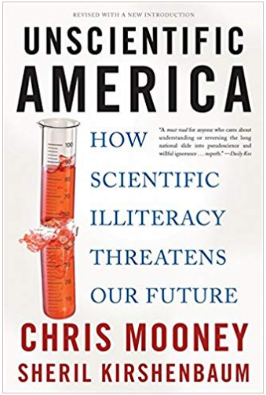
Science & the Scientific Method

Scientific Literacy is in Trouble

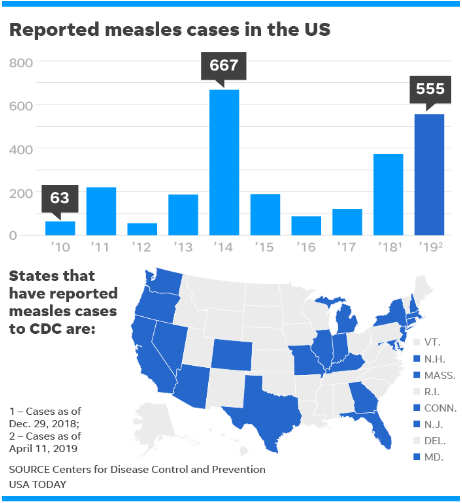
Only 20–25% of North Americans are scientifically literate![1]

- 1 in 5 think the Sun revolves around the Earth
- $< 1/3$ know DNA is the hereditary material
- $\sim$10% know what radiation is
- …



1. Miller (1998) *Public Understanding Sci.* **7**: 203–223.

Scientific Literacy is in Trouble

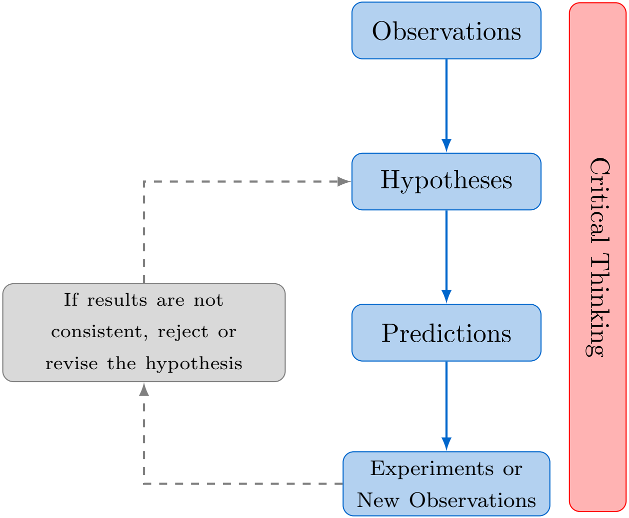


Why Is Scientific Literacy Important?

Science is playing an increasing role in our day-to-day lives

- Climate change
- Alternative energy sources
- Health care
- Impact of development
- Disease spread (vaccines, HIV, resistant bacteria)
- Stem cell research
- Forensics
- ...

The Scientific Method



The Scientific Method

Critical Thinking

In science, critical thinking often means: being able to separate **data** from **interpretation of data**

- Don't rely on other people's word or interpretation, no matter who they are
- Only trust data (and only do this if you are convinced methods were appropriate)

Should do nothing short of changing
the way you interact with the world

CBC

The New York Times

WIKIPEDIA

The Free Encyclopedia

The Daily

OPEN ACCESS Freely available online

PLOS ONE

Assessment of the Extirpated Maritimes Walrus Using Morphological and Ancient DNA Analysis

Brenna A. McLeod[1,2]*, **Timothy R. Frasier**[1], **Zoe Lucas**[2,3]

1 Biology Department, Saint Mary's University, Halifax, Nova Scotia, Canada, **2** Nova Scotia Museum of Natural History, Halifax, Nova Scotia, Canada, **3** The Friends of the Green Horse Society, Halifax, Nova Scotia, Canada

Abstract

Species biogeography is a result of complex events and factors associated with climate change, ecological interactions, anthropogenic impacts, physical geography, and evolution. To understand the contemporary biogeography of a species, it is necessary to understand its history. Specimens from areas of localized extinction are important, as extirpation of species from these areas may represent the loss of unique adaptations and a distinctive evolutionary trajectory. The walrus (*Odobenus rosmarus*) has a discontinuous circumpolar distribution in the arctic and subarctic that once included the southeastern Canadian Maritimes region. However, exploitation of the Maritimes population during the 16th-18th centuries led to extirpation, and the species has not inhabited areas south of 55°N for ~250 years. We examined genetic and morphological characteristics of specimens from the Maritimes, Atlantic (*O. r. rosmarus*) and Pacific (*O. r. divergens*) populations to test the hypothesis that the first group was distinctive. Analysis of Atlantic and Maritimes specimens indicated that most skull and mandibular measurements were significantly different between the Maritimes and Atlantic groups and discriminant analysis of principal components confirmed them as distinctive groups, with complete isolation of skull features. The Maritimes walrus appear to have been larger animals, with larger and more robust tusks, skulls and mandibles. The mtDNA control region haplotypes identified in Maritimes specimens were unique to the region and a greater average number of nucleotide differences were found between the regions (Atlantic and Maritimes) than within either group. Levels of diversity (h and π) were lower in the Maritimes, consistent with other studies of species at range margins. Our data suggest that the Maritimes walrus was a morphologically and genetically distinctive group that was on a different evolutionary path from other walrus found in the north Atlantic.

