



The application of modern and traditional research approaches to investigate Salmonella susceptibility in chickens

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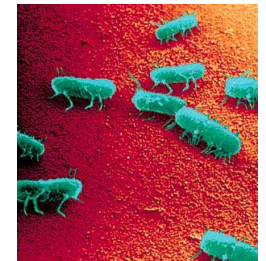
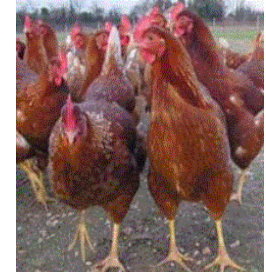
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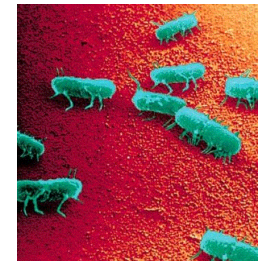
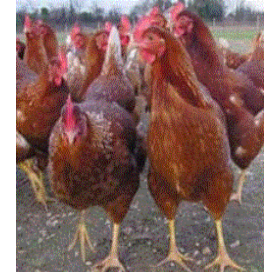
Introduction

- Contamination of poultry products by Salmonella
- Chicken with improved resistance may contribute to solve this problem
- Susceptibility for Salmonella varies between chicken lines
- Host responses to Salmonella are complex
- Mechanisms involved in resistance-associated host responses are unknown



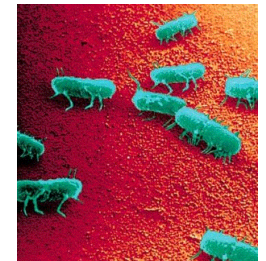
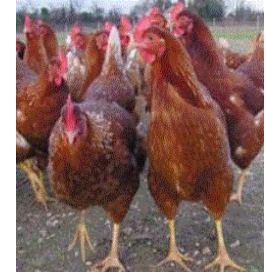
Objective

- Identification of differences in immunological responses of chicken lines that differ in their susceptibility to *Salmonella*
- Identification of other host responses involved in / associated with differences in this susceptibility
- Application of new and traditional techniques
 - Cellular influx and activity
 - Microarray, pathway analysis

