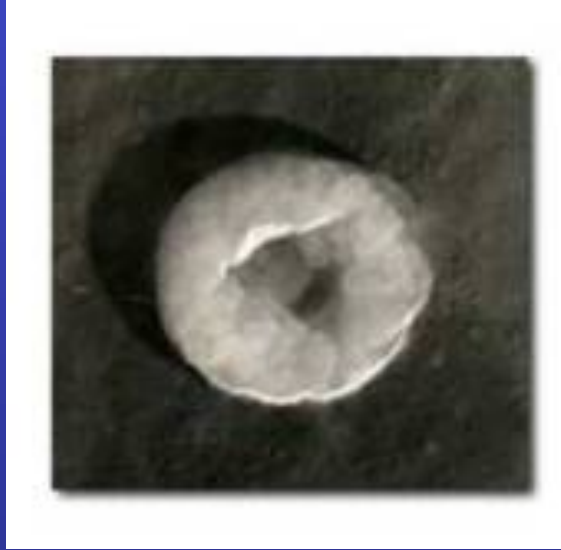
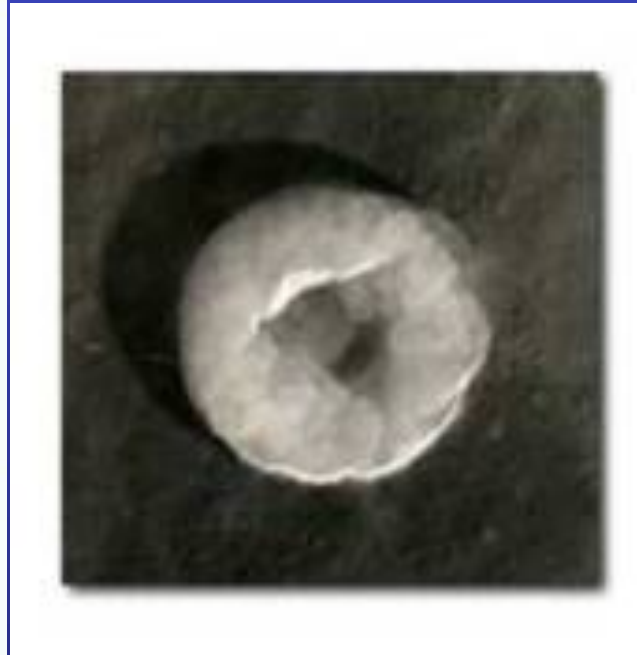


# Genomics of host-parasite co-evolution: a tale of birds and bacteria

[illegible]

Scott V. Edwards  
Department of Organismic  
and Evolutionary Biology  
Harvard University

# House Finches and *Mycoplasma*: a recent host-parasite interaction



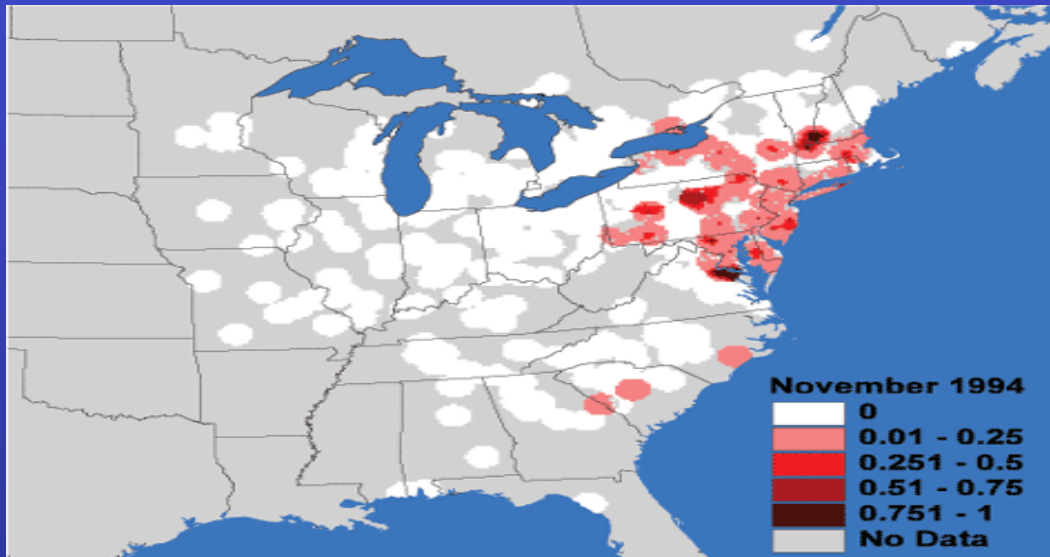
- *Mycoplasma gallisepticum* escaped poultry farms and was found in House Finch populations in the eastern U. S. around 1994
- 9 years later, finches were more resistant to the bacterium and recent parasite strains had attenuated
- Have finches evolved resistance, and if so, how?
- How has the parasite evolved in its new host?



# Recent history of House Finch populations



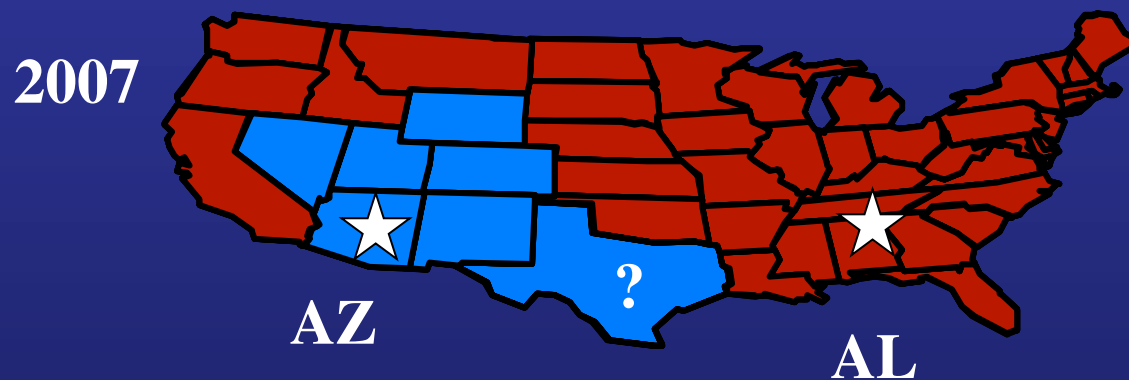
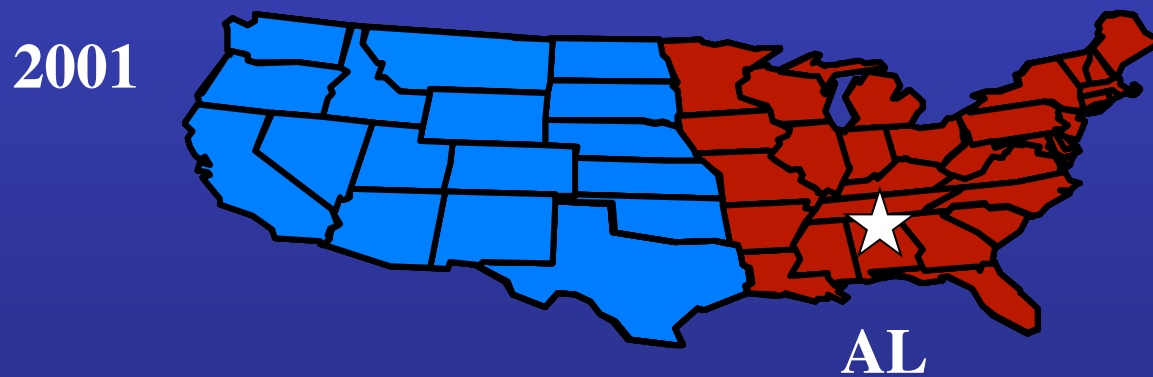
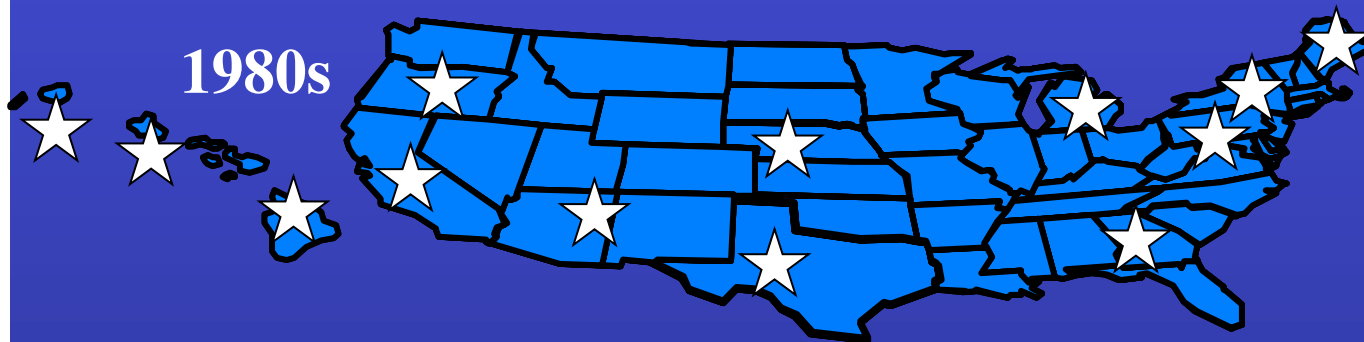
# Rapid spread of *Mycoplasma* in House Finch populations



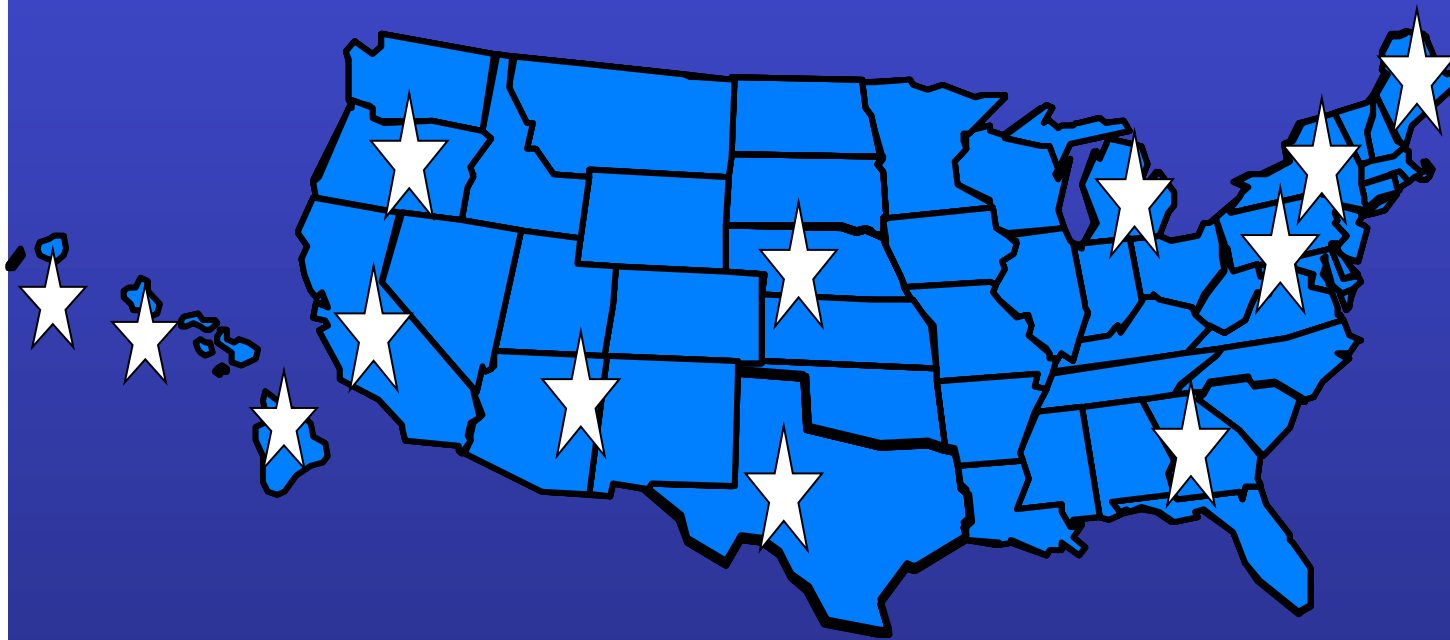
Courtesy Cornell Lab of Ornithology

- *Mycoplasma* is transmitted horizontally
- Spread through the eastern US in 5 years
- Has crossed the Rockies and is spreading

