The Evolution of Virulence in Bacteria and Viruses: an Opinionated Rant

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Microbial Evolution and Co-Adaptation
A Workshop in Honor of Joshua Lederberg
May 21, 2008
Evolutionary biologists have been interested in infectious disease for a very long time.

“A particle of small-pox matter, so minute as to be borne by the wind, must multiply itself many thousandfold in a person thus inoculated; and so with the contagious matter of scarlet fever. It has recently been ascertained that a minute portion of the mucous discharge from an animal affected with rinderpest, if placed in the blood of a healthy ox, increases so fast that in a short space of time "the whole mass of blood, weighing many pounds, is infected, and every small particle of that blood contains enough poison to give, within less than forty-eight hours, the disease to another animal."

C. Darwin

The Variation in Animals and Plants Under Domestication New York: D. Appleton & Co. 1883 (The citations are to articles written in the middle 1860s.)
“The Conventional Wisdom”

Virulence is a primitive character for a parasite

Theoretical base - A dog does not bite the hand that feeds it.

Corollaries and Extrapolations

(i) Evolution (coevolution) will necessarily lead to commensalism, mutualism or the extinction of the host and/or parasite.

(ii) Virulence is an indication of a recent association between a parasite and its host.

The conventional wisdom is an observation, not a mechanism. Why should a parasite be virulent in a novel host? Why should parasite evolution and/or parasite host co-evolution lead to commensalism?
Virulence as Immunological Failings and Screw-ups, “Immunoperversity”

“The pathogenicity and virulence of ________ is the product of a complex interaction between __________ and the host.”
Virulence as Immunological Failings and Screw-ups, “Immunoperversity”

“The pathogenicity and virulence of ________ is the product of a complex interaction between ___________ and the host.”


Also see:


And oodles of Stan Falkow’s papers.
# Immunoperversity and the Virulence of Bacteria

<table>
<thead>
<tr>
<th>Disease</th>
<th>Bacteria</th>
<th>Virulence Site (Red)</th>
<th>Immune Perversity</th>
<th>Damage</th>
</tr>
</thead>
</table>
| Pneumonia                | *S. Pneumoniae*  
*H. Influenzae*  
*N. Meningitides* etc | Lungs                | Induction of pro-inflammatory cytokines, edema, fibrin deposition | Recruitment of fluid and cells into air spaces |
| Duodeal Ulcers           | *Helicobacter pylori*         | Gastic and duodenal mucosa | Chronic inflammation                                      | Mucosal atrophy                                 |
| Septicemic plague        | *Yersinia pestis*             | Circulatory system – systemic | Endotoxins and other factors – systemic inflammatory response | Acute shock                                     |
| Normally commensal       |                               |                      |                                                            |                                                  |
# Immunoperversity and the Virulence of Bacteria

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<thead>
<tr>
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<th>Virulence Site (Red)</th>
<th>Immune Perversity</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic fever</td>
<td><em>Streptococcus pyogenes</em></td>
<td>Heart, joints, skin and brain</td>
<td>M-protein activation of anti-cardiac myocin antibodies</td>
<td>Damage to heart muscle, depositions joints &amp; skin</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Lungs</td>
<td>Persistent bacteria induce Release of high levels of TNFα</td>
<td>Recruitment of fluid into airspaces, and tissue necrosis</td>
</tr>
<tr>
<td>Meningitis</td>
<td><em>S. Pneumoniae</em>&lt;br&gt;<em>H. Influenzae</em>&lt;br&gt;<em>N. Meningitides</em> etc</td>
<td>Meninges</td>
<td>Induction of pro-inflammatory cytokines and chemokines</td>
<td>Breakdown of blood-brain barrier, neutrophil recruitment intracranial pressure, brain damage</td>
</tr>
</tbody>
</table>
Immunoperversity and the Virulence of Viruses

**HIV-AIDS**

**Influenza**
Hypotheses for the Evolution of Virulence

1- Direct selection “Trade off”

The morbidity and mortality of the infection is positively associated with the rate of horizontal (infectious) transmission of the parasite. Virulence determinants evolved in response to selection for virulence,
Hypotheses for the Evolution of Virulence

