Host-parasite interactions: Evolutionary genetics of the House Finch-Mycoplasma epizootic

Scott V. Edwards
Department of Organismic and Evolutionary Biology
Harvard University
Cambridge, MA USA
http://www.oeb.harvard.edu/faculty/edwards
House Finches and *Mycoplasma*: a strong host-parasite interaction

- *Mycoplasma gallisepticum* escaped chickens and invaded House Finches in the eastern U.S., ~1994
- 10 years later, finches are more resistant and recent bacterial strains are attenuated
- Natural selection (?) on House Finches by disease
  - Higher survival rates found in:
    - Females versus males
    - Smaller versus larger males
    - Bright males versus dull males
  - Can we identify the genes contributing to survival or susceptibility?
Multi-pronged approach to a recently established host-parasite interaction

1. Genetic structure of pre-epizootic house finch populations (AFLPs)
2. Large-scale screen for parasite-induced gene expression in house finches
3. Shifts in allele frequency between pre- and post-epizootic house finches
4. Molecular evolution and host range expansion of the *Mycoplasma* parasite
Recent history of House Finch populations

- Historic range
- ~1870 bottleneck?

- 1940 ~200 birds
Mycoplasma are obligate parasites and have some of the smallest genomes of any non-virus sequenced.
- *Mycoplasma gallisepticum* escaped chickens and invaded House Finches in the eastern U.S., ~1994

- 8 years later, finches are more resistant to the bacterium and recent strains are attenuated

- Have finches evolved resistance?
Population and phenotypic consequences of 1994 epidemic

Males decline after epidemic

Increased redness in males and decreased size after epidemic

AFLPs: House Finch are moderately structured with little evidence for genetic bottlenecks

Distribution of variation (AMOVA)
- Among individuals w/in pops.
- Among pops. w/in subspecies (native range)
- Among subspecies (native range)
- Original range
- Introduced range

Tripartite structure of House Finch populations suggested by assignment test of AFLP data

Suppression subtractive hybridization

- A PCR method for differentially amplifying transcripts that differ in expression in two cell populations
- Often used in plant studies; a useful alternative to microarrays

Experimental cDNA, split into two pools

1. “tester 1” (control)
2. “tester 2”

- cDNAs
- Differentially expressed cDNAs
- cDNAs shared between control and tester

Normalisation

Hybridisation 1

Hybridisation 2

Fill in ends

Selectively amplify

Ligate primers ( ) to two cDNA pools
Example macroarray results

Probe identical filters with RNA from infected and uninfected birds

Distinct hybridizations - differentially expressed genes

Common hybridizations -- noise

- identical filters
  (A + B, C + D)

- Reciprocally subtracted probes
  (A vs. B, C vs. D)
Sequencing suggests change in expression for heat shock and immune system genes

Additional upregulated genes
- Granzyme A

Additional downregulated genes
- Mhc class II

Preliminary network of genes induced by infection

Healthy Finch

Mycoplasma infected Finch

↑

HSP90, TIM1, Granzyme A, Mhc class II, invariant chain

↓

apoptosis, mitochondrial degradation, elongation factor 1α, COI, COIII, NADH4

Host modulation or parasite subversion of immune response?

Museum specimens permit temporal comparison of genetic diversity pre- and post-epidemic House Finch populations.
Mhc class I crystal structure

peptide binding region (PBR)

α1 domain

peptide

α2 domain

β2 microglobulin
Both increases and decreases in diversity are predicted by evolution of resistance in house finches.

*Mhc* class II molecules

Finch with conjunctivitis

homozygote

heterozygote

Healthy Finch

Foreign pathogen
Little evidence for change in heterozygosity ($\theta$) at an $Mhc$ class II locus between pre- and post-epidemic samples.

However, rapid shifts in frequency observed at some peptide-binding codons

* MHC class II peptide binding codon

The *Mycoplasma gallisepticum* genome: ~0.99 Mb

Variation in genome size among House Finch (HF) and Turkey (TK) isolates of *Mycoplasma*.

**SmaI**

- **HF GA 1995**: 965-988 kb
- **TK GA 1973**: 935-950 kb

**Eagl**

- **HF GA 1995**: 965-988 kb
- **TK GA 1973**: 935-950 kb

* 48.5 kb
‡ 23.1 kb

Courtesy Wendy Smith, unpubl. data
Recent host shift of *Mycoplasma gallisepticum* to house finches (HF) - but how recent?

Maximum likelihood tree, 
~5200 bp *RpoB* and *fusA* genes

Courtesy Wendy Smith, unpubl. data

**Mycoplasma gallisepticum**

0.05 substitutions/site

TK = turkey
CK = chicken
HF = House Finch
Empirical conclusions

Pre-epizootic House Finch structure
  - AFLPs suggest ‘significant’ but mild population differentiation

Parasite - induced gene expression
  - House Finches show up- and down-regulation of key immune system genes upon experimental infection

Diachronic allele frequency shifts in house finch populations
  - Little evidence for reductions in diversity but some evidence for allele frequency shifts at key immune system genes

Parasite evolution
  - DNA sequence information provides a detailed view of *Mycoplasma* history
Conservation implications

A double invasion
- Range expansions in both hosts and parasites result in novel evolutionary pressures

Microbial host range expansion
- Adaptation of *Mycoplasma gallisepticum* to a novel host could result in yet further increases in host range in wild birds

Implications for infectious disease biology
- Pathogens can spread across the country in a matter of years
- A number of unresolved issues in the role of genetic diversity in regulating parasite expansion
Acknowledgments

**AFLPs, macroarray analysis**
- Zhenshan Wang, U. Washington
- Kristy Farmer, Geoff Hill, Auburn U.

**MHC evolution**
- Christopher Hess, U. Washington

**Mycoplasma evolution**
- Wendy Smith & Colin Dale, U. Utah and Auburn U.

**Funding**
- NSF