Citation: McLeod BA, Frasier TR, Lucas Z (2014) Assessment of the Extirpated Maritimes Walrus Using Morphological and Ancient DNA Analysis. PLoS ONE 9(6): e99569. doi:10.1371/journal.pone.0099569

Editor: Ulrich Joger, State Natural History Museum, Germany

Received November 14, 2013; **Accepted** May 2, 2014; **Published** June 12, 2014

Funding: The research was funded by ExxonMobil (www.exxonmobil.com), The Friends of the Green Horse Society (http://www.greenhorsesociety.com), and the Nova Scotia Museum of Natural History (http://naturalhistory.novascotia.ca). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Regarding any competing interests due to funding from ExxonMobil, the authors confirm that this does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

* E-mail: bmysticetus@gmail.com

Introduction

The biogeography of a species is a result of a complex series of past and current climate changes, ecological interactions, anthropogenic impacts, physical geography, and evolution. To fully understand the contemporary biogeography of a species, and to make projections for future survival, it is necessary to understand the species' history. It is important to examine specimens from regions of localized extinction, as the loss of a species from such regions may represent the loss of unique adaptations and the loss of a potentially distinct evolutionary trajectory for the species. Of particular importance are margins of the species range, which are often identified as areas of increased genetic differentiation and isolation as well as morphological adaptation to 'new' habitats, or niche evolution [1].

The walrus (*Odobenus rosmarus*) is a large pinniped with a discontinuous circumpolar distribution in the arctic and subarctic. The species is easily recognizable by its large tusks and robust size, and is the only species within its family, Odobenidae. There are currently three recognized subspecies. The Atlantic walrus (*O. rosmarus rosmarus*, Illiger, 1815) is found throughout the Eastern Canadian Arctic to Franz Josef Land, the Barents and Kara Seas. The Pacific Walrus (*O. rosmarus divergens*, Linnaeus, 1758) is found in the Bering and Chukchi Seas. The third subspecies (*O. rosmarus laptevi*, Chapskii, 1940) is found in the Laptev Sea. However, the distinction of this last group has been debated and recent morphological and molecular data suggest that *O. r. laptevi* be considered synonymous with *O. r. divergens* [2]. Following centuries of extensive exploitation, the current status and population size of each subspecies are poorly known and currently being investigated. Hunting still occurs in most regions (Canada, US, Russia, and Greenland), and the species may be threatened by habitat disturbance, pollution and climate change [3].

Contemporary walrus distribution and diversity are a result of the species shifting its range through time, which has been tightly associated with climate changes over the past 5–8 million years. While *Odobenus* likely originated in the Pacific Ocean, approximately 5–8 million years ago (mya), individuals from the Pacific founded the Atlantic stock through the Panama seaway [4]. Subsequently, the Pacific stock is thought to have become extinct ~2mya [5] and it is suggested that the current Pacific subspecies originated by recolonization from the Atlantic, through either the

Canadian or Russian Arctic during a subsequent interglacial period [6].

In the North Atlantic, fossil walrus specimens identified throughout the eastern North American seaboard (Virginia, Maine, North Carolina, New Jersey) [7] suggest a more southerly distribution during the Wisconsinan Glaciation/early Pleistocene. However, with climate warming following the last glacial maximum (LGM), the Atlantic walrus distribution shifted northward reaching the Bay of Fundy and the Grand Banks by 12,500–12,800 before present (BP), southern Labrador by 11,500 BP, and the central Canadian Arctic by 9,700 BP [8].

Today ~8 subpopulations of the Atlantic walrus remain. Five of these are found west of Greenland and three are found east of Greenland [9] (but see [10,11] for recommendations on further subdivision)(Fig. 1). Support for the recognition of subpopulation status of these groups (e.g. for management purposes) has been found in both morphological and genetic data for most of these groups (e.g. [9], [12], [13], [2]).

The walrus once inhabited the Canadian Maritimes (waters of the Eastern Canadian provinces of Quebec, New Brunswick, Nova Scotia, and Prince Edward Island), with a population suggested to have been greater than 100,000 during the 17th century [14–16]. Because it was prized for its ivory-like tusks, thick hide and blubber [7], it was heavily hunted during the 16th–18th centuries, particularly around Sable Island, Nova Scotia; the Magdalene Islands; Prince Edward Island; other Gulf of St. Lawrence islands; and off the coast of New Brunswick and Cape Breton [8,17,18]. The species is now extirpated from these areas and has not been common south of 55°N for ~250 years [19,20]. Although there have been very occasional sightings of apparent strays in the region (e.g. [21], [20], [22], [23]), there have been no signs of recolonization and recovery is considered unfeasible [24].

Some data suggest that the Maritimes walrus was distinct from other Atlantic walrus. Although there are no living examples, numerous postglacial walrus specimens have been identified in New Brunswick, Nova Scotia, Newfoundland, and throughout the Gulf of St. Lawrence [19,25–27]. C. R. Harington and the late F. H. Fay conducted preliminary analyses of 92 adult (72 male, 20 female) postglacial walrus from the Maritimes region. They found that this walrus was larger overall, and had very large upper incisors (I3) relative to the Atlantic walrus (pers. comm. to C. R. Harington, 1992, reported by [8]). They suggested the skull specimens, though different in shape, appeared similar to the Pacific walrus which tends to be ~3% taller and ~10% heavier than the Atlantic walrus, and is recognizable by its longer tusks and broader snout [4,28]. In addition, larger size was evident in large average rostral width and condylobasal length (F. H. Fay pers. comm. to ZL).