# Temporal and geographic comparisons of *Mycoplasma*-exposed and unexposed populations



# Temporal and geographic comparisons of *Mycoplasma*-**exposed** and **unexposed** populations



*Mycoplasma*  
exposed

*Mycoplasma*  
unexposed

1980s AFLP study  
museum tissue specimens

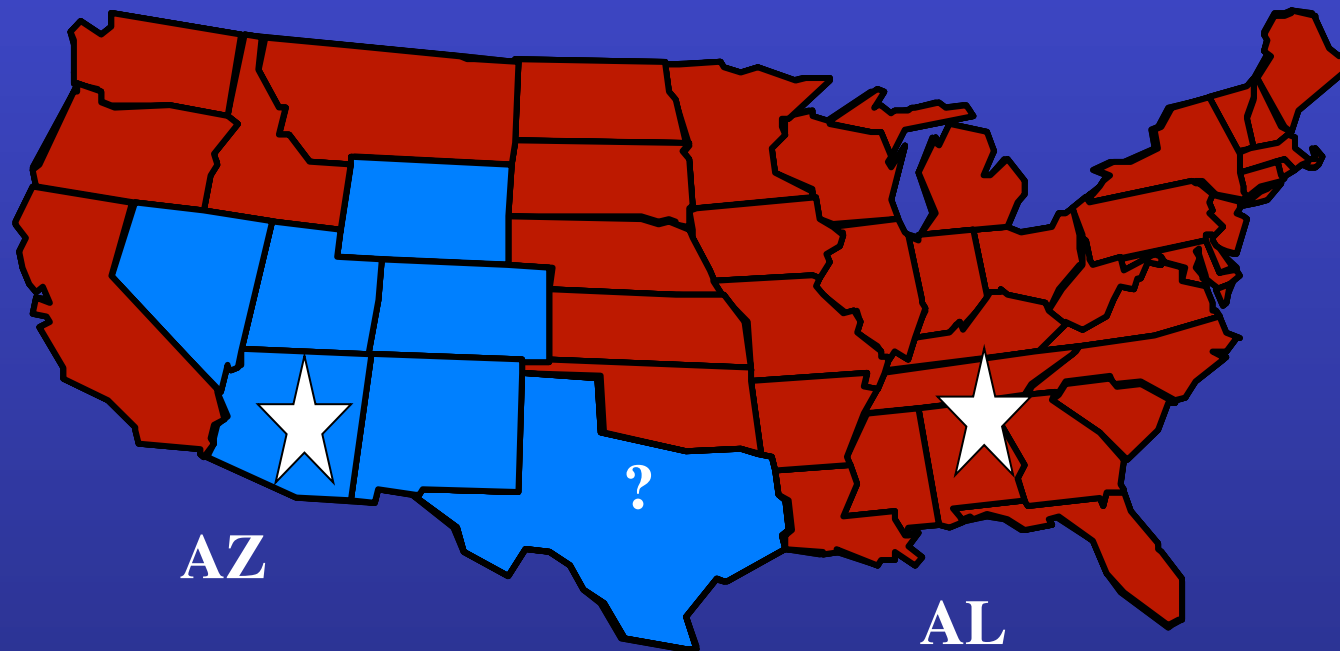
*Mycoplasma*  
exposed

*Mycoplasma*  
unexposed

**2001 Gene expression:**  
population with 5 years exposure



# Temporal and geographic comparisons of *Mycoplasma*-**exposed** and **unexposed** populations



*Mycoplasma*  
exposed

*Mycoplasma*  
unexposed

2007 Gene expression:  
Exposed and unexposed populations

# AFLP survey of House Finches

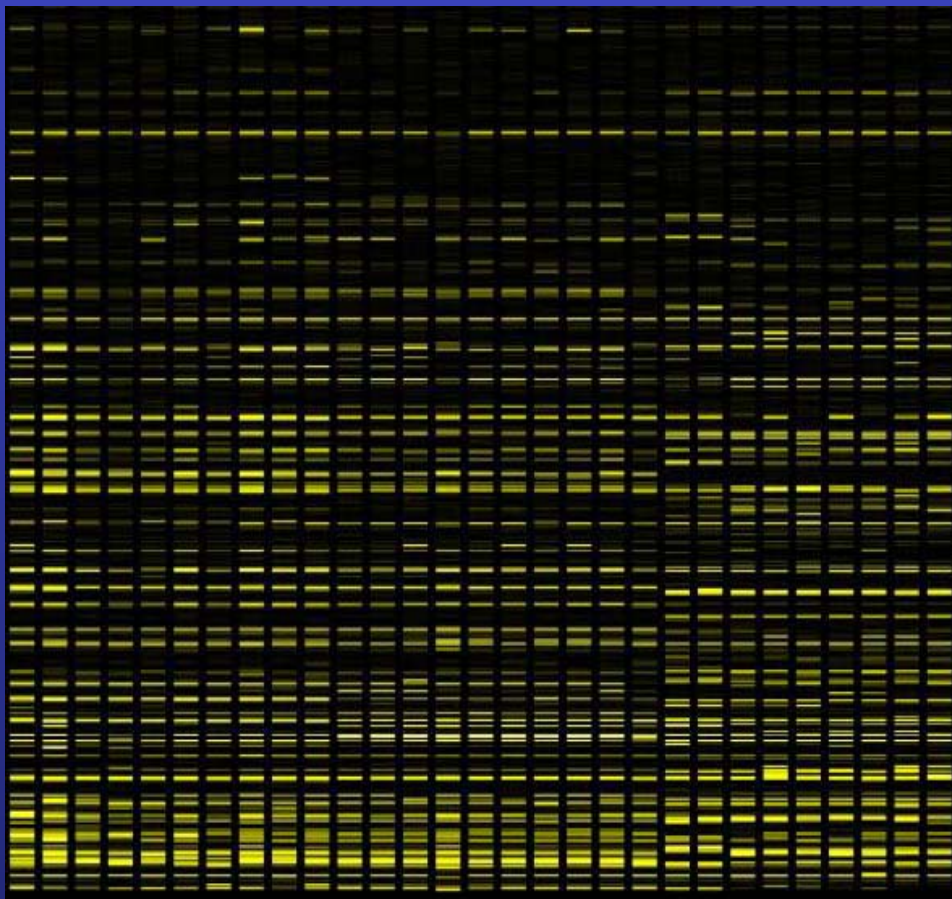
- 163 individuals, 16 populations, 363 markers, 166 (61%) polymorphic

California

Mexico

Purple

Cassin's

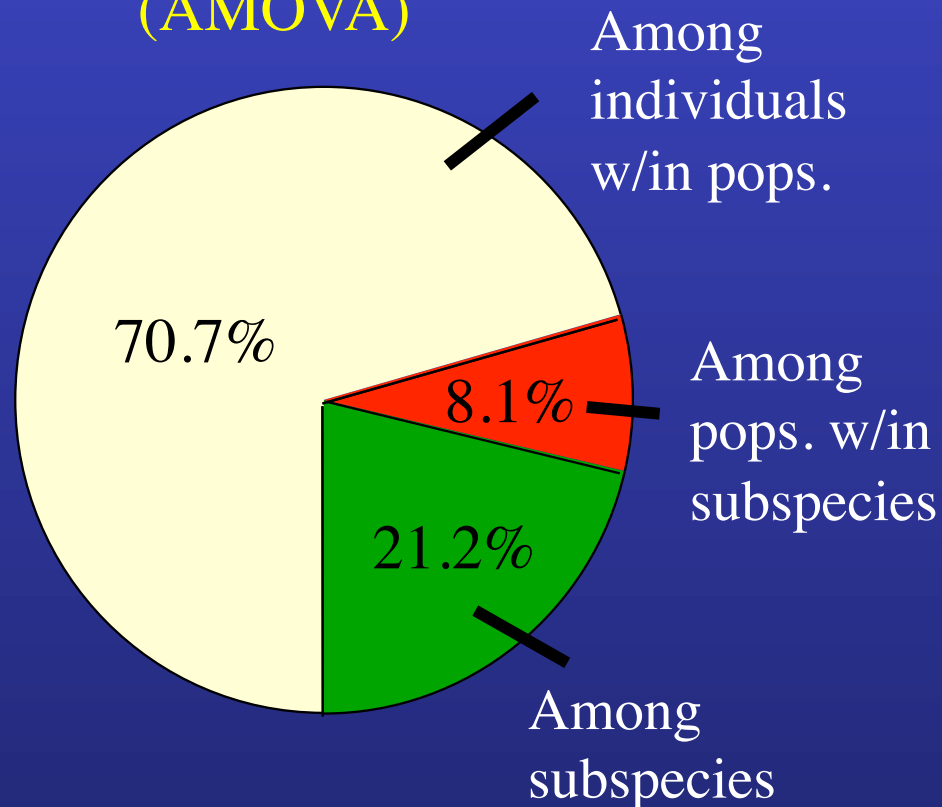


Wang et al. 2003. *Evolution* 57: 2852-2864

# AFLP Results:

House Finches are moderately structured  
with little evidence for genetic bottlenecks

## Distribution of variation (AMOVA)

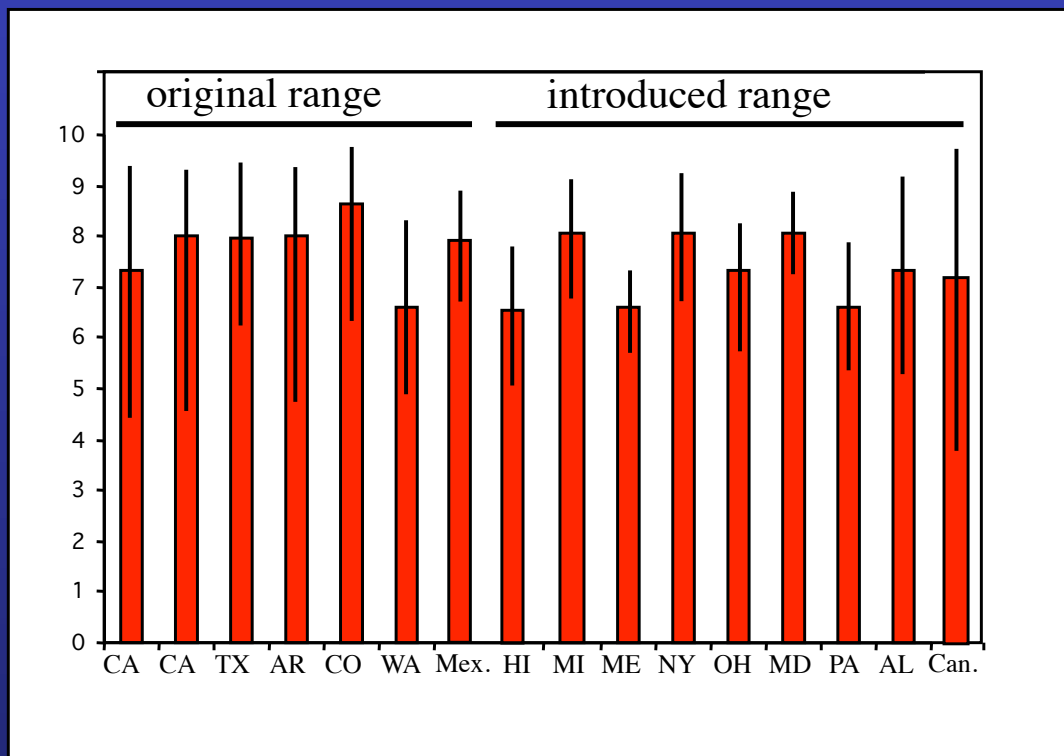


# AFLP Results:

House Finches are moderately structured  
with little evidence for genetic bottlenecks