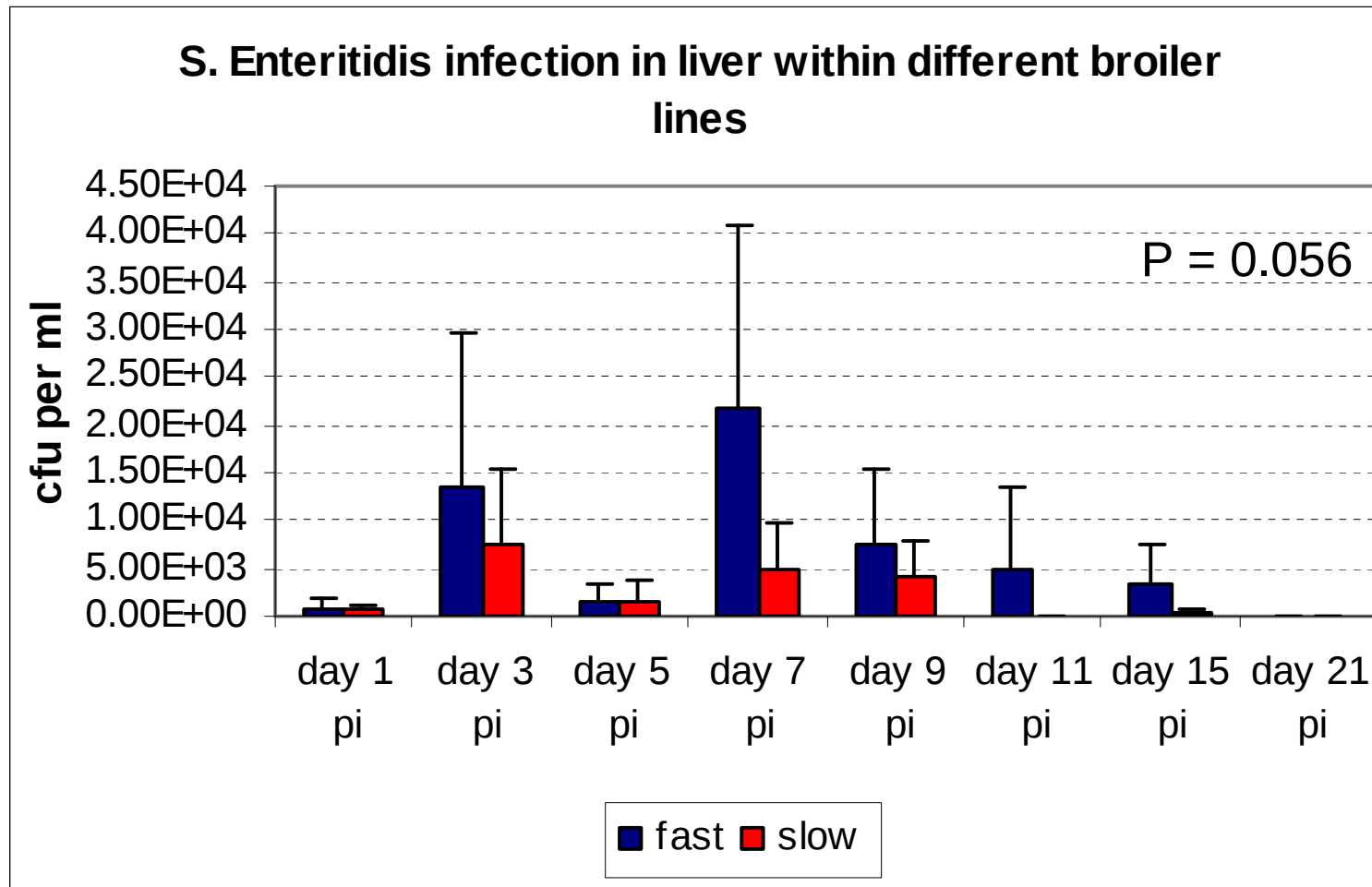


Materials and Methods

- **Two different chicken lines**
 - fast-growing (“highly susceptible”)
 - slow-growing (“less susceptible”)
- **One day-old chickens**
- **Day 1 oral inoculation with 10^5 *Salmonella enteritidis***
 - Colonizing spleen and liver
- **Sampling on 8 time points, day 1 – 21 post infection**
- **Sampling of healthy counterparts**
- **5 animals per day/line/treatment**