We assessed the Maritimes walrus within what is known about global and regional walrus stock structure. The walrus, like several other arctic and subarctic marine mammal species, such as the bowhead (*Balaena mysticetus*) and beluga whales (*Delphinapterus leucas*), appears to have recently inhabited the waters of southeastern Canada. The fact that the area has not been recolonized by walrus may indicate the previous inhabitants were either a distinct, isolated (and perhaps specially adapted) group, or climatic changes over the past 250 years have rendered the habitat now unsuitable. If the group was relatively isolated from more northerly groups, it may have been more genetically and/or morphologically differentiated as well. If this group was adapted for a warmer habitat, its loss represents the loss of evolutionary potential for the species.

For this research, morphological and genetic characteristics of cranial specimens of the extirpated Maritimes walrus collected in Nova Scotia, New Brunswick and Quebec, Canada were compared to those of specimens from current Atlantic and Pacific populations to examine the relationship between the Maritimes group and other walrus subspecies.

Materials and Methods

Morphological Analysis

Morphological data were collected from cranial and mandibular bones held in both public and private collections (n = 278). This included 116 Maritimes (MAR)(*Odobenus rosmarus rosmarus*) specimens (65 cranial/51 mandibular elements), 156 specimens from the Eastern Canadian Arctic (the Atlantic subspecies, ATL) (*Odobenus r. rosmarus*)(82 cranial/74 mandibular elements), and 6 from the North Pacific (the Pacific subspecies, PAC) (*O. r. divergens*) (4 cranial/2 mandibular elements) (Table 1, Fig. 2, Table S1). Most specimens from the ATL have tusk length-at-age and sex information (after [29]). The cranial sample includes 41 males and 30 females while the mandibular sample contains 34 males and 27 females. Ages, as determined by Mansfield [29] ranged from 2.1 – 26+ years. The authors confirm that access to these specimens can be granted to other researchers upon request.

Morphological data were collected using measures previously outlined for seals by the Committee on Marine Mammals [30] and those used previously in *Odobenidae* (after [31], [32], and [2]). Additional measures were used that 1) are commonly used in assessing mammalian cranial morphology; 2) served to capture additional information from skulls in cases where parts of the skull were missing due to fracture or wear (e.g. occipital condyles) and 3) added additional morphological information that we thought was appropriate and informative. Where specimen condition allowed, 18 metric characteristics were examined (Table 2; Fig. 3). These included 5 mandibular measures and 13 cranial measures. Measurements greater than 30.48 cm were taken using vernier calipers (to the nearest 1.0 mm). Measurements less than 30.48 cm were taken using digital calipers (Mitotoyu) (to the nearest 0.01 mm). Tusk circumference and curvilinear tusk length were measured using a flexible measuring tape (to the nearest 1.0 mm). All measurements were taken by one of the authors (BAM).

Morphological Analysis – Data Standardization and Organization

For several of the mandibular and tusk measures, both left and right sides were examined (measures #9–17). Prior to analysis, we examined whether there was any lateral asymmetry by regressing measures from one side on the other and examining the slope of the regression line as well as whether its standard error encompassed 1.0. Lateral asymmetry was identified in most of the measurements, therefore analyses were carried out using the side with the most data available. To quantify the amount of average individual percent directional asymmetry (DA) present we used the formula *%DA = (Right - Left)/(Average of Right and Left)×100* (e.g. [33–35]). This measure allowed us to directly compare the amount of DA present irrespective of dimensions of very different sizes. We also calculated percent absolute asymmetry (%AA) (e.g. [33]); %AA = (maximum value – minimum value)/(average of the two values)×100. This measure allowed us to examine the amount of asymmetry present regardless of directionality, or the amount of "random" asymmetry. To examine whether the %DA and %AA were significantly different between regions, we used student's t-tests with a Bonferroni correction for multiple tests [36].

The goal of this research was to determine if there are differences between animals of ATL and MAR populations; however, such a comparison may be difficult when the sample set

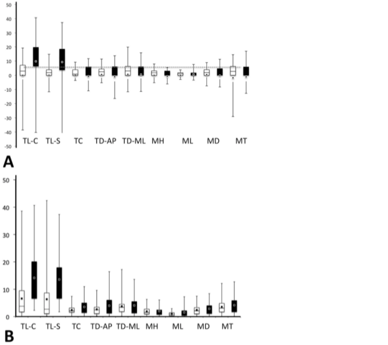


Figure 6. Boxplots of (a) %DA and (b) %AA. Whiskers denote minimum and maximum of the data. Boxes denote the 2nd and 3rd quartiles of the data and the squares denote the data mean. For %DA, negative values indicate a left-side asymmetry while positive values indicate a right-side asymmetry.
doi:10.1371/journal.pone.0099569.g006

every 500 generations, and 1,250,000 (25%) initial steps discarded as burn-in. This tree was visualized using FigTree ver. 1.3.1 [57].

