

*Topic outline:*

*I. Some thoughts about evolutionary biology*

- A. The fact of evolution by natural selection is the unifying principle in biology*
- B. Understanding evolutionary biology has major benefits*

*II. Using evolutionary biology: the case of HIV/AIDS*

- A. Background (virology, epidemiology)*
- B. Evolutionary question #1: Why is HIV/AIDS so hard to treat? AZT as a model*
- C. Evolutionary question #2: Why is HIV fatal?*
- D. Evolutionary question #3: Why are some people resistant?*
- E. Evolutionary question #4: Is a vaccine really possible (or even likely)?*

I. Some thoughts about evolutionary biology

- A. The fact of evolution (“descent with modification”) by natural selection (the process leading to adaptive change) is the unifying principle of the biological sciences

- 1. as Dobzhansky wrote, “Nothing in biology makes sense except in the light of evolution.”

- 2. In spite of the fact that evolution is to biology what atomic theory is to chemistry, evolution is poorly understood – why?

- a. not taught properly in K-12 for “political” reasons – conflict with a few specific religious ideologies (read web article about Kansas . . .)

- b. the word itself covers a whole variety of meanings, which can lead to confusion

- c. meanings include (we’ll discuss each of these in detail)

- i. descent with modification

- ii. change in gene frequencies in populations over time

- iii. speciation

- iv. natural selection

- B. Understanding evolutionary biology has major “payoffs” – it provides us with the means to address major issues, including

- 1. health issues, including epidemiology (more on this shortly); pharmacology

- 2. improving agriculture (pesticide resistance; improving domestic crops and

- livestock)
  - 3. management of economically important resources (forests, fisheries, etc.)
  - 4. conserving biodiversity
  - C. From the home page, read the summary of the “White Paper” on evolution to explore why this is such an important subject
- II. Using evolutionary biology: the case of HIV/AIDS
- This case study will demonstrate the kinds of questions evolutionary biologists ask and the usefulness of those questions even to apparently unrelated fields, as well as introducing important concepts that will be used later in the course.*
- A. Background: epidemiology, virology, etc.
    - 1. In December, 1999, UN AIDS program estimated that 40 million people worldwide were infected with HIV
      - a. epidemic already responsible for more deaths than bubonic plague; by end of 2001, will probably overtake “Spanish influenza” as most deadly epidemic in human history
      - b. Most HIV infections result of one of two different (but related) epidemics dating from 1980's and 1990's: (figs 1.1, 1.2)
        - i. among heterosexual men and women in sub-Saharan Africa and S/SE Asia
          - a) this is by far the larger of the two (nearly 30 million)
          - b) frequency of infection = 10% in general population
          - c) rate of infection is rising
          - d) In most sub-Saharan cities, > 75% of adult deaths due to AIDS
          - e) virus transmitted to new host via heterosexual intercourse
          - f) note that this is also an increasing problem in US
        - ii. among homosexual men and intravenous drug users in the US and Europe
          - a) much smaller

- b) frequency of infection much lower = .56% of general population in NA
- c) rate of infection declining

2. Structure/function of HIV

- a. like all viruses, HIV is an obligate intracellular parasite, incapable of living/reproducing on its own
- b. HIV is a retrovirus with typical retroviral structure (fig 1.3)
  - i. active particle = virion
  - ii. virion structure includes
    - a) gp120 surface protein: on membrane surrounding the capsid
    - b) capsid = "main" virus particle:
      - i) protein coat
      - ii) 2 copies of RNA genome (genetic information for synthesizing a variety of proteins
      - iii) special enzyme = reverse transcriptase (builds DNA from an RNA template)
- c. "life cycle":
  - i. gp120 binds to the CD4 receptor (and a co-receptor) on the cell membrane of human macrophages and T cells
  - ii. capsid enters the cell
  - iii. capsid is lost; reverse transcriptase uses ATP and other host cell machinery to synthesize viral DNA from viral RNA
  - iv. viral DNA enters the nucleus and is incorporated into host DNA
  - v. host cellular machinery transcribes, translates viral DNA into new viral proteins; it also makes new copies of the RNA genome
  - vi. new virions are assembled inside the host cell, then bud off the host cell membrane and enter the bloodstream
  - vii. process repeats
- d. note that, because virus uses host cell machinery, any drug therapies that

disrupt viral life cycle will almost certainly disrupt host cell machinery

3. HIV causes disease because of response of host immune system:

- a. destroys virions in the bloodstream
- b. destroys own infected cells – which are part of the immune system