Results: Colonization liver

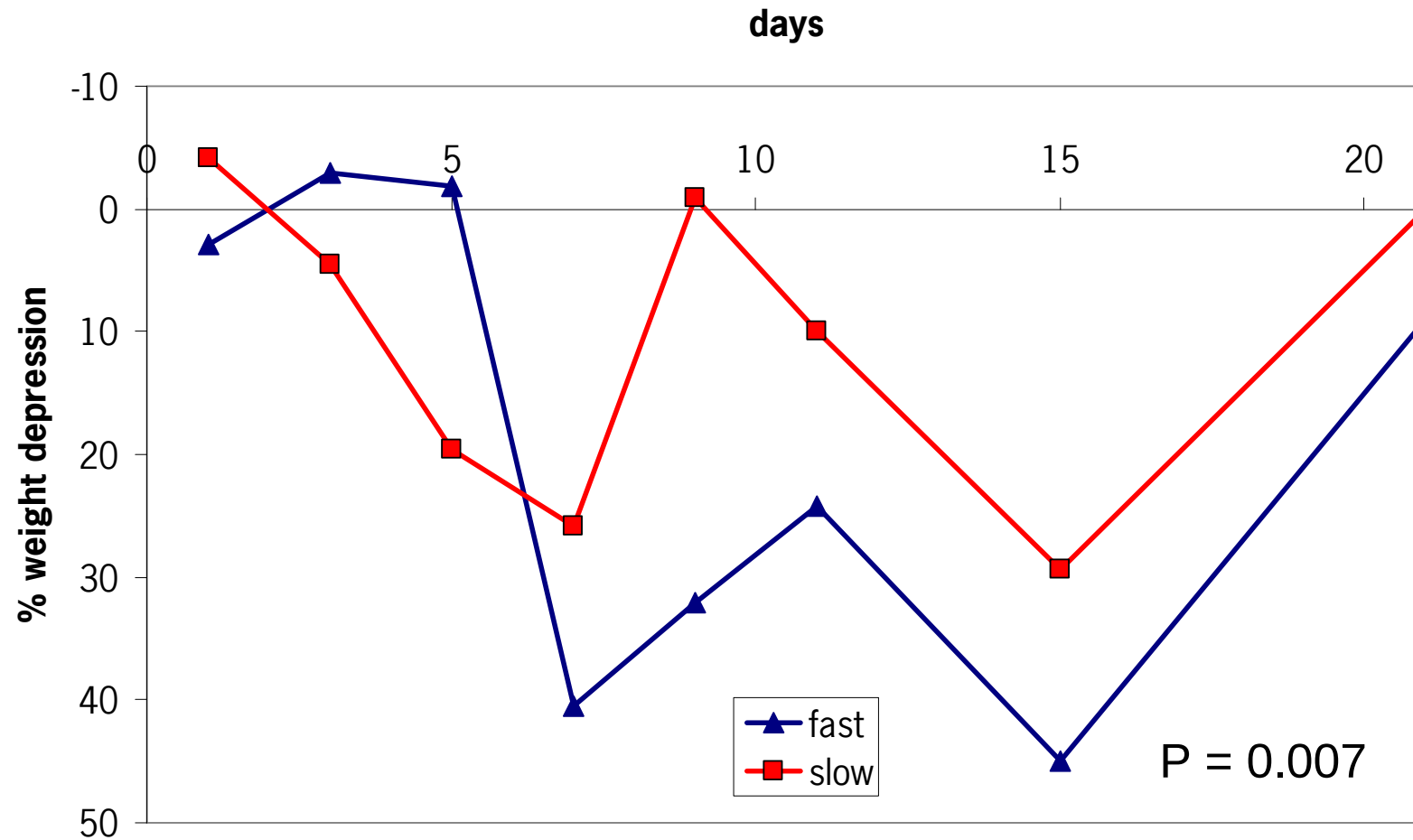


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Results: Grow retardation



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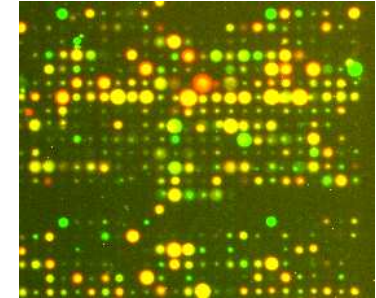
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Conclusion: animal experiment

- The chicken lines differ in Salmonella “susceptibility” as measured by:
 - the number of CFU in the liver
 - the growth retardation

Results: gene expression (1)

- First generation microarrays (4200 cDNAs)
- Fast growing line vs. slow growing line
- Day 1 chicken: control vs. infected
- mRNA from jejunum scrapings (pools)



Upregulated genes in **susceptible** fast-growing chicken line

- Ikaros function in T-cell development
- ZAP-70 role in T-cell receptor signal transduction
- CDH-1 regulation of cell cycle
- GnT-IV upregulated during differentiation/development
- T-cell costimulator expressed after activation of T-cells

Downregulated genes in **susceptible** fast-growing chicken line

- Apolipoprotein B both downregulated in response to
- Cytochrome P450 proinflammatory cytokines

Downregulated genes in **resistant** slow-growing chicken line

- Carboxypeptidase M macrophage differentiation marker
- TNF receptor expressed in macrophages

Conclusion: gene expression (1)

- In the jejunum of the “susceptible” fast-growing chicken line, compared to the “resistant” slow-growing line, more genes were regulated in response to Salmonella that affect T-cell activation
- In the jejunum of the “resistant” slow-growing chicken line, compared to the “susceptible” slow-growing line, more genes were regulated in response to Salmonella that are related to macrophage activation

