

Summary – Are We There Yet?

Problems Associated	Revised K's P's for the Issue
Asymptomatic carriers; false diagnosis	1. Elements of Immunological Proof of Causation 2. Molecular Koch's Postulates
Non-cultivable microorganisms ; Virus	1. Postulates on Viral Diseases 2. Molecular Koch's Postulates 3. Koch's Postulates for 21st Century
Ethical Issues in human and animal Inoculation problem in plants	1. Elements of Immunological Proof of Causation 2. Postulates on Viral Diseases 3. Molecular Koch's Postulates 4. Koch's Postulates for 21st Century