B. Evolutionary question #1: Why is HIV so hard to treat? The case of AZT

1. In general, anti-viral drugs work by inhibiting virus-specific enzymes

2. AZT inhibits reverse transcription

- a. AZT is a thymine “mimic”
- b. when reverse transcriptase “mistakes” AZT for thymine, it adds AZT to the growing DNA strand
- c. AZT doesn’t accept additional nucleotides, so it halts reverse transcription
- d. note that AZT also “fools” DNA polymerase – so it has side effects due to disruption of normal host DNA synthesis as well

3. After a few years, AZT therapy began to lose its effectiveness – why? Classic case of natural selection

- a. HIV has the highest mutation rate of any virus or organisms studied to date:
  - i. during DNA synthesis, RT is highly error-prone (doesn’t make exact DNA “copies” of RNA genome – including the part coding for itself)
  - ii. also lacks “proofreading” function, so “errors” don’t get fixed
  - iii. means that, within an individual, HIV mutates as it replicates
- b. Researchers have demonstrated that, within an individual patient, the reverse transcriptase gene mutates: the gene sequence from HIV late in infection is different from that early in the infection
- c. Analysis of different reverse transcriptase genes from AZT-resistant patients showed that strains late in infection had different amino acid sequences and consequently different conformations at the active site of the enzyme (the site that binds RNA) (fig 1.4)
- d. Apparently, the mutations in the “resistant strains” allow reverse

transcriptase to distinguish AZT from thymine – why would this matter?

- i. within an individual, strains that mistake AZT from thymine don't reproduce
  - ii. strains that don't make mistake do reproduce – and pass on their “resistant” structure to their “offspring”
  - iii. so resistant strains increase in frequency
- e. note that process doesn't end after one “successful” mutant:
- i. mutation happens every generation – in the case of HIV, given the rapid reproduction of HIV and its high mutation rate, can get hundreds of mutants within a single individual
  - ii. some will probably be more prone to mistake AZT for thymine, but some will probably be even less so
  - iii. the mutants that make the fewest mistakes will always be the ones that have the highest reproductive rates – as long as the person is taking AZT
  - iv. so the presence of AZT will continually select for the most resistant strains available
- f. review: AZT resistance happens because
- i. transcription errors by RT lead to mutations in the RT gene
  - ii. mutations produce variability in RT structure and function
  - iii. because of their specific structures/functions, some variants (mutants) are better able than others to survive and reproduce in an environment that includes AZT
  - iv. those virions increase in frequency because they pass their structure/function to their offspring
- g. interesting note: in the absence of AZT, resistant strains are less favored – so withdrawing AZT eventually eliminates resistance
4. General conclusion: because of the high mutation rate of HIV, it's unlikely that we'll ever come up with a therapy to which resistance doesn't evolve, and fairly

rapidly

C. Evolutionary question #2: Why is HIV fatal?

1. The tendency to cause disease = **virulence**
2. In the case of HIV, virulence is a function of its rapid growth (reproductive) rate – so question really is “why does HIV reproduce so quickly that it kills its hosts?”
3. key to answering question = understanding why such a rapid growth rate is advantageous to HIV even though it kills the host – why not use the “strategy” of reproducing slowly and not killing host?
4. IMPORTANT: sometimes, answers to questions about why organisms use one strategy over another is that it's the only strategy available to them – for various biological reasons, they may be **constrained**. E.g.
  - a. perhaps infections of CD4 cells are always fatal because of the role of those cells in the immune system
    - i. turns out not to be the case – human herpes 6 also infects CD4 cells, but only produces mild rash.
  - b. perhaps HIV always reproduces rapidly because there is no genetic variation leading to different growth rates – unlikely explanation because
    - i. late-infection strains grow faster in culture than do early-infection strains from the same individual: so there is variation in growth rates
    - ii. specific genetic variations have been associated with differences in virulence – so it looks like variation in virulence has a genetic basis
5. The other possible answer to the question is that natural selection favors high virulence in HIV – Ewald's “transmission rate hypothesis” (fig 1.5)
  - a. key to understanding this hypothesis (and many others dealing with selection!) is to understand the idea of trade-offs in costs and benefits
    - i. natural selection will favor those combinations of traits that increase survival and reproduction
    - ii. so need to evaluate different “options” or “strategies” organisms might