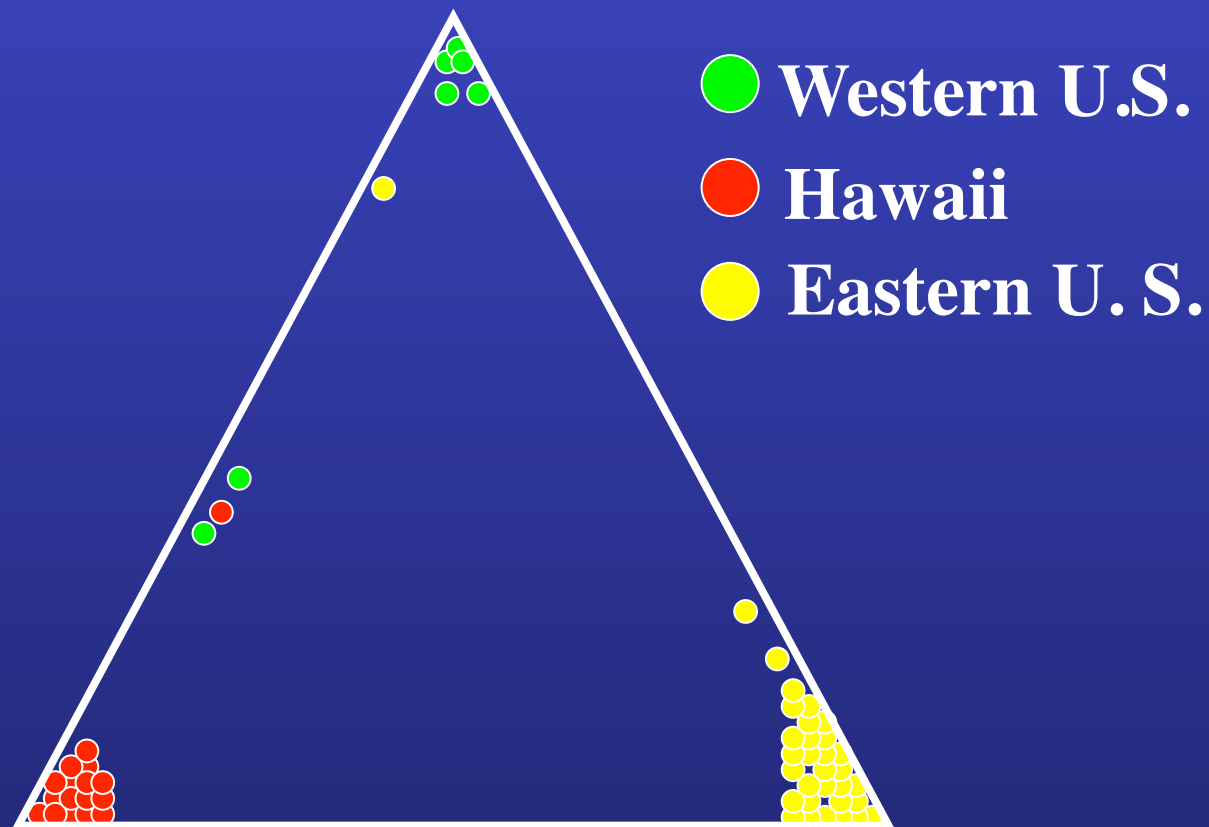
## Nucleotide diversity

(estimated number of substitutions per 1000 sites)



Wang et al. 2003. *Evolution* 57: 2852-2864

# Tripartite organization of House Finch populations suggested by STRUCTURE analysis of AFLP data

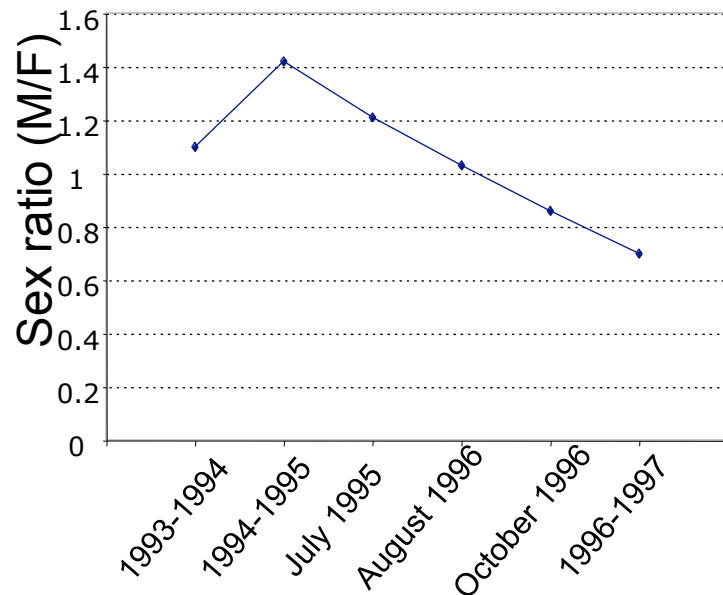


Wang et al. 2003. *Evolution* 57: 2852-2864

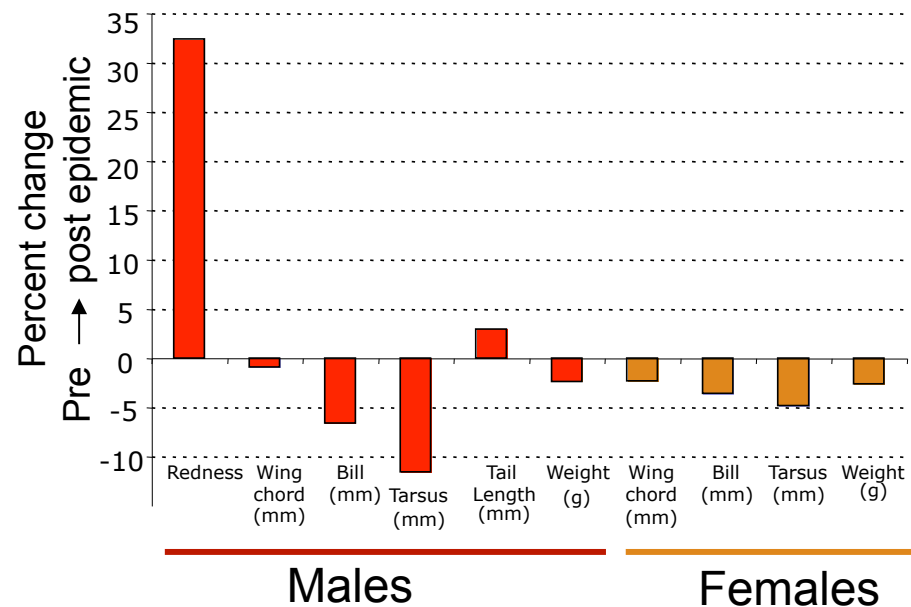
# Population and phenotypic consequences of 1994 epidemic in Alabama



Males decline after epidemic



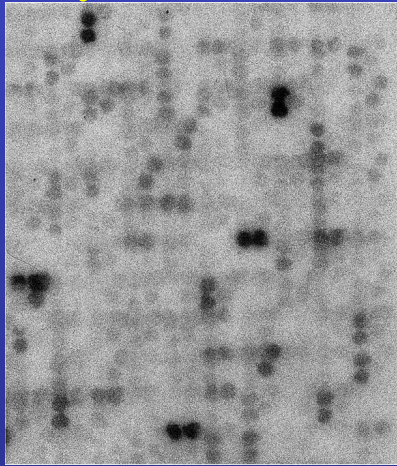
Increased redness in males and decreased size after epidemic



From Nolan, P. M., G.E. Hill and A. M. Stoehr. 1998.  
*Proc. R. Soc. Lond. B*.265: 961-965.

# Temporal and geographic comparisons of gene expression in House Finch populations

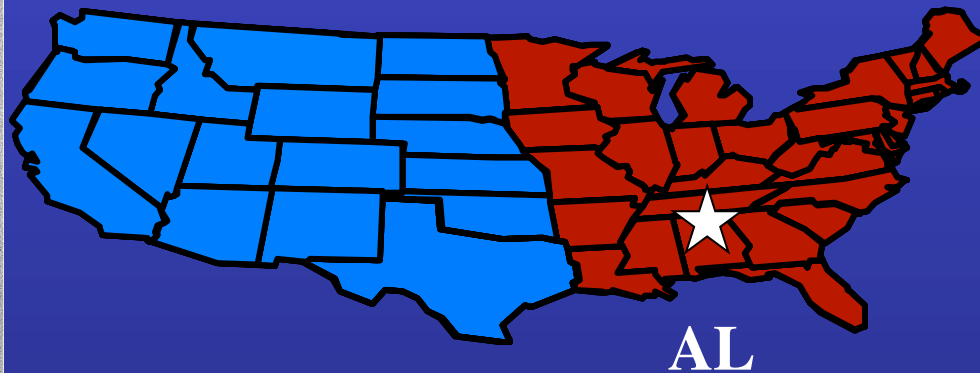
Macroarrays &  
supression-subtractive  
hybridization