Results: Immunological tests day 1

	Line	Control	Infected
CD4 ⁺ T-cells	Fast	14 ($\pm$ 2) ^a	8 ($\pm$ 1)
	Slow	12 ($\pm$ 2)	16 ($\pm$ 2)
CD8 ⁺ T-cells	Fast	38 ($\pm$ 4)	57 ($\pm$ 8)
	Slow	32 ($\pm$ 6)	26 ($\pm$ 3)
Macrophages	Fast	226 ($\pm$ 14)	241($\pm$ 17)
	Slow	213 ($\pm$ 14) ^a	124 ($\pm$ 8)

Macrophage activity	Fast	9 ($\pm$ 5) ^a	64 ($\pm$ 8)
	Slow	33 ($\pm$ 10)	48 ($\pm$ 8)

a: P < 0.05



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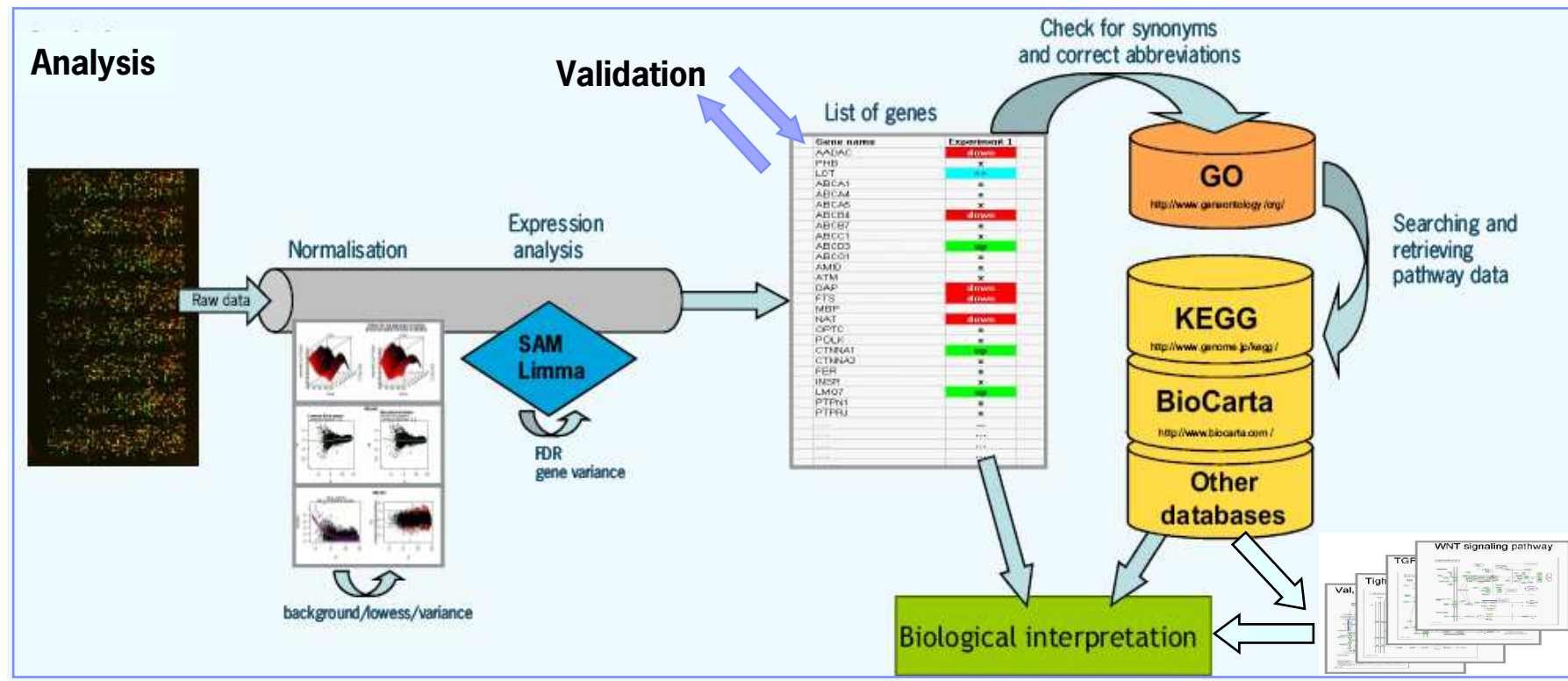
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Conclusion: Immunological tests day 1