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The morbidity and mortality of the infection is positively associated with the rate of horizontal (infectious) transmission of the parasite. Virulence determinants evolved in response to selection for virulence,

2- Shit happens (i) Coincidental evolution
The morbidity and mortality of the infection are coincidental to the parasite’s colonization, replication and persistence in individual hosts. The virulence determinants responsible for invasive disease evolved in response to selection pressures other than virulence in that host.
Hypotheses for the Evolution of Virulence

1- Direct selection “Trade off”
*The morbidity and mortality of the infection is positively associated with the rate of horizontal (infectious) transmission of the parasite.*
*Virulence determinants evolved in response to selection for virulence.*

2- Shit happens (i) Coincidental evolution
*The morbidity and mortality of the infection are coincidental to the parasite’s colonization, replication and persistence in individual hosts.*
*The virulence determinants responsible for invasive disease evolved in response to selection pressures other than virulence in that host.*

3 - Shit happens (ii) Within-host evolution
*Selection within the host favors mutants of normally commensal microparasites that are capable of invading and proliferating in tissues where they cause disease.*
*The factors responsible for invasive disease evolved to their virulent state within the colonized host.*
1- Direct Selection for Virulence

\[ R_0 = \frac{\beta S}{(d+\alpha+\nu)} \]

Number of secondary infections generated by a single infected individual in a wholly susceptible population of density S

- \( d \) – rate of disease-independent mortality
- \( \alpha \) - rate of disease mortality
- \( \nu \) - rate of recovery
- \( \beta \) - Rate of infectious transmission
Direct Selection for Virulence

\[ R_0 = \frac{\beta S}{(d + \alpha + \nu)} \]

Virulence would evolve and be maintained if the transmission rate parameter \( \beta \) is in some way positively associated with the virulence of the microparasite as measured by \( \alpha \). There’s a trade-off between the need for the parasite to replicate and to be infectiously transmitted.

- \( \alpha \) - rate of disease mortality
- \( \nu \) - rate of recovery
- \( \beta \) - Rate of infectious transmission
- \( d \) – rate of disease-independent mortality

Number of secondary infections generated by a single infected individual in a wholly susceptible population of density \( S \).
Direct Selection for Virulence: The myxoma rabbit story

European rabbit (*Oryctolagus cuniculus*) 10 days after infection with the Standard Laboratory Strain of myxoma virus.

Brazilian rabbit (*Sylvilagus brasiliensis*) three weeks after infection with the Standard Laboratory Strain of myxoma virus.

Frank Fenner
Direct Selection for Virulence: The myxoma rabbit story

• Biting insect mediated transmission - need viable rabbits
  • Virulent - high levels of circulating virus - Rabbits that die too quickly to generate as many secondary infections as those that die more slowly
  • Avirulent - Low levels of circulating virus transmit the virus poorly.

In accord with the direct selection for virulence (trade-off) hypothesis, myxoma of intermediate virulence should evolve.
Direct Selection for Virulence: The myxoma rabbit story

Virulence (measured on laboratory rabbits) of myxoma virus strains recovered from the field in Australia expressed as percent

<table>
<thead>
<tr>
<th>Virulence grade</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case Fatality (%)</td>
<td>&gt;99</td>
<td>95-99</td>
<td>70-95</td>
<td>50-70</td>
<td>&lt;50</td>
<td></td>
</tr>
<tr>
<td>Mean survival time days</td>
<td>&lt;13</td>
<td>14-16</td>
<td>17-28</td>
<td>29-50</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>1950-51</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1952-55</td>
<td>13.3</td>
<td>20.0</td>
<td>53.3</td>
<td>13.3</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>1955-58</td>
<td>0.7</td>
<td>5.3</td>
<td>54.6</td>
<td>24.1</td>
<td>15.5</td>
<td>432</td>
</tr>
<tr>
<td>1964-66</td>
<td>0.7</td>
<td>0.3</td>
<td>63.7</td>
<td>34.0</td>
<td>1.3</td>
<td>306</td>
</tr>
<tr>
<td>1970-74</td>
<td>0.6</td>
<td>4.6</td>
<td>74.1</td>
<td>20.7</td>
<td>0</td>
<td>174</td>
</tr>
<tr>
<td>1975-81</td>
<td>1.9</td>
<td>3.3</td>
<td>67.0</td>
<td>27.8</td>
<td>0</td>
<td>212</td>
</tr>
</tbody>
</table>

