Lecture 14

Phylogenetic trees and molecular epidemiology
Wave-Like Spread of Ebola Zaire
Peter D Walsh, Roman Biek, and Leslie A Real

Abstract:
In the past decade the Zaire strain of Ebola virus (ZEBOV) has emerged repeatedly into human populations in central Africa and caused massive die-offs of gorillas and chimpanzees. We tested the view that emergence events are independent and caused by ZEBOV variants that have been long resident at each locality. Phylogenetic analyses place the earliest known outbreak at Yambuku, Democratic Republic of Congo, very near to the root of the ZEBOV tree, suggesting that viruses causing all other known outbreaks evolved from a Yambuku-like virus after 1976. The tendency for earlier outbreaks to be directly ancestral to later outbreaks suggests that outbreaks are epidemiologically linked and may have occurred at the front of an advancing wave. While the ladder-like phylogenetic structure could also bear the signature of positive selection, our statistical power is too weak to reach a conclusion in this regard. Distances among outbreaks indicate a spread rate of about 50 km per year that remains consistent across spatial scales. Viral evolution is clocklike, and sequences show a high level of small-scale spatial structure. Genetic similarity decays with distance at roughly the same rate at all spatial scales. Our analyses suggest that ZEBOV has recently spread across the region rather than being long persistent at each outbreak locality. Controlling the impact of Ebola on wild apes and human populations may be more feasible than previously recognized.
Evolutionary models predict that host immunity will shape the evolution of parasite virulence. While some assumptions of these models have been tested, the actual evolutionary outcome of immune selection on virulence has not. Using the mouse malaria model, Plasmodium chabaudi, we experimentally tested whether immune pressure promotes the evolution of more virulent pathogens by evolving parasite lines in immunized and nonimmunized (?naïve?) mice using serial passage. We found that parasite lines evolved in immunized mice became more virulent to both naïve and immune mice than lines evolved in naïve mice. When these evolved lines were transmitted through mosquitoes, there was a general reduction in virulence across all lines. However, the immune-selected lines remained more virulent to naïve mice than the naïve-selected lines, though not to immunized mice. Thus, immune selection accelerated the rate of virulence evolution, rendering parasites more dangerous to naïve hosts. These results argue for further consideration of the evolutionary consequences for pathogen virulence of vaccination.

Abstract
The habitat of the mycelial saprobic form of Paracoccidioides brasiliensis, which produces the infectious propagula, has not been determined and has proven difficult for mycologists to describe. The fungus has been rarely isolated from the environment, the disease has a prolonged latency period and no outbreaks have been reported. These facts have precluded the adoption of preventive measures to avoid infection.

The confirmation of natural infections in nine-banded armadillos (Dasypus novemcinctus) with P. brasiliensis, in high frequency and wide geographic distribution, has opened new avenues for the study and understanding of its ecology. Armadillos belong to the order Xenarthra, which has existed in South America ever since the Paleocene Era (65 million years ago), when the South American subcontinent was still a detached land, before the consolidation of what is now known as the American continent. On the other hand, strong molecular evidence suggests that P. brasiliensis and other dimorphic pathogenic fungi such as Blastomyces dermatitidis, Coccidioides immitis and Histoplasma capsulatum belong to the family Onygenaceae sensu lato (order Onygenales, Ascomycota), which appeared around 150 million years ago.

P. brasiliensis ecology and relation to its human host are probably linked to the fungal evolutionary past, especially its long coexistence with and adaptation to animal hosts other than Homo sapiens, of earlier origin. Instead of being a blind alley, the meaning of parasitism for dimorphic pathogenic fungi should be considered as an open two-way avenue, in which the fungus may return to the environment, therefore contributing to preserve its teleomorphic (sexual) and anamorphic (asexual) forms in a defined and protected natural habitat.
Origins of HIV/AIDS

- Back to HIV and chimpanzees
- SIV was discovered in 1985 in a captive Asian monkey
- SIVs are found naturally only in **African** primates
Origins of HIV/AIDS

HIV-1 and SIVcpz

HIV-2 and SIVsmm

P.t.s.  SIVrcm  SIVmnd2  SIVdrl  E  B  A  H

HIV-1 and SIVcpz

P.t.t.  S IVrcm  SIVmnd2  SIVdrl  E  B  A  H

HIV-2 and SIVsmm

SIVmnd1  SIVlhoest  SIVsunc  SIVoInc  SIVwrc  SIVcol  SIVsyk  SIVmon  SIVasc  SIVgsn  SIVmus

0.05 substitutions/site
Origins of HIV/AIDS

- HIV-2 introduced at least 8 times from mangabeys
- HIV-1 introduced at least 3 times from chimps
- Two of these “groups” endemic to Cameroon
- Group M is pandemic
• HIV-1 group M causes >99.9% of HIV infections worldwide