- In the jejunum of the “susceptible” fast-growing chicken line T-cell-associated immune responses were regulated in response to Salmonella
- In the jejunum of the more “resistant” slow-growing chicken line macrophage-associated immune responses were regulated in response to Salmonella. Under control conditions the activity of macrophages is higher in the slow-growing line
- There is a clear difference in host immunological response between the two chicken lines
- Results are in agreement with the conclusions of the gene expression data

Results: gene expression (2)

- Second generation microarrays (38000 features)
- Moderate overlap with earlier results
- Much more gene expression differences between the chicken lines -> pathway analysis



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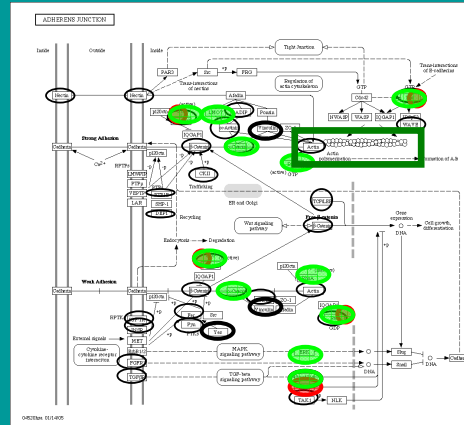


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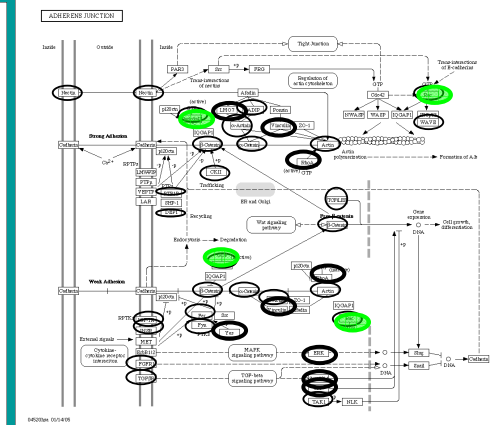
Results: example pathway analysis

