

Evolution of H5N1 in birds

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How & why does H5N1 evolve?

- Summary of current knowledge of evolutionary behavior of avian influenza
- What is driving the observed high diversity of H5N1 (HA clades & reassortment)



Mechanisms of evolution

- Random & selection driven mutation (drift)
 - Primarily in HA & NA
- Reassortment
 - Generates "genotypes"
- Compensatory mutations?
 - Following reassortment &/or in response to drift
 - Within & between gene segments



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Quickly...

- Much of the work I will present on H5N1 results from surveillance in southern China
- Methods, including dated phylogenies & estimates of population diversity, conducted within rigorous statistical framework

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Substitution rates

- Rates in all viruses (various subtypes) equivalent regardless of host
 - avian, human, swine, equine
- HP H5 in poultry no higher than LP (any subtype) in wild duck
- In H5N1 rates of HA & NA (5×10^{-3}) significantly higher than internal genes (2.5×10^{-3})



Vijaykrishna et al. 2008 PLoS Pathogens

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Natural selection

- Averaged across genes, all H5N1 segments under strong negative (or purifying) selection
 - i.e. Proteins are structurally conserved
- **BUT**
- HA under relatively less negative selection
- Limited site-specific selection in the HA
 - Generally antigenic, receptor binding & glycosylation (AA pos. 156) sites
 - These observations consistent over past 5 years



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Reassortment (1)

- Please note: I am talking about gene exchange between different subtype viruses
 - Intra- & inter-clade reassortment of H5N1 viruses documented in Europe/Africa (Salzberg et al. 2007 EID), Indonesia (Lam et al. 2008 PLoS Pathogens) & Vietnam (Nguyen et al. 2008 EID)
- Gs/Gd-like H5N1 virus derived from LPAI H5 virus in natural gene pool (wild duck)
- Moved into domestic poultry as a single particle
 - i.e. **Not** generated in poultry through reassortment



Duan et al. 2008 Virology
Vijaykrishna et al. 2008 PLoS Pathogens

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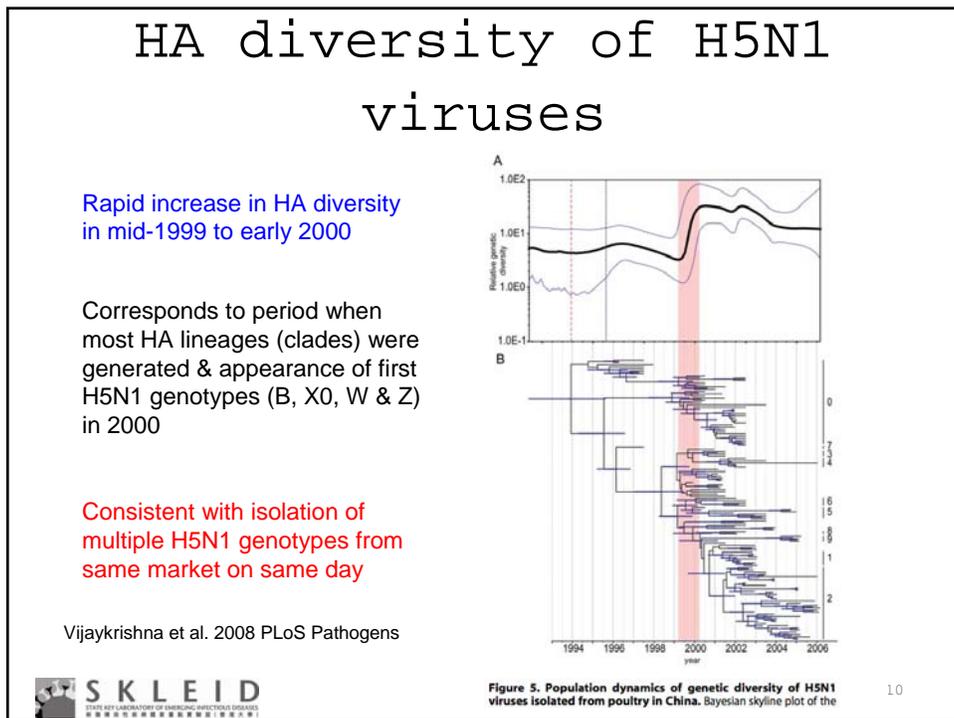
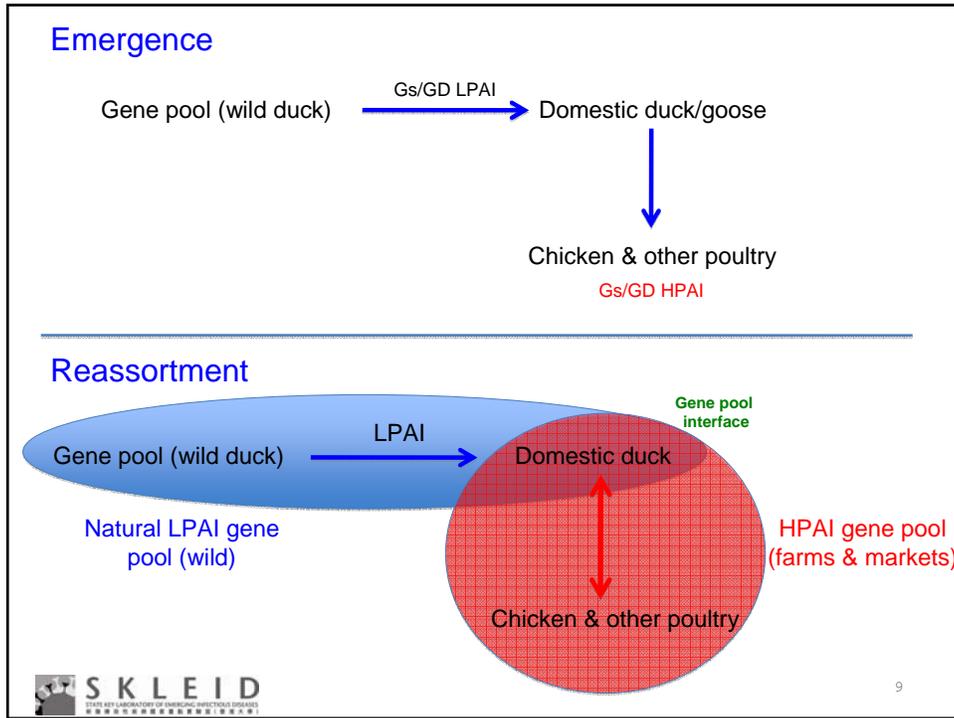
Reassortment (2)