• Slightly harder to pin down its geographical origins because of spread

• Various clues place it at the same seen (probably Cameroon)

• It’s a close relative to other AIDS viruses clearly linked to Cameroon

• Chimpanzees themselves acquired their virus from preying on other primates

• How did the virus get into humans?
Origins of HIV/AIDS

- Divine retribution
- Doesn’t matter— it doesn’t cause AIDS
- Conspiracy theories— e.g. the CIA did it
- Ritualistic use of monkey blood
- Zoonosis (a disease communicable from animals to man under natural conditions)
- Contamination of vaccines

- THE PLAUSIBLE HYPOTHESES ALL HAVE IN COMMON THE INCrimination of simian immunodeficiency viruses (SIVcpz) FROM CHIMPANZEEs
- THE KEY DISCOVERY WAS THE FINDING THAT AFRICAN PRIMATES ARE INFECTED WITH SIMILAR VIRUSES…
After graduating in 1973 Edward Hooper divided his time between Europe and Africa, working as, among other things, a United Nations official, a school-teacher, a storekeeper in a diamond mine, and the BBC correspondent in Sudan and Uganda.
Origins of HIV/AIDS


- There’s an apparent correlation between oral polio vaccine (OPV) sites (1957-1960) and earliest instances of HIV-1 in Democratic Republic of Congo (DRC, ex-Zaire).

- 350/400 chimps sacrificed in experiments at Lindi camp near Kisangani, DRC, and allegedly OPV cultured in their kidneys (Hooper 1999).

- This culturing process is suggested to have facilitated the transfer to humans of chimpanzee simian immunodeficiency virus (SIVcpz).

- There’s a precedent: early polio vaccines are known to have been contaminated with the simian virus SV40.
Origins of HIV/AIDS

Aids: the big mistake?

‘Hooper believes it likely Aids entered the population through trials of an experimental oral polio vaccine’

Salk, Sabin and Koprowski were intelligent, thoughtful people who eradicated a horrible disease. This is a charade, an assassination.

The idea that such a devastating, global disease might have been caused by scientists is anathema to doctors and scientists like Hooper says that, unless we face up to the possibility that something like this can happen, we face doing nothing to deal with it to the human race.

‘There are implications for medical and veterinary research taking place right now,’ he says. ‘We are on the verge of putting animal cells, even animal organs, inside human patients and immunosuppressing them so they won’t reject the material. We do not know all the retro-viruses present in pigs and baboons, and we do not know what we are about to open another Pandora’s box.’

‘If so, the effect won’t be felt by us or our children and their children, but by the animals and their children.’

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In the main, he says, the scientific reaction to his theory has been fair. ‘For the most part, they’ve said there isn’t enough evidence yet to prove the theory, but there is a case to answer.’

And before long, we may know. Wistar Institute, under whose auspices Koprowski worked, has recently — in eight years of resistance — released a dozen archive samples of Chimpanzee and polio virus for independent testing. Researchers will test for SIV and to determine which was of primatid origin.
Origins of HIV/AIDS

Chimp caught in a liana net by pygmies, at one of Rollais's base camps in the north of Province Orientale, 1958. (Credit: C. Rollais)

Agnes Flock vaccinating a “sea of Africans” with CHAT in the Ruwizi Valley, 1958. (Credit: R. Phillips)
Origins of HIV/AIDS

Non-invasive sampling of SIVcpz from the supposed “source” (and a big blank space on the map of SIVcpz distribution)
Professors W.D. Hamilton

W.D. HAMILTON is a good candidate for the title of most distinguished Darwinian since Darwin.

Other candidates would have to include R.A. Fisher whom Hamilton revered as a young student at Cambridge. Hamilton reeeved Fisher in his penetrating biological intuition and his ability to reason it in mathematics. But like Darwin and unlike Fisher, he was also a superb field naturalist and explorer. A subject of all his 20th-century successors, Darwin would have just enjoyed talking to Hamilton. Partly because they could have swapped jungle tales and beetle sex, partly because both were gentle and deep, but mostly because Hamilton’s theories were responsible for clearing up so many of the very problems that had intrigued and tantalized Darwin.

William Donald Hamilton was a fellow Society Research Professor in the Department of Zoology at Oxford, and a Professional Fellow of New College. He was born in Cairo in 1923, spent a happy childhood botanising and collecting butterflies in Kent, was educated at Tonbridge and St. John’s College, Cambridge, where he read genetics.

For his PhD he moved to London, where he was jointly enrolled at University College and LSE. He became a strong supporter of the A virus was originally introduced into the human population in an oral polio vaccine tested in Africa in the 1950s. Hamilton went on to show that these viruses are not only common but that they can be inherited. "It is not a bad idea to think of this in evolutionary terms," he said. "It is not a bad idea to think of this in evolutionary terms."