Adherence junction pathway

Control vs infected

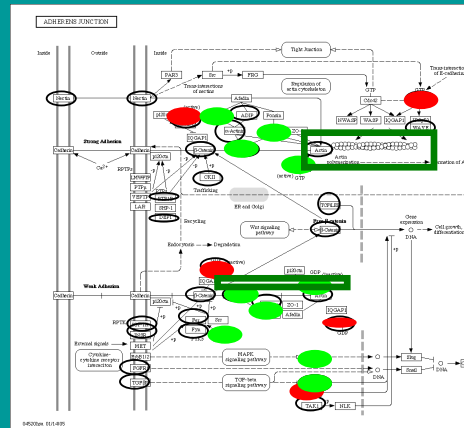


Fast-growing

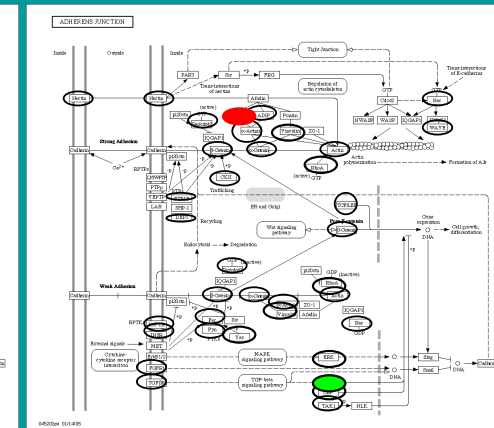


Slow-growing

Slow vs. Fast



Control



Infected

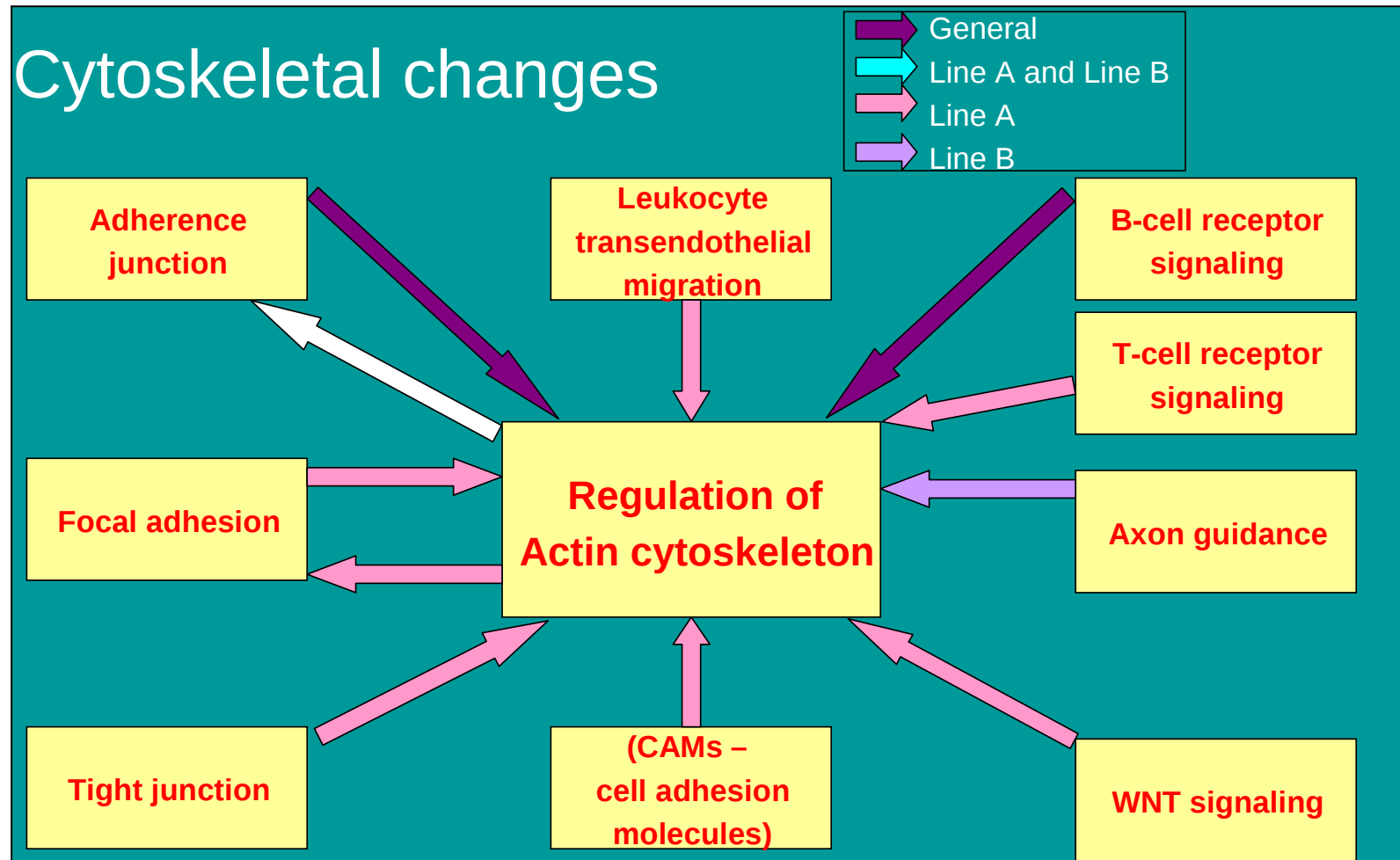


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Results: networks of pathways