Direct Selection for Virulence

While direct selection may be the most popular hypothesis for evolution and maintenance of virulence and there are other examples, save possible in the eyes of the authors of the reports, none are as compelling as the myxoma rabbit story.

For a recent critique of the evidence in support of the direct selection - “trade-off” hypothesis for the evolution of virulence and its implications for “virulence management” see Ebert and Bull (2003) Trends in Microbiology 11: 15-20
Direct Selection for Virulence

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If virulence is due to some form of immunoperversity and is maintained by direct selection, virulent parasites must exploit that immunoperversity to their own ends.
Shit-happens: (i) Coincidental evolution of virulence

Criteria

1- The virulence of the infection is not positively associated with the rate of infectious transmission and may be negatively associated.

2- The morbidity and mortality of the infection can be attributed to the host’s, insufficient, inappropriate or overzealous response to the replication of the parasite and or its products (e.g. endotoxins or exotoxins).
Coincidental evolution of virulence: *E. coli* O157:H7 Shiga toxin-encoding prophage

Roughly equal frequencies of *E. coli* K-12 or *E. coli* O157:H7 carrying and not carrying Stx-encoding prophage were mixed in distilled water with *Tetrahymena pyriformis* from axenic cultures and the changes in density of the bacterial and protozoa populations followed respectively by plating and microscopically.

*Tetrahymena pyriformis.*

Protozoa-Mediated Selection for *E. coli* carrying Shiga toxin encoding prophage

In the presence but not absence of grazing *Tetrahymena*, *E. coli* carrying the Shiga toxin – encoding prophage have an advantage over non-lysogens.
Protozoa-Mediated Selection for *E. coli* carrying Shiga-toxin encoding prophage

Naturally occurring *E. coli* O157:H7 with the Stx prophage also have a fitness advantage over prophage-free cells in the presence but not absence of Tetrahymena.

Experiments with Stx1 and Stx2 negative prophage suggest that some, but possibly not all, of the fitness advantage in the presence of Tetrahymena can be attributed to Stx2.

The Stx-encoding prophage enhances survival in food vacuoles.
Shit-happens (ii) Within-host evolution of virulence

Criteria

1- The virulence of the infection is not positively associated with the rate of infectious transmission of the microparasite and may be negatively associated.

2- The morbidity and mortality of the infection requires the evolution of invasiveness of microparasite within the colonized host

3- The evolved strain is unlikely to be transmitted.

Within-host evolution of virulence

A number of bacteria that are normally commensal occasionally cause invasive disease.
Within-host evolution of virulence

Inspirational observations

Despite vast numbers of colonizing bacteria, invasive infections can be attributed to the products of very few and possibly single bacteria.

Bruce Stocker

Meynell, G. G., 1957 The applicability of the hypothesis of independent action to fatal infections in mice given *Salmonella typhimurium* by mouth. J Gen Microbiol **16**: 396-404.


Richard Moxon

Also:


Within-host Evolution of Virulence

The Moxon Murphy Experiment

Blood - bacteremia
between $10^4$ and $10^5$ 97% of infections were either $\text{Str}^s$ or $\text{Str}^r$ rather than mixed

Mixtures of $\text{Str}^s$ and $\text{Str}^r$

$\text{H. influenzae}$

Nasopharyngeal cultures
100< primarily $\text{Str}^s$ or $\text{Str}^r$
$>10^5$ mostly mixed

CNF - 98% from monoclonal bacteremias had the same Str marker of the bacteria in the blood. Of 19 rats with mixed bacteremias 13 had monoclonal $\text{Str}^s$ or $\text{Str}^r$ infections.