Male advertisement is an honest boast of health: Hamilton, centre, on his last expedition, to the Congo in January
Origins of HIV/AIDS

Western blot analysis of Kisangani chimpanzee urine samples

6   15   19   21  25   28   36  40  48   51

gp160
gp120
p66
p51
gp41
p31
p24
p17
Origin of AIDS: Contaminated polio vaccine theory refuted

Michael Worobey¹, Mario L. Santiago², Brandon F. Keele², Jean-Bosco N. Ndjango⁴, Jeffrey B. Joy⁶, Bernard L. Labama⁵, Benoît D. Dhed'a⁴, Andrew Rambaut⁷, Paul M. Sharp⁸, George M. Shaw²,³ and Beatrice H. Hahn²

Despite strong evidence to the contrary¹,²,³,⁴,⁵, speculation continues that the AIDS virus, human immunodeficiency virus type 1 (HIV-1), may have crossed into humans as a result of contamination of the oral polio vaccine (OPV)⁶,⁷,⁸. This 'OPV/AIDS theory' claims that chimpanzees from the vicinity of Stanleyville — now Kisangani in the Democratic Republic of Congo — were the source of a simian immunodeficiency virus (SIVcpz) that was transmitted to humans when chimpanzee tissues were allegedly used in the preparation of OPV⁶,⁷. Here we show that SIVcpz is indeed endemic in wild chimpanzees of this region but that the circulating virus is phylogenetically distinct from all strains of HIV-1, providing direct evidence that these chimpanzees were not the source of the human AIDS pandemic.
Phylogenetic position
Expected for source population

Origins of HIV/AIDS
Worobey et al. (2004): Nature
Ancestor estimated
At 1930ish

Vaccines used after 1957

Origins of HIV/AIDS

Timing the Ancestor of the HIV-1 Pandemic Strains

B. Korber, M. Muldoon, J. Theiler, F. Gao, R. Gupta, A. Lapedes, B. H. Hahn, S. Wolinsky, T. Bhattacharya

Ancestor estimated
At 1930ish

Vaccines used after 1957
Summary

- The SIVcpz from the alleged HIV-1 group M source region (according to OPV/AIDS theory) is not the sister lineage to group M
- Confirms other lines of evidence (dating, ZR59, archival vaccine testing, group N recombination, genetic diversity in Kinshasa)
- A new region where we can expect possible emergence of HIV-1
- Sampling is continuing to study natural history of SIVcpz in wild chimps
Revisiting an old hypothesis...Haiti and AIDS

Haiti's Aids and voodoo challenge
By Nick Caistor
In Port-au-Prince, Haiti

Fighting Aids in Haiti has meant confronting traditional beliefs in magic and tackling a culture in which many children start having sex around the age of 12.

The Caribbean nation is one of the countries hit hardest by Aids outside of sub-Saharan Africa.

In the early 1980s Haitians were held responsible for the spread of the disease in the United States and other developed countries.

But Dr Marie Deschamps, co-director of the Gheskio infectious diseases clinic in the Haitian capital Port-au-Prince says the truth was the opposite - it was foreigners who brought the disease into Haiti.

"From what we observed, in the beginning it was mostly bisexuals, foreign men who came from the United States, from Canada and from France. They would come to Haiti to meet with the bisexuals here just for money," she said.

A voodoo stall - many Haitians believe illness is caused by magic.
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- Korber et al (2000) Science…Haitian subtype B sequences branch off earlier; could be older epidemic or possibly a sampling artifact

- Robbins et al (2003) JV…close similarity in TMRCA of US and Haitian B subtype consistent with the commonly help assumptions of an epidemiological link b/w Haitian and US homosexuals which led to HIV’s spread b/w the countries
Questions:

• Did HIV-1 move from Haiti to US, or US to Haiti?

• When did these events take place?
Approach:

• Full-length *env* alignment of published B and D subtype sequences (117 B plus 5 D)

• Bayesian MCMC phylogenetic approach

• Archival Haitian-linked samples, Pitchenik *et al*, *AIM*, 1983
Molecular epidemiology from a criminal case showing the phylogenetic fingerprint of a founder effect.

In this case, viral variants (R) from a woman infected intentionally with HIV-positive blood form a confined subclade embedded within the greater viral diversity found in the blood “donor” (D) Adapted from Metzker et al. 2002, PNAS.
**Background.** The circumstances surrounding the emergence of HIV-1 group M, subtype B (the predominant strain of AIDS virus in Europe, Haiti, the US, and the rest of the Americas) remain unclear. Here we report a set of fossil HIV-1 sequences that provide definitive evidence of where, when, and how subtype B emerged.