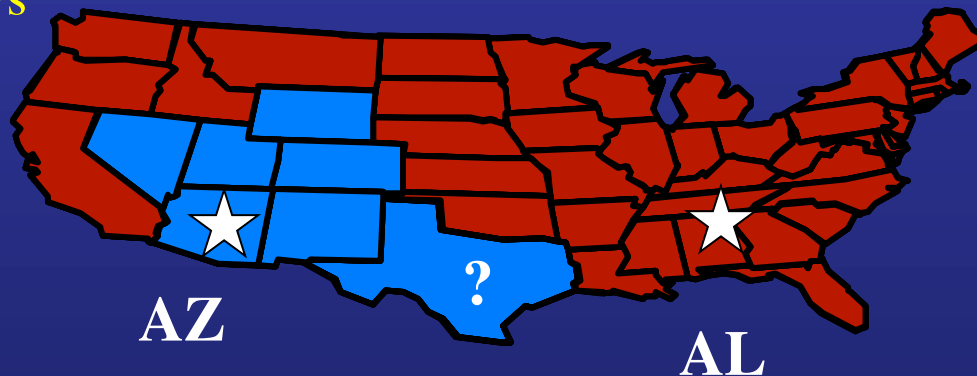
*Mycoplasma*  
exposed  
areas

*Mycoplasma*  
unexposed  
areas

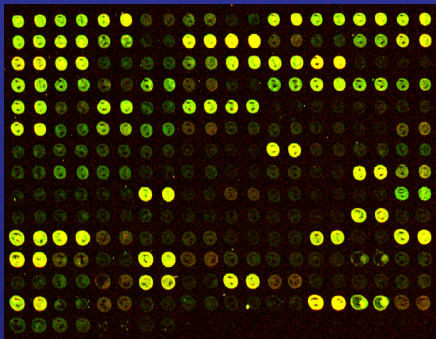
2001



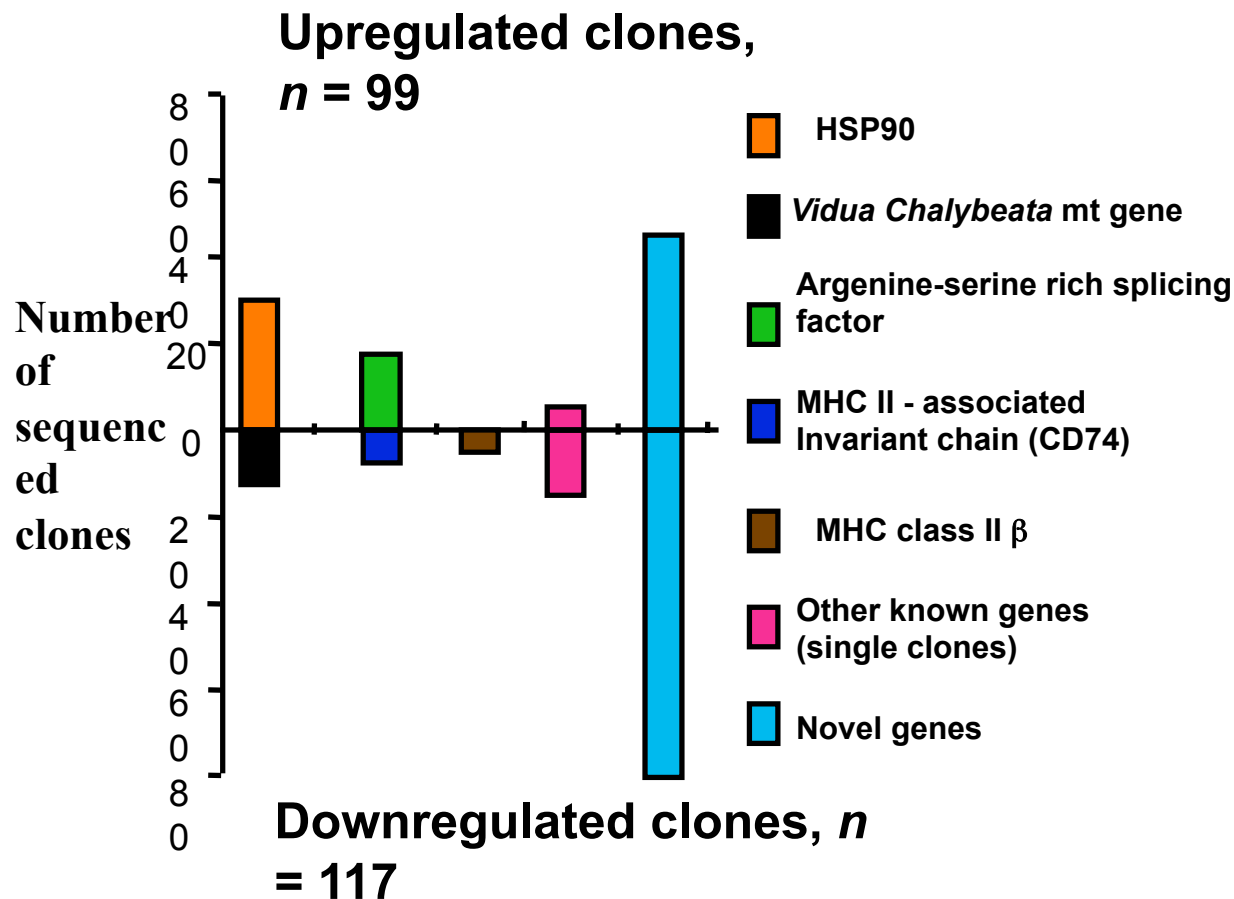
2007



Two-color microarrays



# Macroarray and sequencing suggests change in expression for heat shock and immune system genes



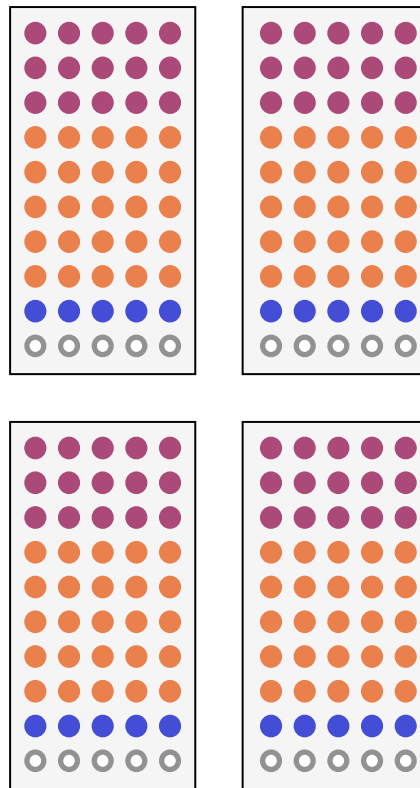
Wang, Z., et al. (2006) *Mol Ecol* 15, 1263-73.

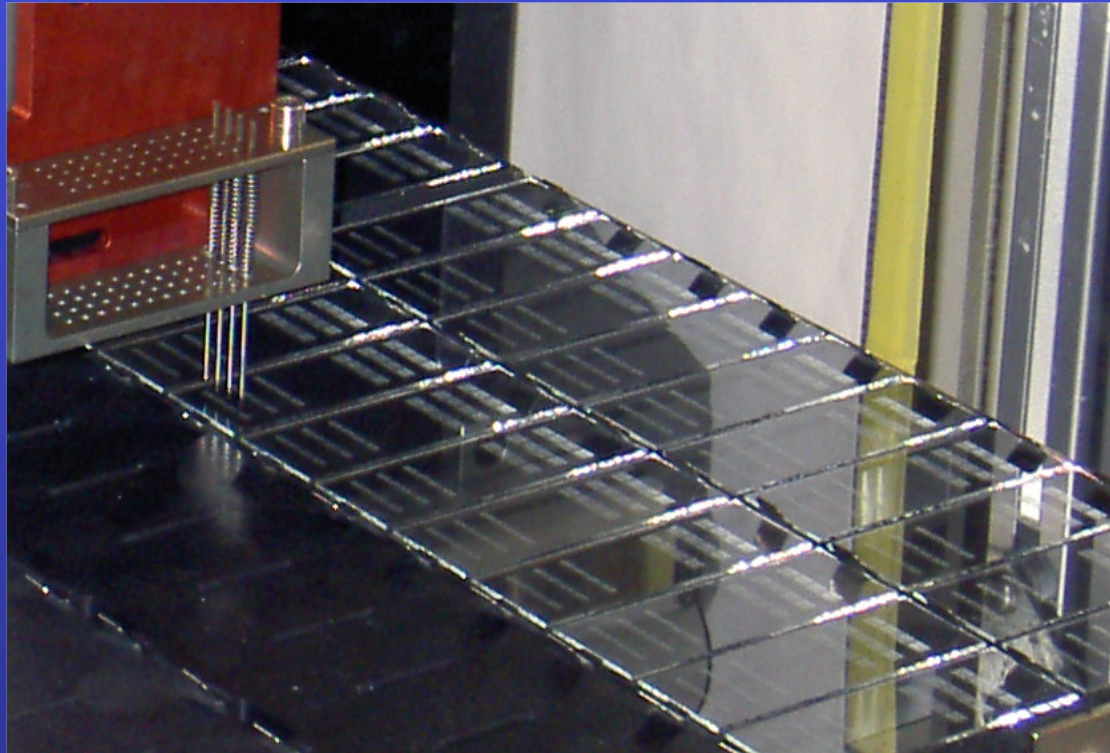


# Custom House Finch cDNA microarrays

**Printed 4X on each slide**

- 220 clones differentially expressed in macroarray experiment
- 780 random additional Finch clones (also from macroarray)
- 5 House Finch housekeeping genes
- 10 *Escherichia coli* housekeeping genes





Printing House Finch microarrays  
at the Harvard Center for  
Systems Biology

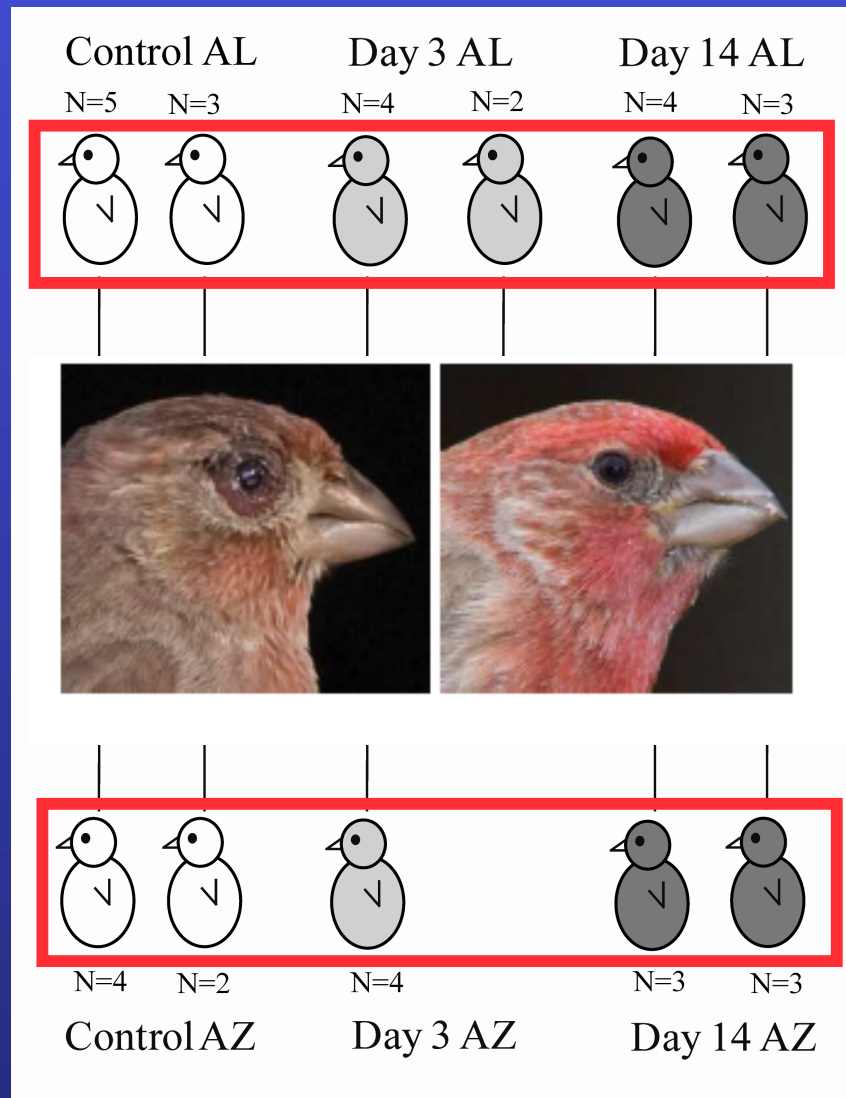
# Microarray hybridization protocol

Dye

Cy5

Cy3

Cy5



AL = Alabama (exposed to MG 7 years prior)

AZ = Arizona (unexposed to MG)

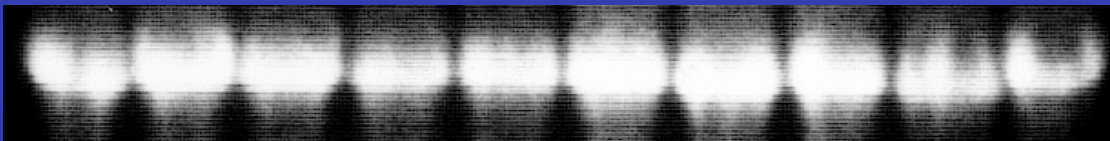
# Confirmation of gene expression patterns