Results

Morphological Analysis

Regression analysis suggested that lateral asymmetry was evident in 6 of the 9 tusk and mandible measures for which left and right sides were examined. Both ATL and MAR showed lateral asymmetry for all tusk measures. While MAR did not show signs of asymmetry in mandibular measures, ATL had slight asymmetry for mandible thickness. Because of this asymmetry, right sides were not exchanged for missing left sides when standardizing the datasets. As a result, the sides with a higher count (left sides for all measures) were used. To further examine asymmetry in the specimens, we also examined %DA and %AA separately for each of the regions (Table 3; Fig. 6) and whether average %DA and %AA within each region was significantly different. After incorporating a Bonferroni correction, only two measures were significantly different between the regions: straight and curvilinear tusk length (Measures #9 and #10). We found that MAR average individual %DA was ~10%, with right tusks longer than left. For the remaining measures, %DA ranged from 0.017%–1.79% while %AA ranged from ~0.978%–4.181% with the MAR exhibiting greater %AA for all measures.

Parametric and non-parametric tests of means of each of the morphological variables showed that 13 of the 16 comparisons were significantly different ($p<0.05$) between the ATL and MAR groups (Fig. 7). Skull measurements corresponding to overall skull length (CBL, ONL) and width (RW, IW) were significantly different across regions, with MAR longer and wider than ATL, while other measures (NL, N-Oc) were not. Mandible length,

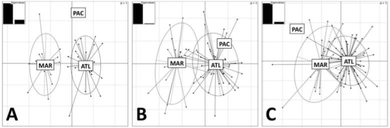


Figure 8. Discriminant function analysis scatterplots for skull (a), tusk (b), and mandible (c) data. Embedded is also a plot of the first two eigenvalues of the discriminant analysis. Circles encompass the range of 2/3 of the data points.
doi:10.1371/journal.pone.0099569.g008

KJ522887 - KJ522911, KJ522920 - KJ522922) and 8 within the historic MAR specimens (Genbank accession KJ522912 – KJ522919). Haplotypes from this study were then compared to those available from Lindqvist et al. [2]; however, because our sequences were shorter than those of Lindqvist et al. [2], three of their haplotypes were collapsed into one (Sequence IDs ATL13/ATL14, a unique haplotype, became identical to ATL11 and ATL21, two other haplotypes). Across the two studies, 3 of our ATL haplotypes were shared with the Lindqvist et al. [2] Atlantic specimens. These were the same as the following sequence IDs from Thule, NW Greenland: ATL07/ATL17, ATL09/ATL10/ATL16, and ATL6/ATL08. However, no haplotypes were shared between historic MAR specimens and any other Atlantic specimens.

Haplotype diversity (h), nucleotide diversity (π), and the average number of nucleotide differences both within and between regions were calculated for samples from PAC, Laptev Sea (LAP), ATL, and MAR (Table 4). Both h and π were found to be the lowest in the MAR, while they were the highest in the PAC. In addition the MAR had the lowest number of nucleotide differences between sequences.

Haplotypes from PAC, LAP, and ATL (both east and west of Greenland) appear to cluster together within the network, with the exception of two PAC haplotypes that fall out in the network closer to ATL haplotypes from east of Greenland (Fig. 9). Although the samples from the MAR did not share any haplotypes with the PAC/LAP group or the ATL group, the MAR sequences are more similar to the ATL than the PAC/LAP specimens. Many of the ATL haplotypes are highly similar, with most differing from each other by only a single basepair. In addition, the MAR haplotypes appear to branch off in two separate lineages from a single ATL haplotype. Haplotypes that are most similar (and closest) to the MAR haplotypes within the network originate from animals sampled around Southeast Baffin Island and Nottingham Island.

Similar to the median joining network, the phylogenetic tree shows the ATL and PAC/LAP subspecies grouping within separate and distinct clades, with the MAR specimens grouping within the ATL clade (Fig. 10).

Discussion

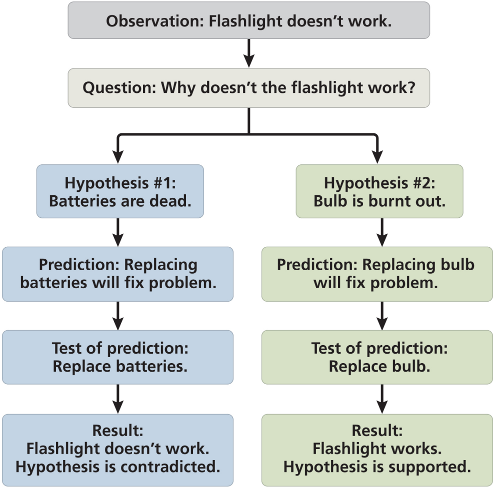
The morphological analyses presented here indicate that the Maritimes walrus was a physically distinctive group from other populations of walrus in the North Atlantic, west of Greenland. Comparisons of morphological measures across ATL and MAR indicated that most of the cranial and mandibular measures were significantly different between the two groups, with MAR values being greater in all cases. The discriminant analysis of principal components (DAPC) also identified the ATL and MAR as distinctive groups, with complete isolation of skull features. The MAR group appears to have been comprised of larger animals, with larger and more robust tusks, skulls and mandibles. This agrees with previous suggestions that the group was morphologically distinct, and more robust in overall size (pers. comm. to

Table 4. Haplotype (h) and nucleotide diversity (π) and corresponding standard error (in parentheses) for PAC, LAP, ATL and MAR regions.