Moxon and Murphy PNAS 75, 1534 (1978)
Within-Host Evolution of Virulence

*Haemophilus influenzae* type b Eagan strain

Sm+ and Nal+ have no discernable differences in fitness in the nasal passages, in the blood stream or in broth.

Margolis and Levin. JID 2007: 196 (Oct 1): 1068-1075
Within-Host Evolution of Virulence

Repeat of Moxon-Murphy

Margolis and Levin. JID 2007: 196 (Oct 1): 1068-1075
Within-Host Evolution of Virulence

Bacteria Invade at Random

If lambda is the expected number of bacteremias then

$$\lambda = -\ln(P(k = 0))$$

$$10^7 \text{ inoculum } \lambda = -\ln\left(\frac{24}{25}\right) = 0.19$$

Then the distribution of rats with different numbers of invasion events (k=0,1,2):

$$P(k; \lambda) = e^{-\lambda}\frac{\lambda^k}{k!}$$

If w bacteria cross over during each invasion events then the probability of either all Nal-r or all Str-r in the blood is then:

$$P(\text{all Nal-r or all Str-r}; k) = \frac{1}{2} w_k + \frac{1}{2} w_k$$

Margolis and Levin. JID 2007: 196 (Oct 1): 1068-1075
Within-Host Evolution of Virulence

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>$10^8$ dose (14 bacteremic rats)</th>
<th>$10^7$ dose (5 bacteremic rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rats with 1 type</td>
<td>Rats with 2 types</td>
</tr>
<tr>
<td>Observed</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

Expected from the statistical model
if the no. of bacteria is

<table>
<thead>
<tr>
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<th>$10^8$ dose (14 bacteremic rats)</th>
<th>$10^7$ dose (5 bacteremic rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 bacteria</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Rats with 1 type have either nalidixic acid–resistant mutant or streptomycin-resistant mutant bacteria in their blood cultures. $P$ values where the model does not significantly differ from observed values are in bold type.

Only one bacterium is responsible for establishing the blood population.

Margolis and Levin. JID 2007: 196 (Oct 1): 1068-1075
Within-Host Evolution of Virulence

Test of the within-host evolution hypothesis

Margolis and Levin. JID 2007: 196 (Oct 1): 1068-1075
Alas, within-host evolution is one of but not the sole explanation for the blood invasive *H. influenzae* being the product of single cells.

Margolis and Levin. JID 2007: 196 (Oct 1): 1068-1075
Within-Host Evolution of Virulence

Competition between the evolved (invasive) and ancestral strain in the nasopharynx

Table 2. Selection rate of ancestral strains and the evolved blood isolate (Em091).

<table>
<thead>
<tr>
<th>Strain (Str-r/Nal-r)</th>
<th>Nasal wash $n = 25$</th>
<th>Nasal epithelium $n = 25$</th>
<th>Blood $n = 6$</th>
<th>In vitro $n = 6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fm154/Em6 (ancestral)</td>
<td>$-0.199 \pm 0.159$</td>
<td>$-0.087 \pm 0.205$</td>
<td>$0.070 \pm 0.615$</td>
<td>$-0.151 \pm 0.262$</td>
</tr>
<tr>
<td>Em091/Em092 (blood/nasal)</td>
<td>$0.129 \pm 0.189$</td>
<td>$0.256 \pm 0.190$</td>
<td>$0.069 \pm 0.599$</td>
<td></td>
</tr>
</tbody>
</table>

There is no evidence for the evolved blood-invasive strain being more fit than the ancestral strain in colonizing the nasopharynx.

Margolis and Levin. JID 2007: 196 (Oct 1): 1068-1075
What about the People? Co-evolution

“(T)he struggle against disease and particularly infectious disease has been a very influential evolutionary agent …”

How much has infectious disease-mediated selection shaped and continues to shape human evolution?

How do we account for immunoperversity?

Haldane, J. B. S., 1949 Disease and evolution. La Ricerca Scientifica 19: 68-76.
Even under the best of conditions, human evolution is slow compared to that of microbes.