## *Confirmation of macroarray data by Northern blots*

infected individuals    uninfected individuals

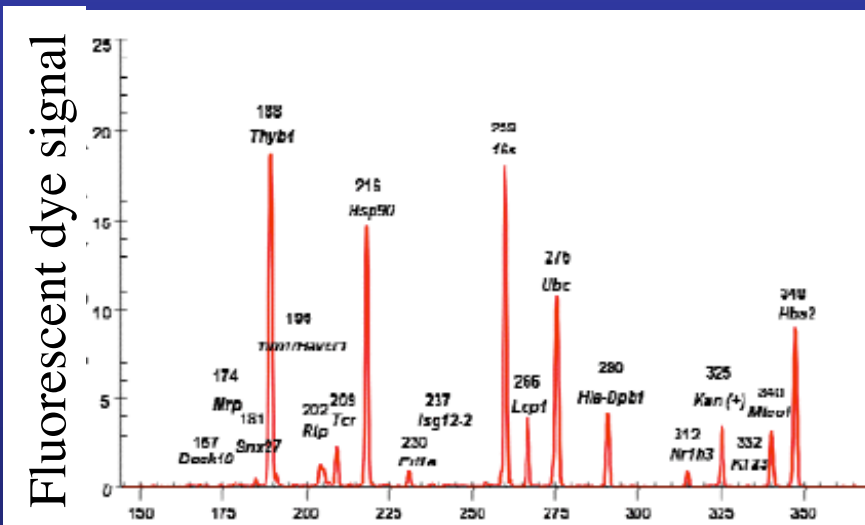


HSP90



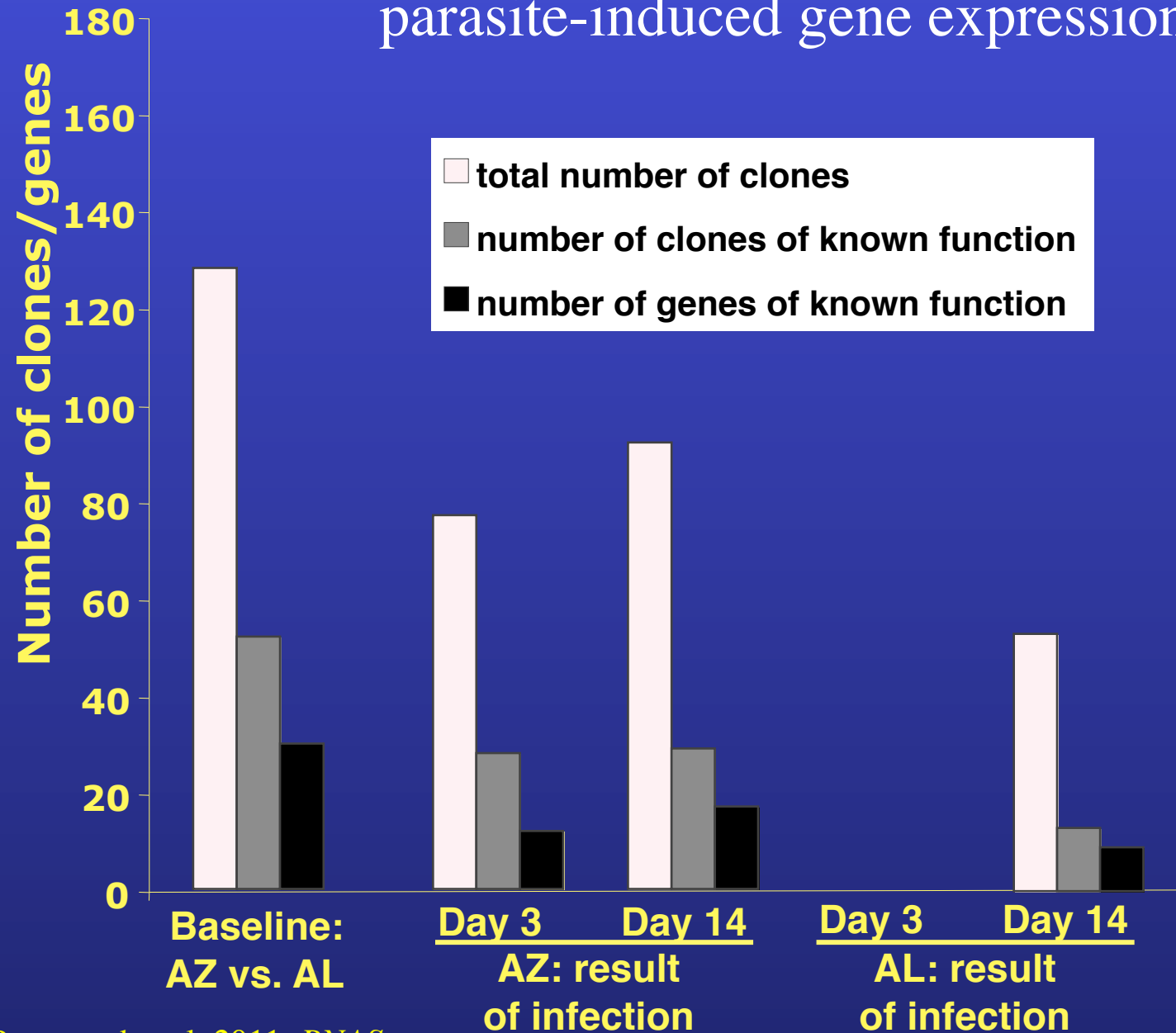
18SrRNA

## *Confirmation of microarray data using Q-PCR*



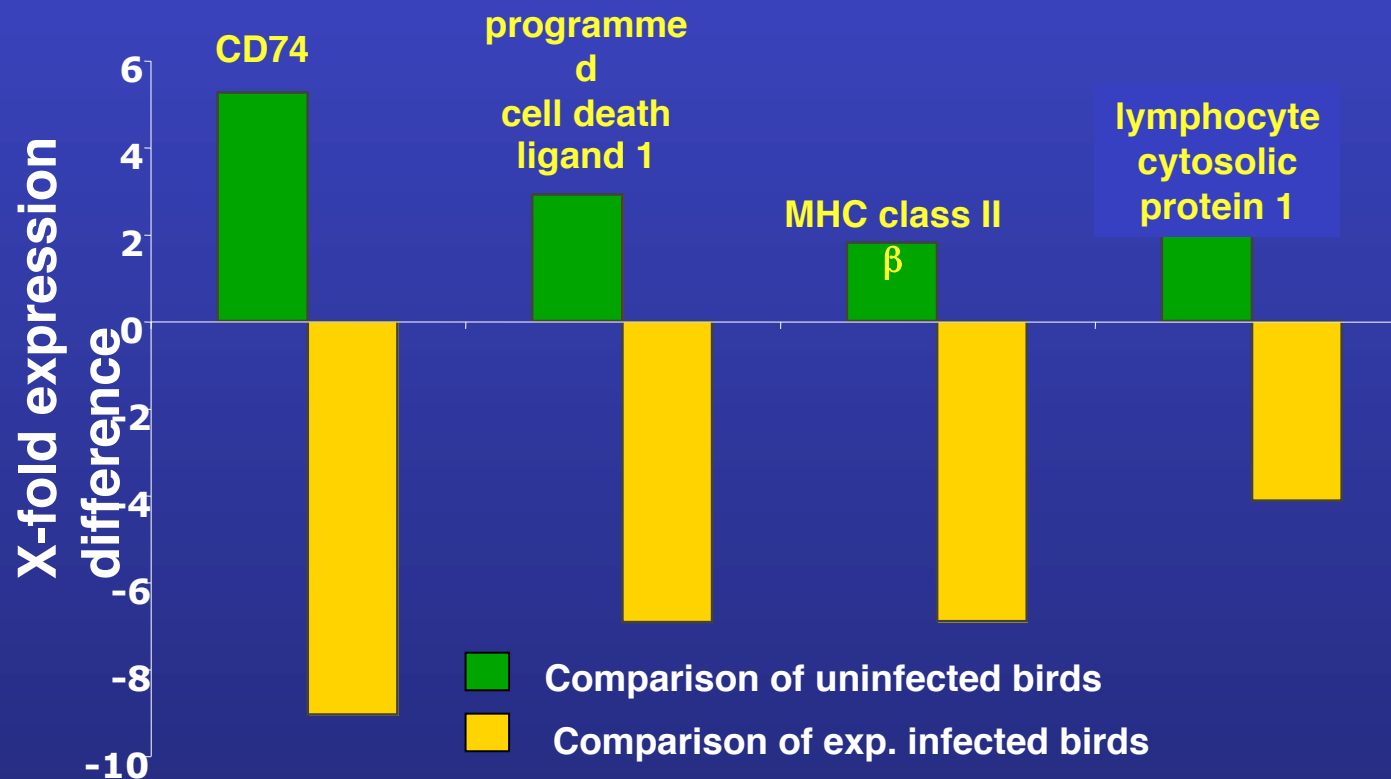
Q-PCR Fragment size (bp)