Region	PAC	ATL	MAR	LAP
	$h=0.967$ (0.036)	$h=0.844$ (0.024)	$h=0.439$ (0.114)	$h=0.800$ (0.164)
	$\pi=0.02574$ (0.00340)	$\pi=0.00666$ (0.00031)	$\pi=0.00332$ (0.00121)	$\pi=0.00468$ (0.00096)
PAC	8.675			
ATL	13.980	2.230		
MAR	14.469	4.644	1.119	
LAP	7.463	12.957	13.850	1.600

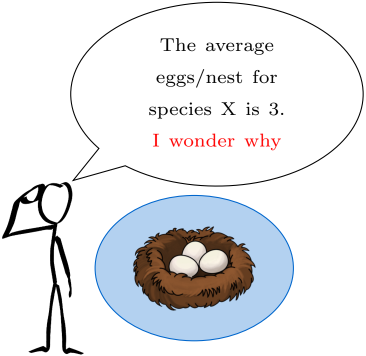
Below this is the average number of nucleotide differences within (on diagonal) and between regions.
doi:10.1371/journal.pone.0099569.t004

How Science Works



How Science Works

Observations/
facts



How Science Works

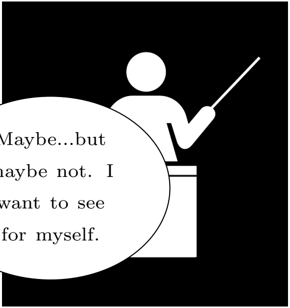
3 is the optimal number of population growth

That's the way I wanted it

How Science Works

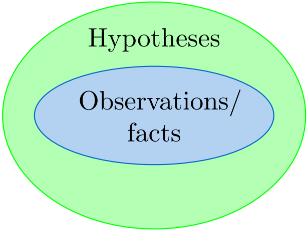
That's the way I wanted it

3 is the optimal number of population growth



Maybe...but maybe not. I want to see for myself.

How Science Works



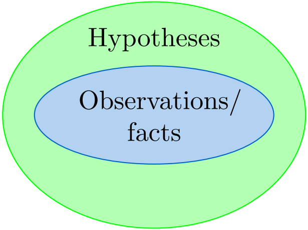
Hypotheses

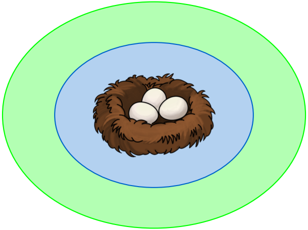
- *Explanatory* statements about *why* things are the way they are
- Should lead to clear *predictions*
- Lead to understanding of multiple observations
- *Must be falsifiable* (testable)
- Should have more than 1 (process of elimination)
- Are an infinite # of hypotheses for each observation, but not all equally likely

How Science Works

Hypothesis #1: Egg production in species X is limited by available calcium

Predictions:
1. Adding Ca to diet will increase egg production
2. Reducing Ca in diet will decrease egg production

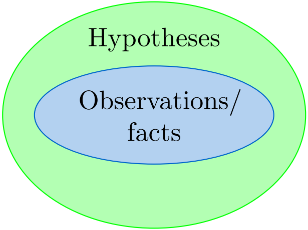


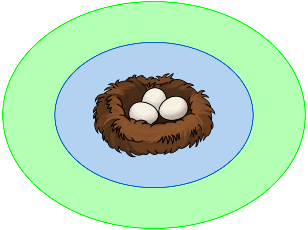


How Science Works

Hypothesis #2: Egg production in species X is limited by energetic resources

Predictions:
1. Adding resources to diet will increase egg production
2. Reducing resources in diet will decrease egg production

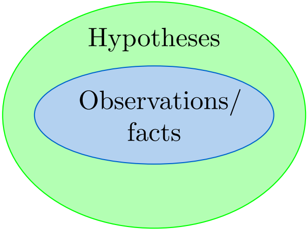


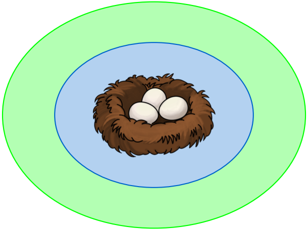


How Science Works

Hypothesis #3: Individuals lay different #s of eggs, but then aliens add/remove them to make it 3

Predictions:
1. If we actually watch them lay the eggs, should see different #s
2. If watched 24 hours a day, should see aliens adjusting egg counts

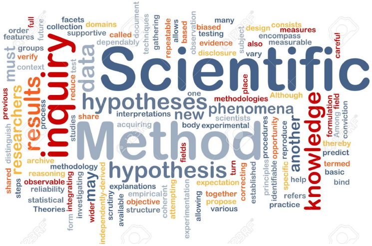




How Science Works

Investigate problem

- Literature
- Experimentation
- Observation



How Science Works

Work through all reasonable hypotheses (that you can)

- Ideally, only one is “left standing”, but not all are mutually exclusive

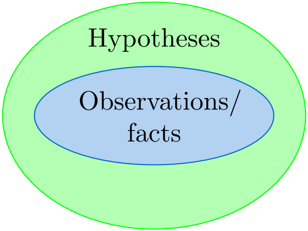
Karl Popper
(1902–1994)