- Reassortant H5N1 viruses (e.g. genotype Z) generated locally in domestic duck **after** Gs/GD virus introduction
 - New gene segments derived from natural gene pool BUT in poultry first detected in domestic duck, not chicken or other poultry
- Frequent gene flow from natural gene pool to domestic duck



Duan et al. 2008 Virology
Vijaykrishna et al. 2008 PLoS Pathogens

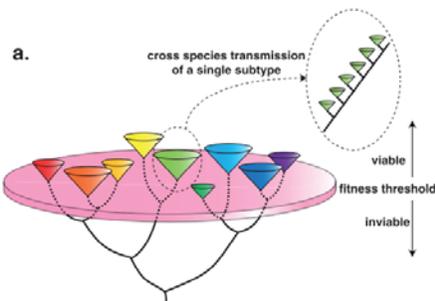
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Possible mechanism..

- This diversity may have arisen from movement of virus through large, immunologically naïve populations of **diverse species in markets**
- Coupled with **relatively high** rates of HA substitution rates & positive selection
- We believe this may select for H5N1 viruses that are adapted to multiple hosts & may account for ecological success of this virus

AIV fitness landscapes



Dugan et al. 2008 PLoS Pathogens

HA, NA & NS genes

Genetically divergent lineages are much less likely to successfully reassort i.e. **large fitness cost associated with reassortment of these genes**



PB2, PB1, PA, NP & M genes

Little or no change in fitness associated with frequent reassortment of **functionally equivalent** gene segments

Consistent with observed behavior of H5N1 reassortment

Intra-subtypic competition

- Demonstrated for H6 subtype viruses in wild duck
 - Eurasian H6 virus introduced to Nth America
 - Introduction drives diversification of Nth American lineages which eventually go extinct
 - Eurasian viruses become established in Nth America
- Appears to also affect H5N1 evolution

Bahl et al. 2008 under review



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Inter-subtypic competition

- May also be a factor BUT
- Multiple subtypes isolated from same markets on same day
- Evidence of extensive co-infection in poultry & wild populations
 - Argues against **strong** inter-subtypic competition



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Other matters

- Data on why is H5 so 'stable'
 - It is a dynamic population of viruses that continue to evolve
- Data on how human viruses fit in
 - Thankfully they don't. **There has been NO indication of H5N1 reassortment with human viruses**

Remaining areas

- Extent & affect of quasispecies
- Compensatory mutations
- Affect of vaccination on evolution
- Interaction of different subtypes
- Importance of statistically robust methods in evolutionary studies