# Microarray results: naïve (Arizona) birds show more parasite-induced gene expression

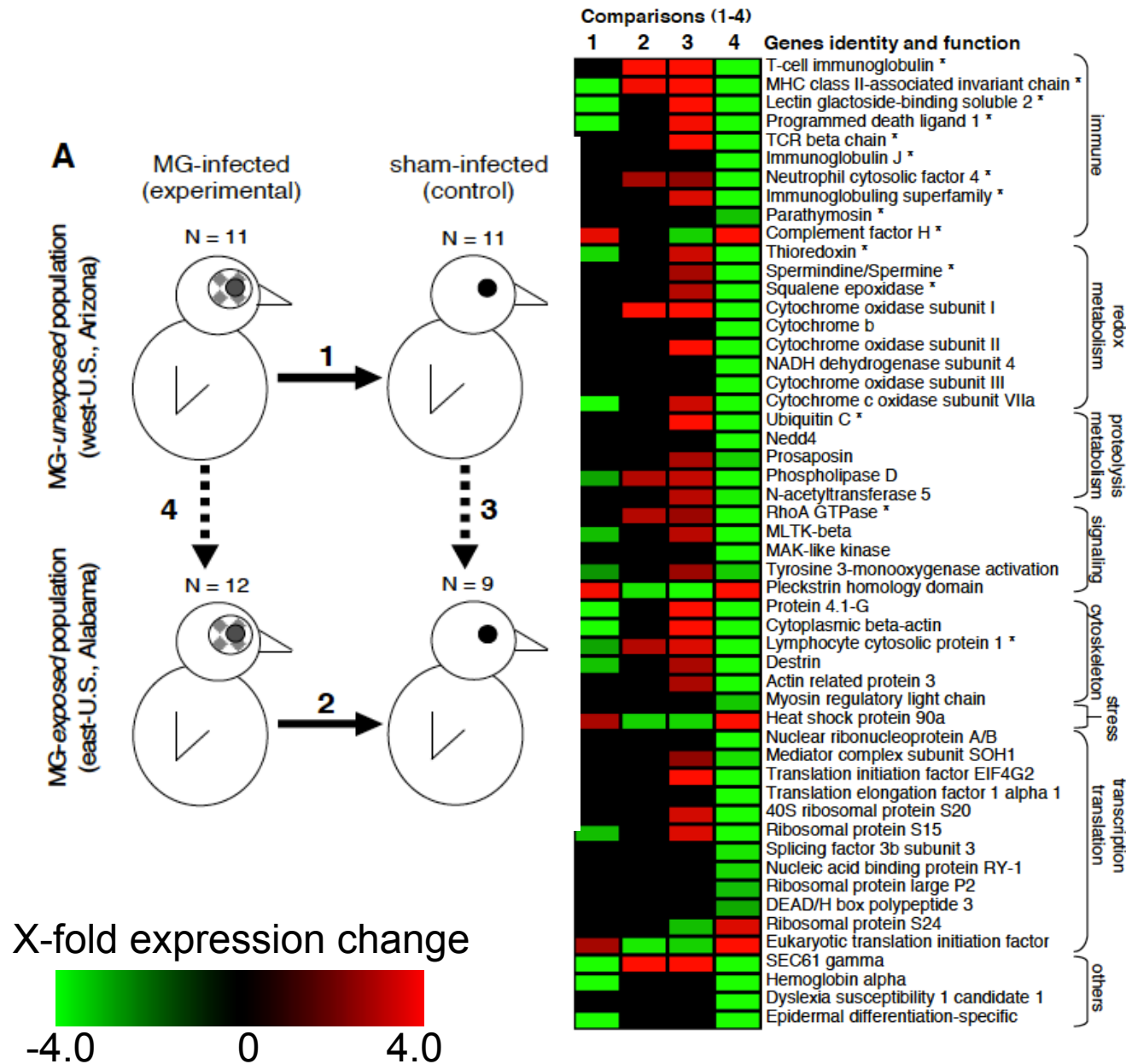


# Immune system: geographic differences in gene expression

All bars are Arizona levels *relative* to Alabama levels

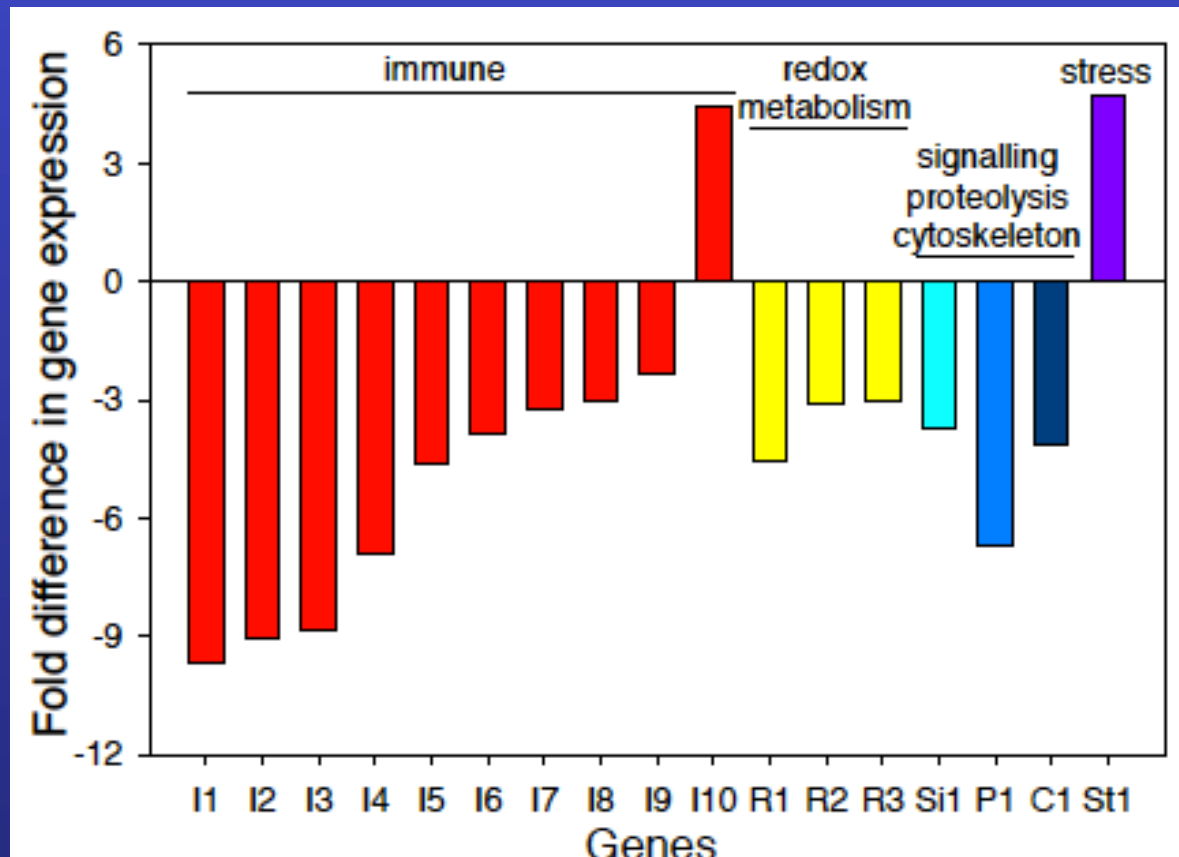


# Arizona vs. Alabama: contrasting responses to infection



# Gene expression response to infection: variation among gene categories

All bars are Arizona levels *relative* to Alabama levels

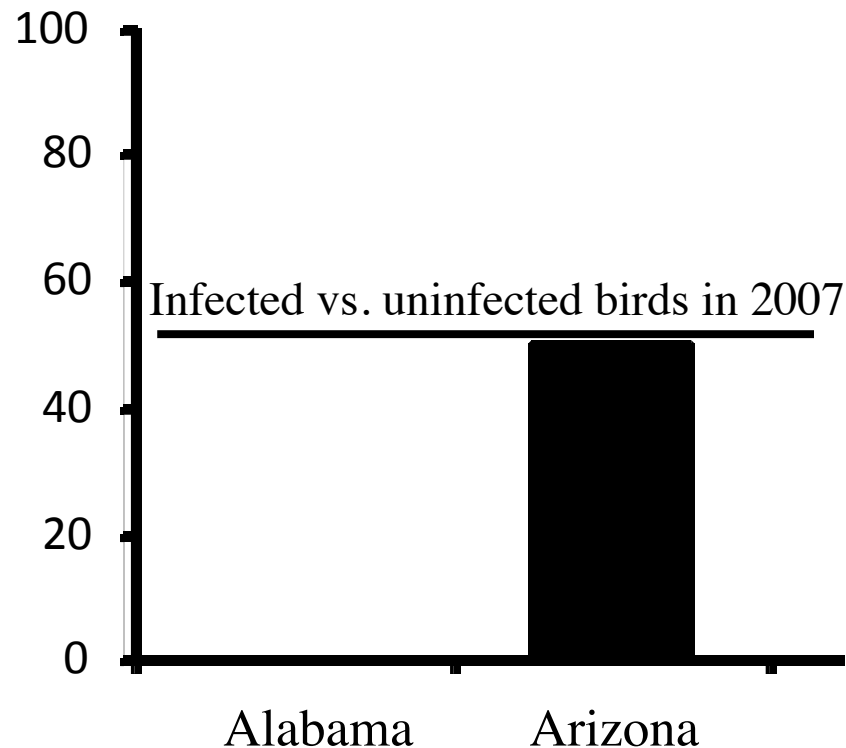




# Change in expression response to infection in Alabama birds between 2001 and 2007

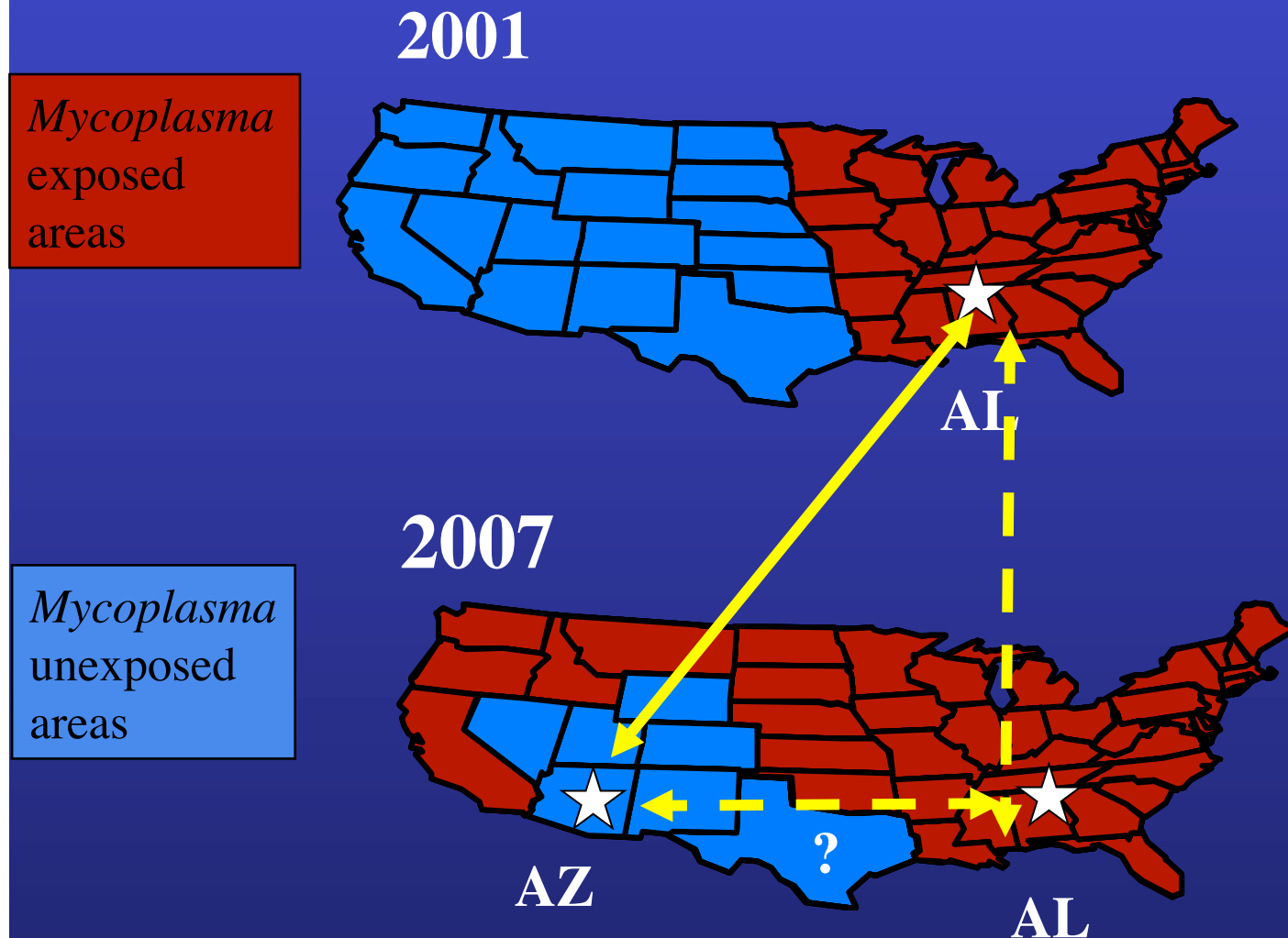


Percent of expression differences consistent with response of Alabama birds in 2001

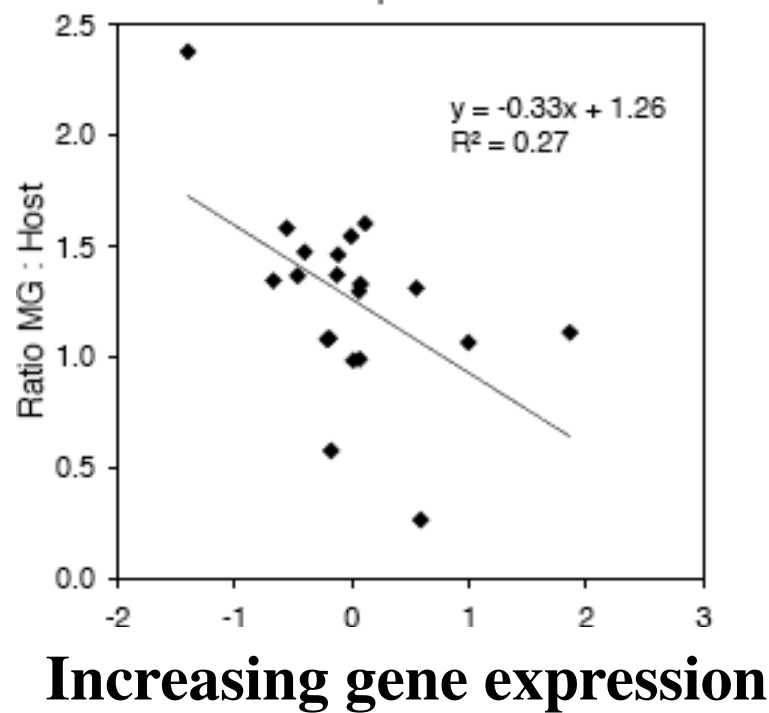
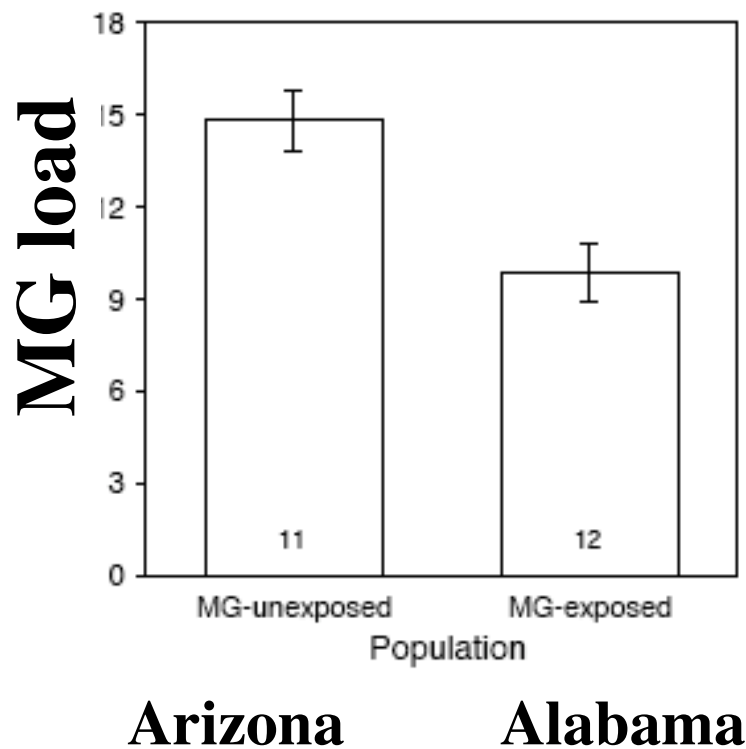


Expression responses of previously exposed birds from AL in 2007 had diverged from those of AL birds in 2001

Signature of microevolution of gene expression in Alabama  
Recapitulation of gene expression response of AL 2001 by AZ 2007