<table>
<thead>
<tr>
<th></th>
<th>Haploid</th>
<th>No Dom</th>
<th>Dominant</th>
<th>Recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Po Po</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Selection s</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Final Pt</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Generations</td>
<td>460.512</td>
<td>921.024</td>
<td>510.507</td>
<td>500360.5</td>
</tr>
<tr>
<td>Years *</td>
<td>9.21E+03</td>
<td>1.84E+04</td>
<td>1.02E+04</td>
<td>1.00E+07</td>
</tr>
</tbody>
</table>

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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Selection s</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Final Pt</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Generations</td>
<td>690.6755</td>
<td>1381.351</td>
<td>790.5754</td>
<td>100490.7</td>
</tr>
<tr>
<td>Years *</td>
<td>1.38E+04</td>
<td>2.76E+04</td>
<td>1.58E+04</td>
<td>2.01E+06</td>
</tr>
</tbody>
</table>

* Assumes 20 years per generation
The efficacy of infectious disease-mediated selection in extant populations is not all that great

The maximum intensity of selection would be the fraction of the population infected.
The realized intensity of selection would depend on:
• The relative numbers of progeny produced by infected and uninfected individuals.
• The extent to which the variation in that number of progeny can be attributed to largely additive genetic variation.

Most of the plethora of the mechanisms that protect us from bacterial and viral infections evolved long before we ascended (?) to Homo sapien and are general in their function and adaptive in their response.
Direct evidence for disease-mediated natural selection in humans is not easy to come by

**Polymorphism**
The ole sickle cell story - may be aberrant
- Intense selection $\sim 100\%$ infected
- Childhood mortality ($s \sim 0.20$)
- A sick erythrocyte

**Rare variants**
Tay-Sachs, Cystic fibrosis … have been postulated to be maintained by balancing disease-mediated selection. But the selection coefficient favoring the normal homozygotes would be small $s = q/(1-q) \sim 0.02$ and without a specific extent disease to study it would be hard to demonstrate a disadvantage for the normal homozygotes.
Inherited Variation in Infection Mortality

Risk of dying of different causes between the ages of 16 and 58 for adoptees with one or more biological or adoptive parent who died of the same cause before age 50 or 70.

Table 4. Effect of the Death of a Biologic or Adoptive Parent on the Rate of Adoptee Mortality from Concordant Causes Assessed by the Proportional-Hazards Regression Model.*

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Parent Dead before the Age of 50</th>
<th>Parent Dead before the Age of 70</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO. †  RR  95 PERCENT CL</td>
<td>NO. †  RR  95 PERCENT CL</td>
</tr>
<tr>
<td>All causes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>779  1.71†  1.14–2.57</td>
<td>813  1.85†  1.17–2.92</td>
</tr>
<tr>
<td>Adoptive</td>
<td>913  0.71  0.37–1.36</td>
<td>917  0.80  0.55–1.16</td>
</tr>
<tr>
<td>Natural causes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>739  1.98†  1.25–3.12</td>
<td>771  1.49  0.92–2.39</td>
</tr>
<tr>
<td>Adoptive</td>
<td>889  0.96  0.48–1.90</td>
<td>878  0.96  0.65–1.41</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>641  5.81§  2.47–13.7</td>
<td>436  5.00§  1.73–14.4</td>
</tr>
<tr>
<td>Adoptive</td>
<td>840  0.73  0.10–5.36</td>
<td>537  1.00  0.34–2.97</td>
</tr>
<tr>
<td>Vascular causes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>585  4.52§  1.32–15.4</td>
<td>464  1.92  0.78–4.73</td>
</tr>
<tr>
<td>Adoptive</td>
<td>822  3.02  0.72–12.8</td>
<td>627  1.50  0.65–3.46</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic</td>
<td>593  1.19  0.16–8.99</td>
<td>463  0.87  0.26–2.88</td>
</tr>
</tbody>
</table>
| Adoptive       | 818  5.16¶  1.20–22.2            | 578  1.49  0.56–3.97             

“If a biologic parent died of infection before age 50 or 70, the mortality rate for adoptees increased five fold.”