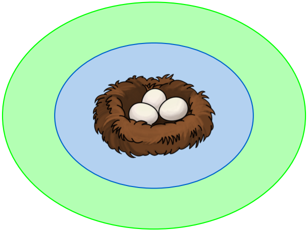
How Science Works

Hypothesis #2:
Resources

Hypothesis #1:
Calcium

Hypothesis #3:
Aliens



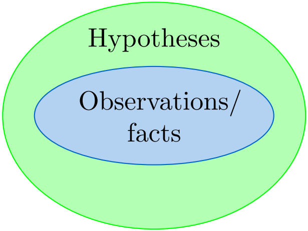


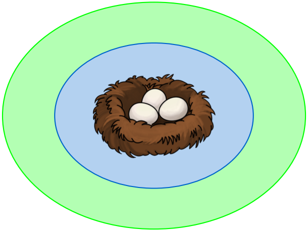
How Science Works

Hypothesis #2:
Resources

~~Hypothesis #1:
Calcium~~

~~Hypothesis #3:
Aliens~~





How Science Works

Submit your work for peer review

- Reasonable hypotheses
- Appropriate methods
- Appropriate interpretation
- …

How Science Works

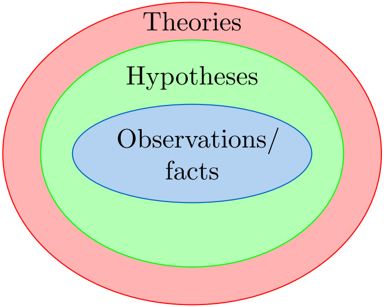
Submit your work for peer review

- Reasonable hypotheses
- Appropriate methods
- Appropriate interpretation
- ...

Adds to our collective understanding of the world

Now other people can focus on other hypotheses

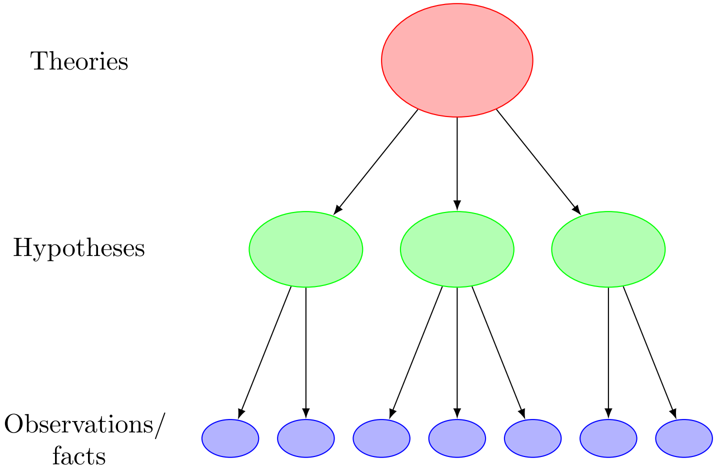
How Science Works



Theories

- General/major *explanatory statements* that explain many *hypotheses*
- Lead to understanding of multiple hypotheses

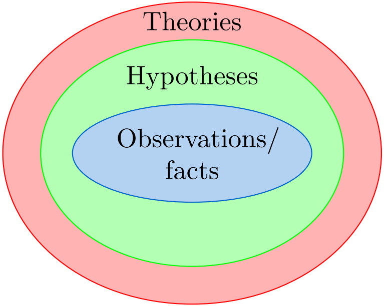
How Science Works

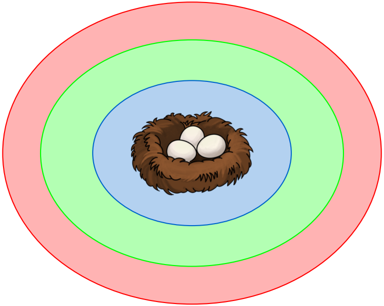


How Science Works

Theory: Natural selection - individuals have evolved to maximize their reproductive success given their environment (egg # maximizes reproductive "output" given the resources available)

Hypothesis #2: Resources





Theory vs Fact in Science

THEORY

General Public:
"An idea. Something not yet proven".
e.g., "Evolution is *only* a theory"

Theory vs Fact in Science

THEORY

General Public:
"An idea. Something not yet proven".
e.g., "Evolution is *only* a theory"

In Science:
"A statement of what are held to be the general laws, principles, or causes of something known or observed".

Theory vs Fact in Science

Fact: Gravity exists - things fall

Theory of Gravity: Our explanation/understanding of why and how things fall

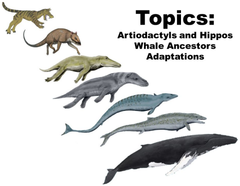
---

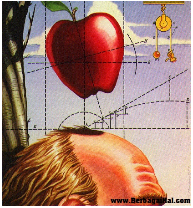
Fact: Evolution occurs - things evolve

Theory of Evolution: Our explanation/understanding of why and how things evolve (*e.g.*, natural selection)

Theory vs Fact in Science

Some other theories in science:



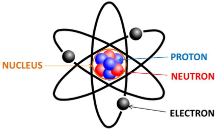


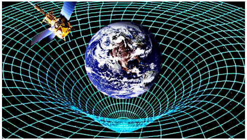
Theory of gravity

Theory of evolution

Theory of relativity

Atomic theory





Theory vs Fact in Science

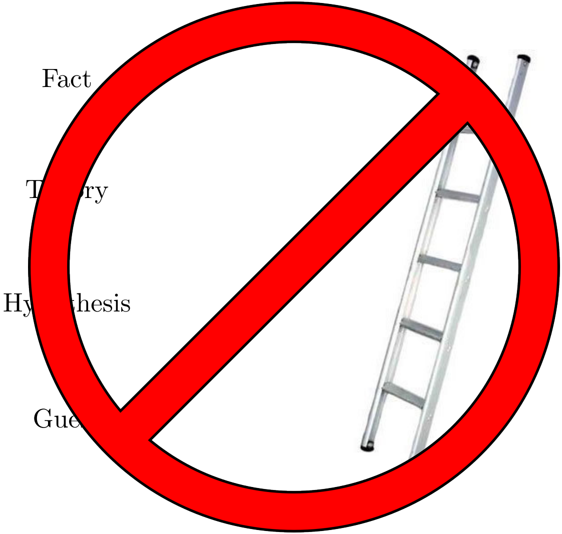
Fact

Theory

Hypothesis

Guess

Theory vs Fact in Science



Theory vs Fact in Science

Facts and theories are different things, NOT different rungs on a hierarchy

Facts are the world's data, theories are structures of ideas that explain and interpret facts

Theory vs Fact in Science

"Evolution is *only* a theory" is not a valid criticism

- Indicates a misunderstanding of terminology
- Suggests theories can become something else if more evidence/support is obtained (which they can't)

Nature of Science

Scientifically, can we be 100% sure of a hypothesis/theory?

Nature of Science

Scientifically, can we be 100% sure of a hypothesis/theory?

Absolutely not! Why not?

Nature of Science

Scientifically, can we be 100% sure of a hypothesis/theory?

Absolutely not! Why not?

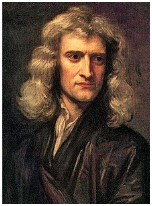
World is very complicated

- Old hypotheses/theories constantly revised
- New hypotheses/theories constantly developed

Current understanding influenced by

- Previous knowledge
- Culture
- Technology
- ...

Example: Gravity



Newton first described gravity scientifically

- Explained the majority of observations that most people had

As telescopes developed, some people noticed some orbits didn't quite agree with Newtonian expectations.

Einstein developed a new theory that explained things better

- Einstein's theory will undoubtedly be revised

Nature of Science

Can't ever "prove" a hypothesis

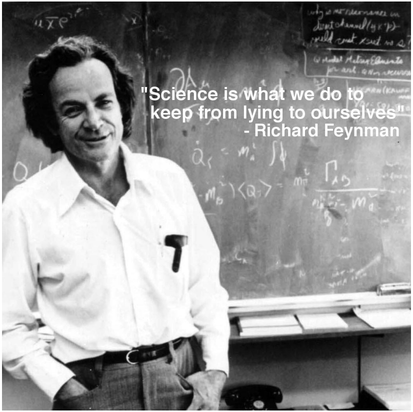
Can only reject all other potential explanations (that we can think of)

No amount of experimentation can ever prove me right; a single experiment can prove me wrong.

Nature of Science

If done correctly, science is self-correcting

Only way we have of improving our understanding of the world



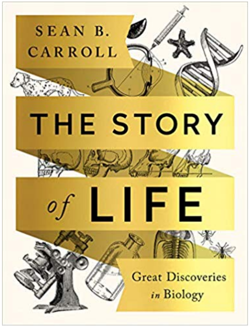
Nature of Science

If done correctly, science is self-correcting

Only way we have of improving our understanding of the world

Questions?

Case Studies

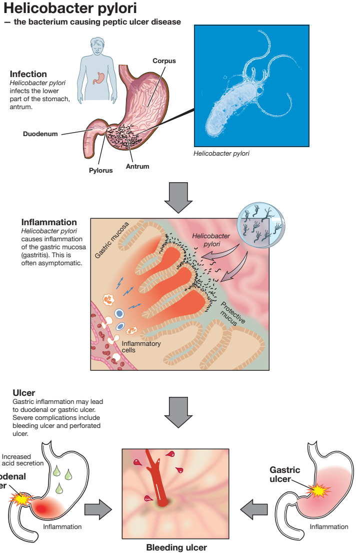


Case Study #1

Cause of gastritis and ulcers

Robin Warren (left) &
Barry Marshall (right)

Nobel Prize in 2005



Case Study #2

Vaccines and autism

EARLY REPORT

Early report

Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children

A J Wakefield, S H Murch, A Anthony, J Linnell, D M Casson, M Malik, M Berelowitz, A P Dhillon, M A Thomson, P Harvey, A Valentine, S E Davies, J A Walker-Smith

Wakefield et al. (1998) *The Lancet* **351**: 637–641.

Case Study #2

Vaccines and autism

We did not prove an association between measles, mumps, and rubella vaccine and the syndrome described. Virological studies are underway that may help to resolve this issue.

If there is a causal link between measles, mumps, and rubella vaccine and this syndrome, a rising incidence might be anticipated after the introduction of this vaccine in the UK in 1988. Published evidence is inadequate to show whether there is a change in incidence[22] or a link with measles, mumps, and rubella vaccine.[23] A genetic

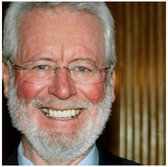
Case Study #2

Vaccines and autism

ARTICLES

Autism and measles, mumps, and rubella vaccine: no epidemiological evidence for a causal association

Brent Taylor, Elizabeth Miller, C Paddy Farrington, Maria-Christina Petropoulos, Isabelle Favot-Mayaud, Jun Li, Pauline A Waight



Taylor et al. (1999) *The Lancet* **353**: 2026–2029.