# Patterns of gene expression correlate with *Mycoplasma* load of hosts

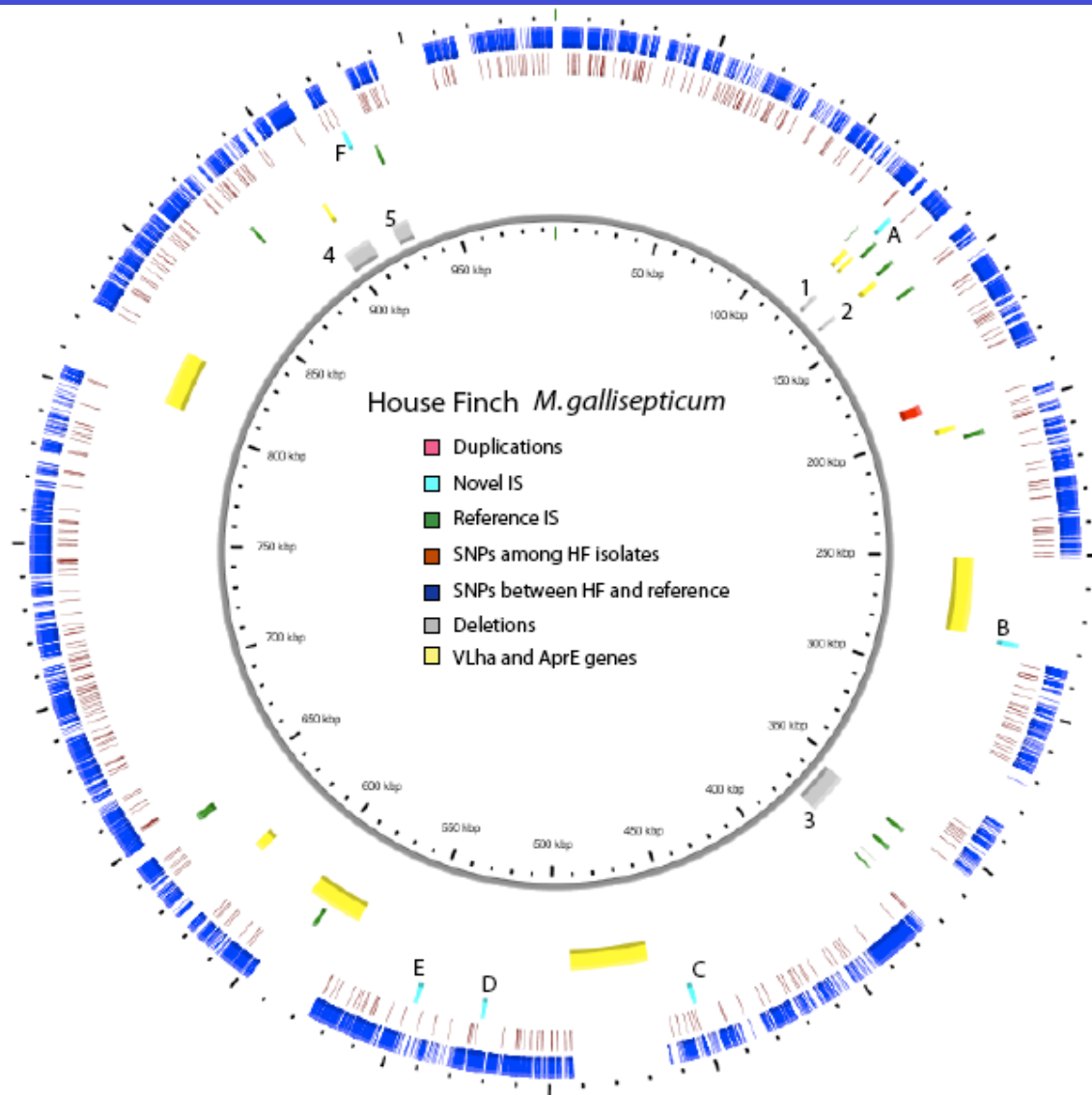


# The *Mycoplasma gallisepticum* genome: ~0.99 Mb

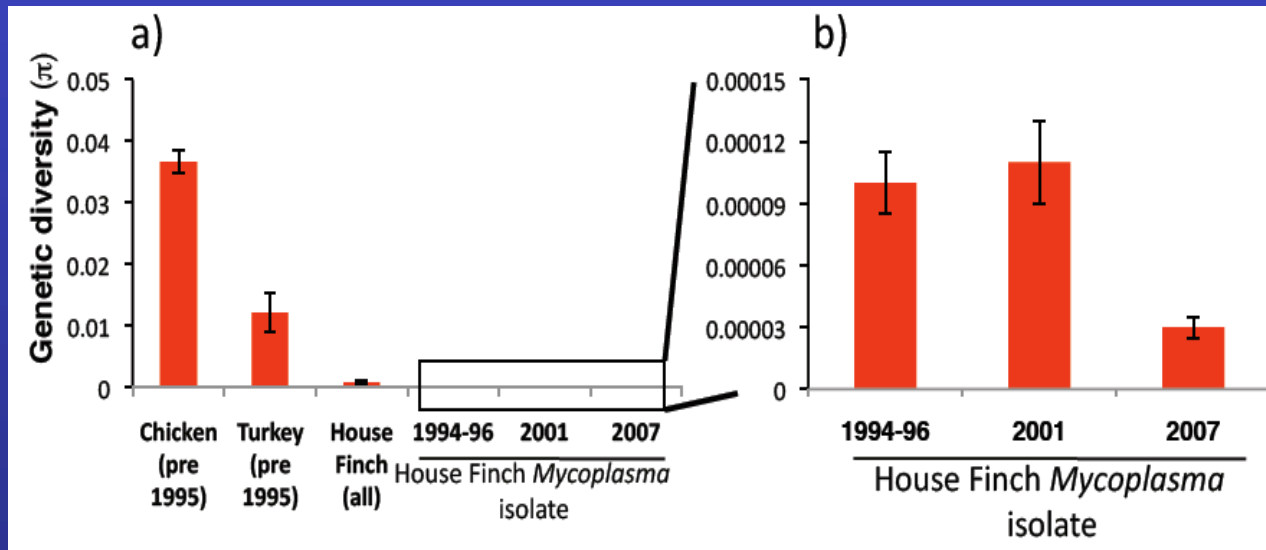


Papazisi, L., et al. (2003) *Microbiology* 149, 2307-16.

# House Finch *Mycoplasma* genome

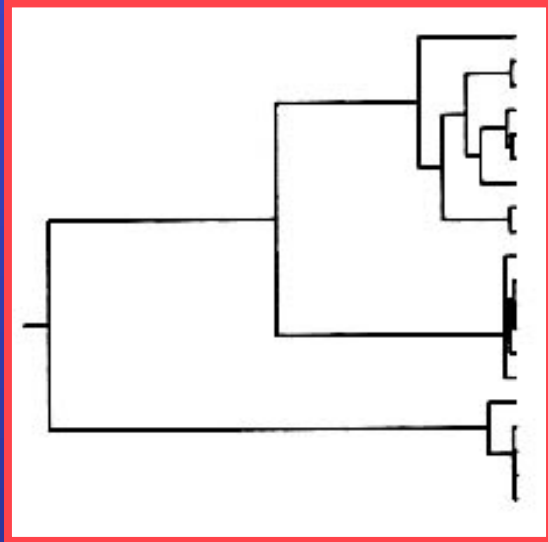


# House Finch *Mycoplasma*: Genetic diversity over time



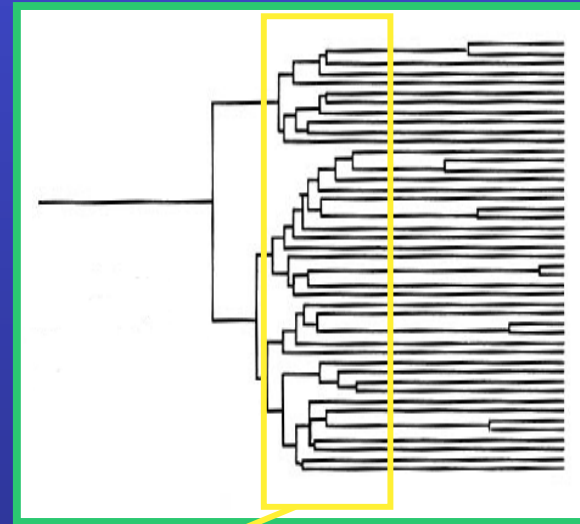
# Signatures of stable and expanding populations