- use in terms of costs and benefits to reproduction – selection will favor the strategy with the greatest ratio of benefit to cost
- b. the transmission rate hypothesis proposes two possible strategies: rapid reproduction (high degree of virulence and fatality) and slow reproduction (low degree of virulence, low fatality)
  - c. what are the costs and benefits of rapid growth for virion?
    - i. benefit = increased prevalence in bloodstream = increased chance of being transmitted to new host during intercourse
    - ii. cost = killing enough CD4 cells that host is too ill to engage in intercourse
  - d. balance between costs and benefits will vary depending on sexual behavior:
    - i. when people have few sexual partners, chances of transmission to new host are rare for any episode of intercourse **even if many virions are present in the bloodstream**
      - a) benefit of having many virions present is very low – transmission to new host is unlikely
      - b) cost of having many virions present is very high: host is likely to die before switching sexual partners and transmitting virus
    - ii. when people have many sexual partners, chances of transmission to a new host are pretty much a function of the number of virions in the bloodstream:
      - a) so benefit of having many virions is high: the more virions present, the greater the chances of transmission to new host during a given episode of intercourse
      - b) cost is low: because individuals have many partners, virus is likely to be transmitted before host dies, even if host dies relatively quickly
6. According to Ewald, the increase in virulence in HIV was due to changing sexual practices – both in heterosexuals in Africa and Asia and in homosexuals in the U.S. and Europe – in the 1980's and 1990's: an increase in promiscuity

- selectively favored the more virulent strains
7. Note that this hypothesis is now being tested by “natural experiments”:
    - a. the rate of partner change among homosexual men in the US and Europe is declining
    - b. HIV-2, a much more benign form of HIV, is spreading from its historical center of incidence in West Africa, where transmission due to partner exchange are low, to India, where partner exchange may be much more frequent
    - c. what does the hypothesis predict will happen?
- D. Evolutionary question #3: Why are some people resistant to HIV?
1. Two patterns of resistance have been confirmed:
    - a. some people repeatedly exposed to the virus are not infected
    - b. some people who are infected live much longer than expected
  2. Molecular basis for resistance has been hypothesized: resistant individuals have variants of the co-receptor protein that prohibit HIV entry into the cell
    - a. co-receptor genes from 3 long-term survivors sequenced; one was different from the others = Δ32 (for 32 base deletion).
    - b. demonstrated in lab that HIV can't enter cells with that allele
  3. Pattern of resistance interesting: allele is not randomly distributed among human populations
    - a. Δ32 present in ~ 9% of Caucasians, but absent from people of Japanese, African descent
    - b. non-random distribution suggests that selection in the past has favored the allele in some populations but not others: e.g., same allele confers resistance to bubonic plague (currently being tested) – this is one example of a very important pattern that we'll discuss more later!
  4. At least two other alleles are also correlated with resistance; together, these are important for trying to find effective treatments.

- E. Evolutionary question #4: Is a vaccine against HIV likely (or possible)?
1. Background: how vaccines work
    - a. T cells recognize foreign proteins = **epitopes** as non-self; presence of epitopes triggers immune response
    - b. vaccines = epitopes from killed or incomplete virions; these “pre-prime” the immune system so when “real” virus invades, the immune system is ready to respond
  2. The target epitope for HIV = gp120 (the surface protein that helps bind to host cells)
    - a. we know that gp120 genes mutate – many gp120 strains exist
    - b. to be effective, a vaccine would need to work against multiple strains
    - c. so question is – how variable is gp120, and how likely is it that new forms will continue to arise?
  3. To answer the question, really need to look at evolutionary history of the virus – because we need to understand how it changes over time, not just how many variants are present currently (fig. 1.6)
    - a. to reconstruct the **phylogeny** = evolutionary history of HIV, investigators take samples of HIV and related viruses (SIV's) from people and other primates from all over the world and reconstruct its evolutionary history using the basic assumption that strains that have the greatest similarity shared the most recent common ancestor
    - b. Fig 1.6 a and b show results of recent analysis:
      - i. 3 major viral lineages branch from a single common SIV ancestor
        - a) HIV-2 related to an SIV infecting mangabeys
        - b) HIV-1 related to an SIV (SIV<sub>cpz</sub>) infecting chimps – likely transmitted to humans via hunting, eating chimps
      - ii. closer look at HIV-1 relationships shows two important features:
        - a) at least three different subgroups fall out, each most closely related to

a different SIV – implies at least 3 different transmission events from chimps to humans

b) sequence divergence among HIV-1 strains is very high

c) together, these findings suggest that diversity among HIV-1 strains is not only high now, but may actually increase over time

4. So, answer to the question about vaccines is that, given both existing diversity among HIV strains and its evolutionary history, it's likely to be harder to develop an HIV vaccine than it is to develop one for colds.

#### F. Conclusions from evolutionary analysis of HIV/AIDS

1. Efforts to develop a vaccine may be futile – effort might be better directed elsewhere.

2. Resistant alleles should become more frequent, and may offer some help in designing medicines.

3. If the transmission rate hypothesis is correct, the best defense against HIV in the long run will be changing behavior: this will have two effects –

a. decreasing rates of infection

b. selecting for more benign strains of the virus

4. Multiple drug resistance is likely to be a continuing problem, so there will be a continuous need for new drug therapies.