**Methods.** We recovered complete HIV-1 *env* gene sequences from specimens collected in 1982-83 from five Haitian AIDS patients, all recent immigrants to the US who were among the first recognized AIDS victims. We tested the hypothesis of a Haitian origin for subtype B by conducting a detailed Bayesian Markov chain Monte Carlo phylogenetic analysis, including a relaxed molecular clock analysis, using an alignment of the fossil sequences plus 117 previously published subtype B *env* sequences from 19 different countries.

**Results.** The hypothesis of a US or other non-Haitian origin of subtype B is strongly rejected (*P* < 0.001) in favor of a Haitian origin (*P* = 0.999). HIV-1 moved from Africa to Haiti in a single patient in or around 1966 [1962-1970]. It then spread there for some years before first successfully dispersing elsewhere. Almost all non-Haitian subtype B infections around the world can be traced to a single migration of the virus out of Haiti in or around 1969 [1966-1972], a key turning point in the history of the AIDS pandemic. One exception is the subtype B epidemic in Trinidad and Tobago, which emanated from a separate, single-patient introduction from Haiti.

**Conclusions.** Our findings establish Haiti as the country with the oldest HIV/AIDS epidemic outside sub-Saharan Africa. Because of its 40-year history, the HIV-1 epidemic in Haiti exhibits a greater range of viral genetic diversity than the rest of the world’s subtype B strains combined, a fact relevant to vaccine design. The timing of the Haitian origin of the epidemic supports the idea that the genesis of subtype B occurred with the return of one of the many Haitian professionals who worked in the Congo in the 1960s. The timing of the subsequent single-patient initiation of the US and worldwide epidemic shows conclusively that HIV-1 was circulating in the US for over a decade before the recognition of AIDS in 1981. Our results suggest that the global spread of HIV-1 involves more inertia than previously supposed, with major outbreaks hinging on rare, single transmission events. They also provide compelling independent corroboration of an early-twentieth-century M group ancestor.
Schematic diagrams of phylogenetic patterns expected under various hypotheses for the origin and spread of subtype B. (a) If the virus reached Haiti first, then Haitian HIV-1 sequences are expected to branch off from the root part of the subtype B subtree before sequences from elsewhere. Alternatively, (b) the Haitian epidemic could have been imported from the US or elsewhere; (c) both the US and Haitian epidemics could have begun effectively simultaneously then remained distinct; or (d) high levels of migration could have obscured where the virus arrived first.
The 50% majority rule consensus tree from the Bayesian MCMC (MrBayes) analysis. Posterior probabilities for each node are shown. The branch lengths represent the mean value observed for that branch among the post-burnin sampled trees. This is the full version of the abridged tree depicted in Fig. 2 of the main paper, which collapsed the Trinidad and Tobago clade and dominant clade into blue and yellow triangles, respectively. The same color coding is used here. The fossil sequences are labeled in larger bold text. All the other strains are listed with by their LANL designations (N subtype.country.year.strain) (2). The country codes are as follows: AR = Argentina; AU = Australia; BR = Brazil; CA = Canada; CD = Democratic Republic of the Congo; CO = Colombia; DE = Germany; EC = Ecuador; ES = Estonia; FR = France; GA = Gabon; GB = United Kingdom; HT = Haiti; JP = Japan; KR = South Korea; NL = Netherlands; TH = Thailand; TT = Trinidad and Tobago; UG = Uganda; US = United States of America; ZA = South Africa. The Haitian fossil sequence H6 clusters with the dominant clade sequences according to this analysis, but its position is unresolved and is consistent with it being basal to dominant clade sequences as suggested by the relaxed molecular clock analysis.
The abridged consensus tree from the Bayesian MCMC (MrBayes) analysis. Posterior probabilities for each node are shown. With the exceptions noted in the main text, the Haitian sequences occupy all the deepest branches within subtype B. The 11 sequences of the Trinidad and Tobago clade and the 96 sequences of the dominant international clade are represented by the blue and yellow triangles, respectively. The Haitian or Haitian-linked sequences are shown in green, with the fossil sequences labeled in larger bold text. Haitian sequence H6 clusters with the dominant clade sequences according to this analysis.
The consensus tree of the relaxed molecular clock analysis, with the Haitian fossil sequences bulleted. For computational and comparative purposes (10), we restricted this analysis to North American and Caribbean sequences only. The tips of the tree correspond to year of sampling, and the branch lengths reflect the mean of the posterior probability density. The posterior probability density for the TMRCA for subtype B in Haiti is depicted in dark green, and the 95% highest probability density (HPD) is shown by the horizontal bar and light green shading. The TMRCA means and 95% HPDs for the other key nodes were as follows: subtype B/D ancestor = 1954 [1946-61]; subtype D ancestor = 1966 [1961-71]; Trinidad and Tobago subtype B ancestor = 1973 [1970-76]; US/Canada subtype B ancestor = 1969 [1966-72]. Sequence H6 falls outside of the dominant clade according to this analysis.