Long internal branches  
Short external branches



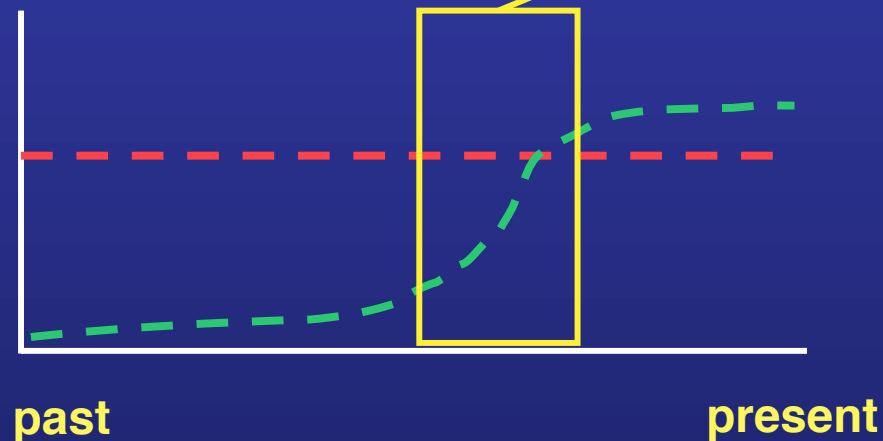
Stable population

Short internal branches  
Long external branches

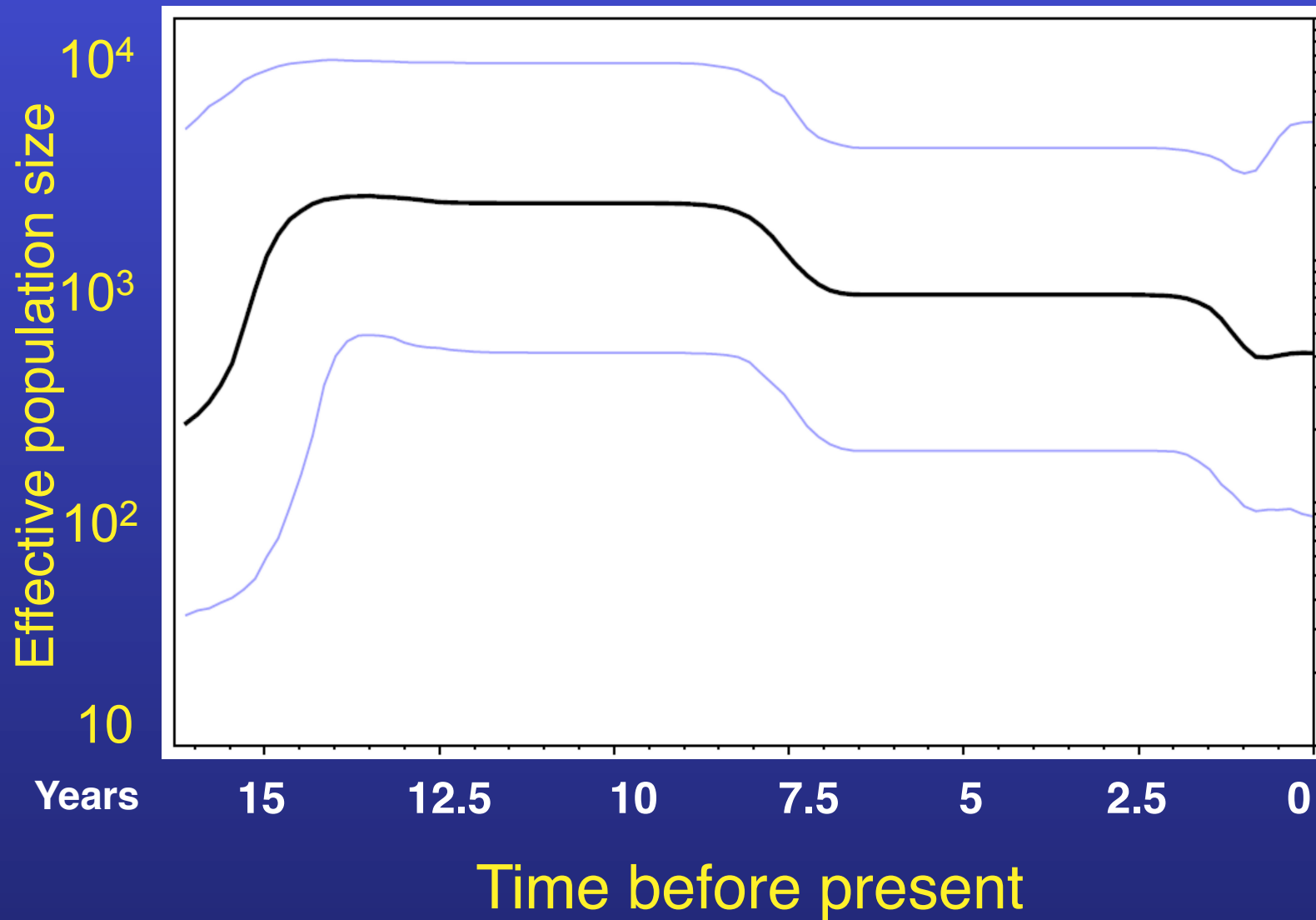


Expanding population

Genetic  
diversity ( $\theta$ )  
or  
population  
size



# Inferred *Mycoplasma* expansion 17 years ago





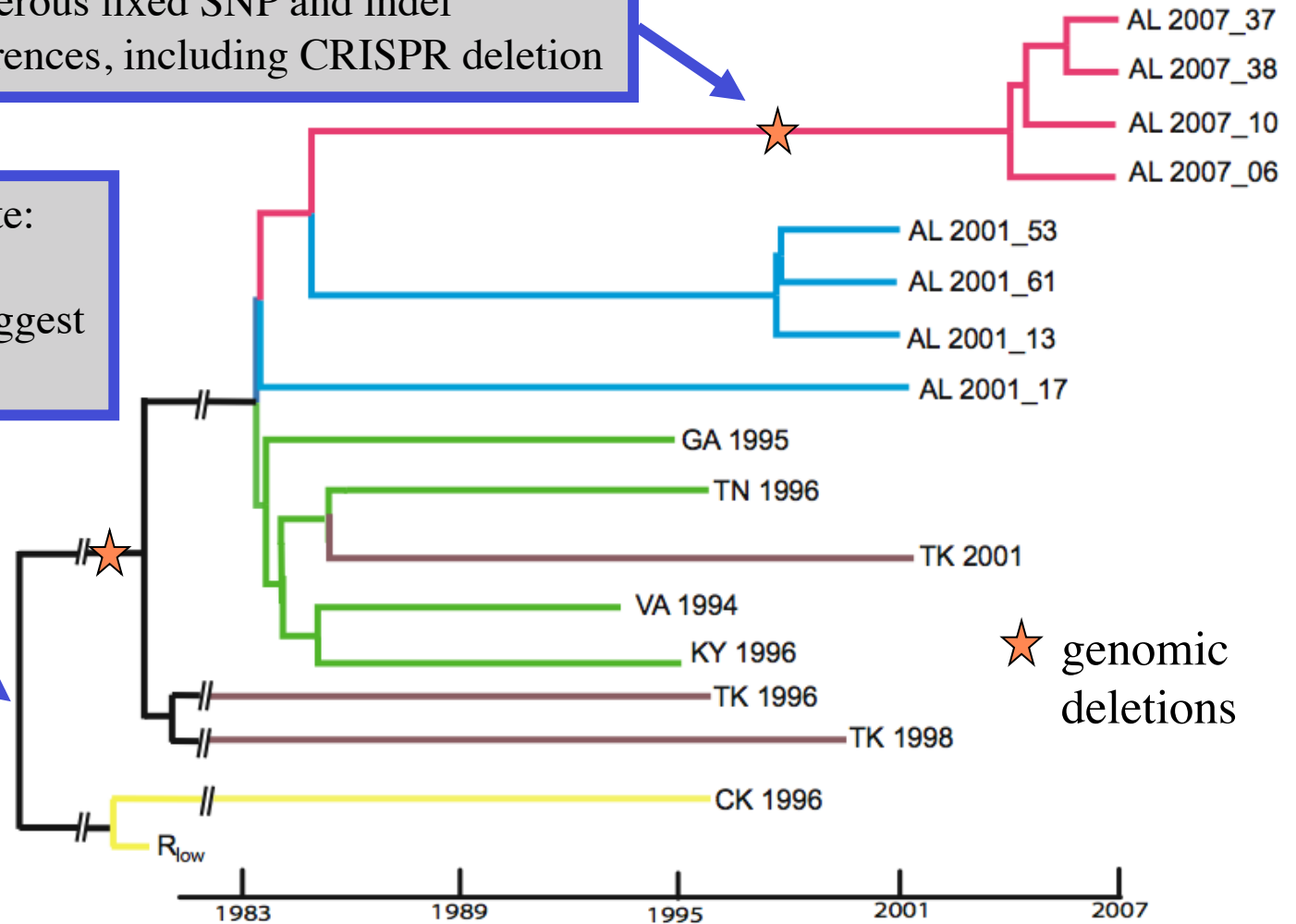


# Implications of a serially-sampled phylogeny of 17 *Mycoplasma* strains

Numerous fixed SNP and indel differences, including CRISPR deletion

Estimated substitution rate:  
 $\sim 10^{-5}$  per site per year;  
 24 homoplasious sites suggest recombination

Ancient coalescence of chicken and finch strains (611 yrs.; 95 % c.i. 484-753 yrs.)

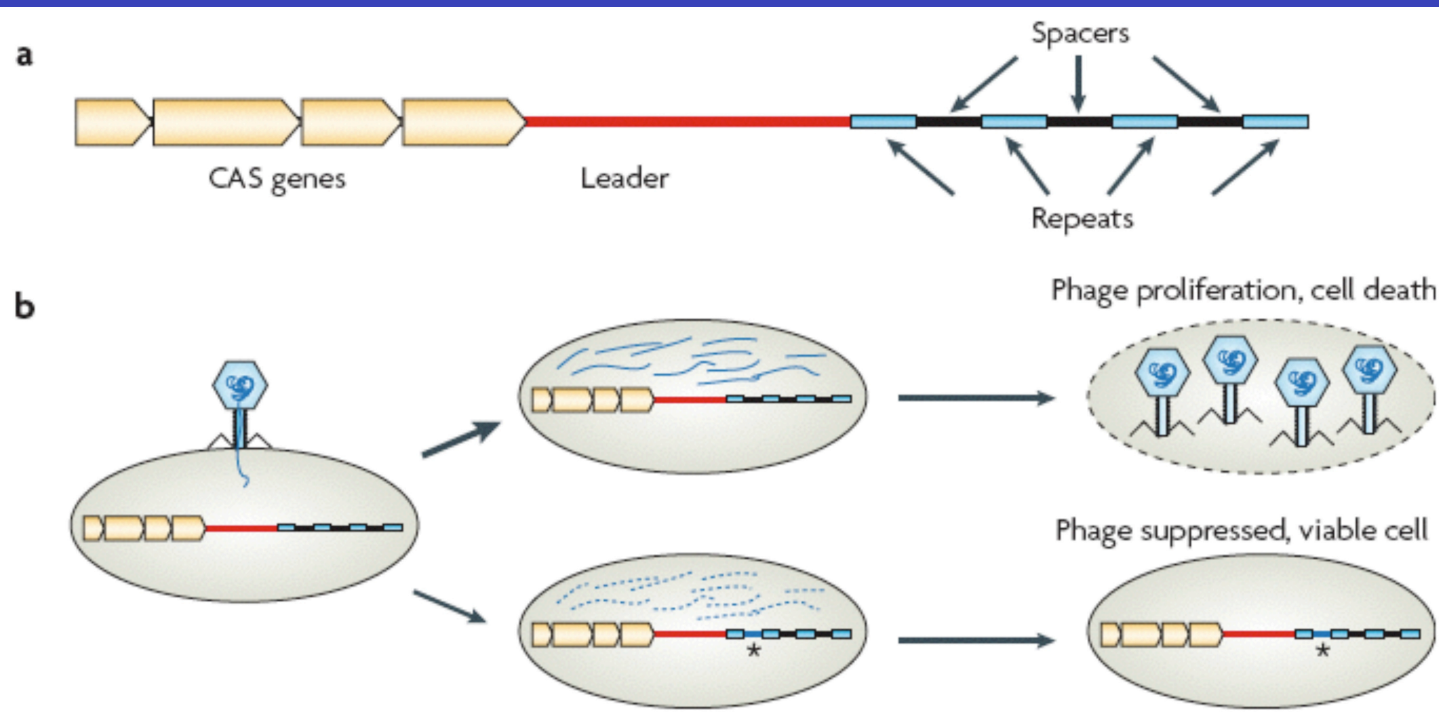


731,536 aligned sites

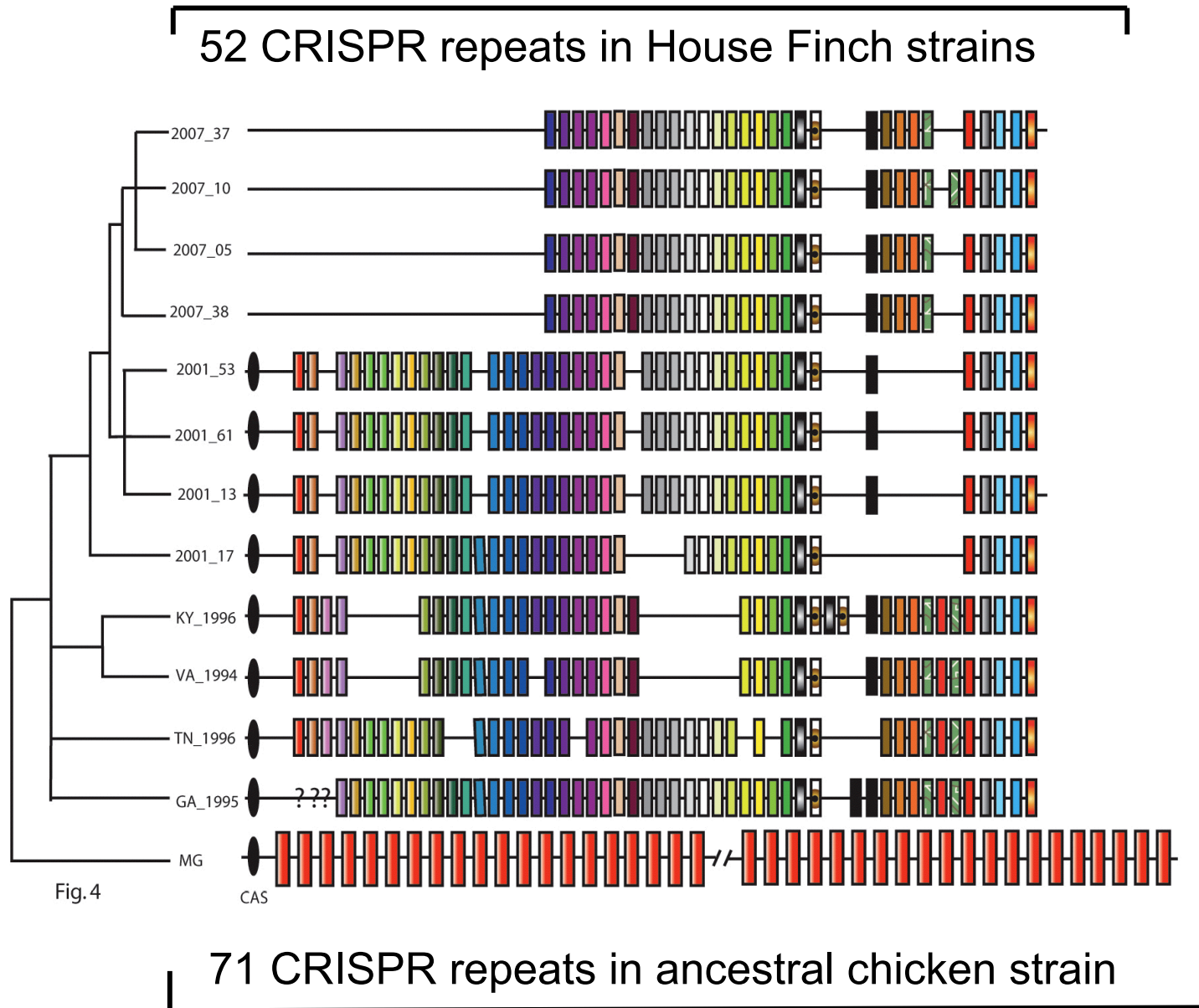
Phylogeny obtained using BEAST, strict clock, 10 million cycles, sampling every 1000 cycles

# CRISPR loci: acquired protection against phage

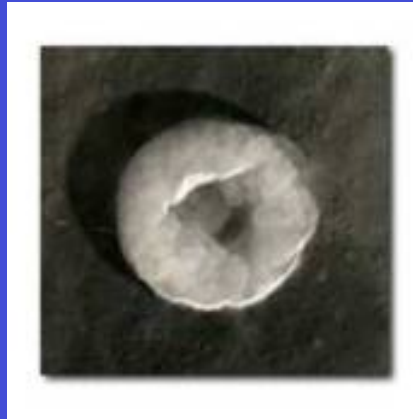
Clustered, interspaced short palindromic repeats



# Rapid evolution of CRISPR loci in *Mycoplasma*

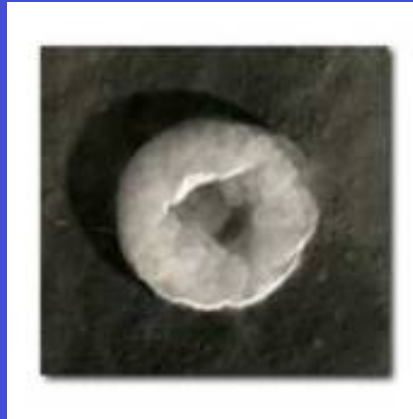


# Host parasite co-evolution in House Finches and *Mycoplasma*:



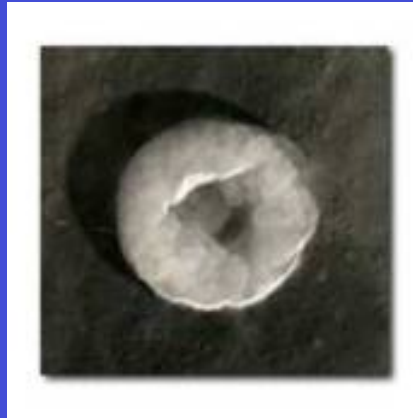
- Evidence for microevolution in house finches and population-dependent expression response to *Mycoplasma* infection

# Host parasite co-evolution in House Finches and *Mycoplasma*:



- Parasite host-switch accompanied by bottleneck, population expansion, genomic deletions and loss of CRISPR system

# Host parasite co-evolution in House Finches and *Mycoplasma*:



- Serial sampling of both host and parasite allows detailed investigations via next generation sequencing approaches

# Acknowledgments

## *House Finch gene expression*

- Camille Bonneaud, Harvard
- Susan Balenger, Geoff Hill, Auburn University

## *Mycoplasma evolution*

- Nigel Delaney, Chris Marx, Harvard
- Allen Rodrigo, Peter Tsai, U. Auckland/NESCent
- Geoff Hill, Auburn University

## *House Finch genomics*

- Niclas Backström, Harvard

## *Funding*

- NSF