Te Pas et al. Advances in Bioinformatics Volume 2008 (2008), Article ID 719468

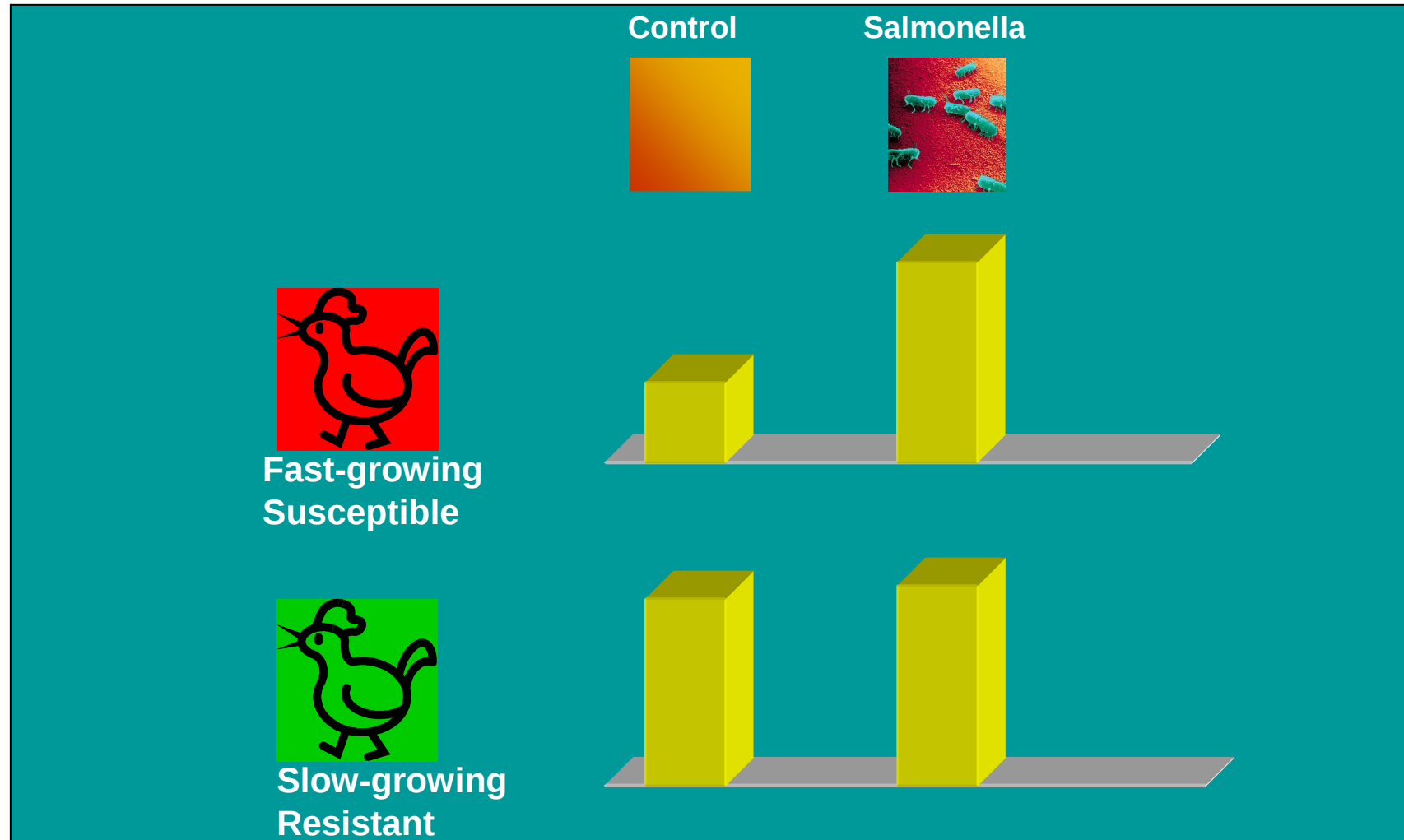


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Conclusion: networks of pathways



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General conclusions

- The two chicken lines differ in their immunological response to Salmonella
- The two chicken lines differ in their response to Salmonella in many other (non-immunologic) pathways or networks of pathways
- Data generated with new and traditional techniques are confirmatory and complementary
 - Integration functional and positional information
- Different selection backgrounds are accompanied with differences in basal expression levels of a number of pathways

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