Case Study #2

Vaccines and autism

Early report

Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children

A J Wakefield, S H Murch, A Anthony, J Linnell, D M Casson, M Malik, M Berelowitz, A P Dhillon, M A Thomson, P Harvey, A Valentine, S E Davies, J A Walker-Smith

RETRACTED

Summary

Background We investigated a consecutive series of children with chronic enterocolitis and regressive developmental disorder.

Methods 12 children (mean age 6 years [range 3–10], 11 boys) were referred to a paediatric gastroenterology unit with a history of normal development followed by loss of acquired skills, including language, together with diarrhoea and abdominal pain. Children underwent gastroenterological, neurological, and developmental assessment and review of developmental records. Ileocolonoscopy and biopsy sampling, magnetic-resonance imaging (MRI), electroencephalography (EEG), and lumbar puncture were done under sedation. Barium follow-through radiography was done where possible. Biochemical, haematological, and immunological profiles were examined.

Findings Onset of behavioural symptoms was associated, by the parents, with measles, mumps, and rubella vaccination in eight of the 12 children, with measles infection in one child, and otitis media in another. All 12 children had intestinal abnormalities, ranging from lymphoid nodular hyperplasia to aphthoid ulceration. Histology showed patchy chronic inflammation in colon in 11 children and reactive ileal lymphoid hyperplasia in seven, but no granulomas. Behavioural disorders included autism (nine), disintegrative psychosis (one), and possible postviral or vaccinal encephalitis (two). There were no focal neurological abnormalities and MRI and EEG tests were normal. Abnormal laboratory results were significantly raised urinary methylmalonic acid compared with age-matched controls (p=0·003), low haemoglobin in four children, and a low serum IgA in four children.

Interpretation We identified associated gastrointestinal disease and developmental regression in a group of previously normal children, which was generally associated in time with possible environmental triggers.

Lancet 1998; **351:** 637–41

See Commentary page

Inflammatory Bowel Disease Study Group, University Departments of Medicine and Histopathology (A J Wakefield FRCS, A Anthony MB, J Linnell PhD, A P Dhillon MRCPath, S E Davies MRCPath) **and the University Departments of Paediatric Gastroenterology** (S H Murch MB, D M Casson MRCP, M Malik MRCP, M A Thomson FRCP, J A Walker-Smith FRCP,), **Child and Adolescent Psychiatry** (M Berelowitz FRCPsych), **Neurology** (P Harvey FRCP), **and Radiology** (A Valentine FRCR), **Royal Free Hospital and School of Medicine, London NW3 2QG, UK**

Correspondence to: Dr A J Wakefield

Introduction

We saw several children who, after a period of apparent normality, lost acquired skills, including communication. They all had gastrointestinal symptoms, including abdominal pain, diarrhoea, and bloating and, in some cases, food intolerance. We describe the clinical findings, and gastrointestinal features of these children.

Patients and methods

12 children, consecutively referred to the department of paediatric gastroenterology with a history of a pervasive developmental disorder with loss of acquired skills and intestinal symptoms (diarrhoea, abdominal pain, bloating and food intolerance), were investigated. All children were admitted to the ward for 1 week, accompanied by their parents.

Clinical investigations

We took histories, including details of immunisations and exposure to infectious diseases, and assessed the children. In 11 cases the history was obtained by the senior clinician (JW-S). Neurological and psychiatric assessments were done by consultant staff (PH, MB) with HMS-4 criteria.[1] Developmental histories included a review of prospective developmental records from parents, health visitors, and general practitioners. Four children did not undergo psychiatric assessment in hospital; all had been assessed professionally elsewhere, so these assessments were used as the basis for their behavioural diagnosis.

After bowel preparation, ileocolonoscopy was performed by SHM or MAT under sedation with midazolam and pethidine. Paired frozen and formalin-fixed mucosal biopsy samples were taken from the terminal ileum; ascending, transverse, descending, and sigmoid colons, and from the rectum. The procedure was recorded by video or still images, and were compared with images of the previous seven consecutive paediatric colonoscopies (four normal colonoscopies and three on children with ulcerative colitis), in which the physician reported normal appearances in the terminal ileum. Barium follow-through radiography was possible in some cases.

Also under sedation, cerebral magnetic-resonance imaging (MRI), electroencephalography (EEG) including visual, brain stem auditory, and sensory evoked potentials (where compliance made these possible), and lumbar puncture were done.

Laboratory investigations

Thyroid function, serum long-chain fatty acids, and cerebrospinal-fluid lactate were measured to exclude known causes of childhood neurodegenerative disease. Urinary methylmalonic acid was measured in random urine samples from eight of the 12 children and 14 age-matched and sex-matched normal controls, by a modification of a technique described previously.[2] Chromatograms were scanned digitally on computer, to analyse the methylmalonic-acid zones from cases and controls. Urinary methylmalonic-acid concentrations in patients and controls were compared by a two-sample t test. Urinary creatinine was estimated by routine spectrophotometric assay.

Children were screened for antiendomyseal antibodies and boys were screened for fragile-X if this had not been done