Emerging Science for Environmental Health Sciences: The microbiome

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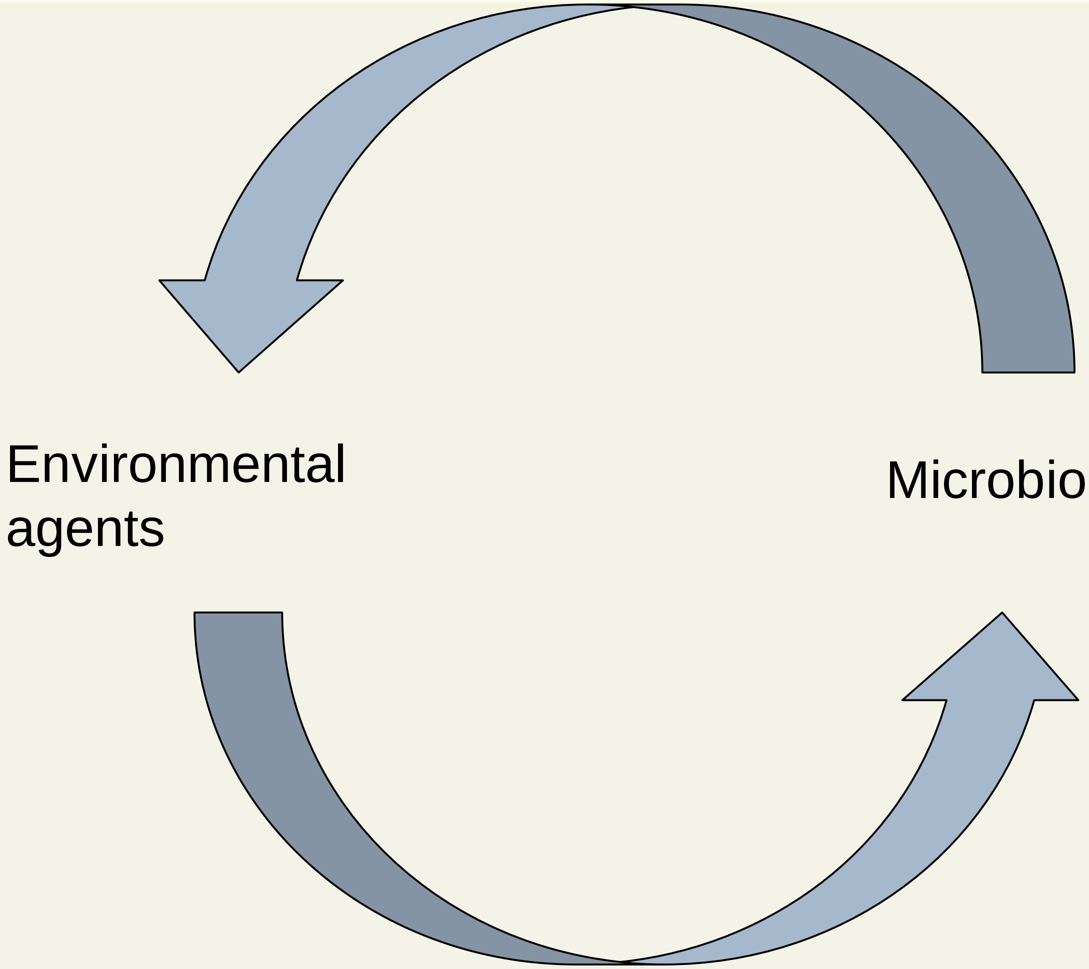
Many ways of thinking about the interactive roles of the microbiome in environmental health

- Ecosystem microbiomes mediate fate and transport of environmental toxicants
- Host microbiomes participate in metabolism and toxicokinetics
- Ecosystem and host microbiomes are targets for environmental toxicants (antimicrobials as envrionmental contaminants)
- Horizontal gene transfer among microbes is responsive to environmental toxicants (resistance genes as environmental contaminants)
- Host microbiomes are involved in acute and chronic diseases

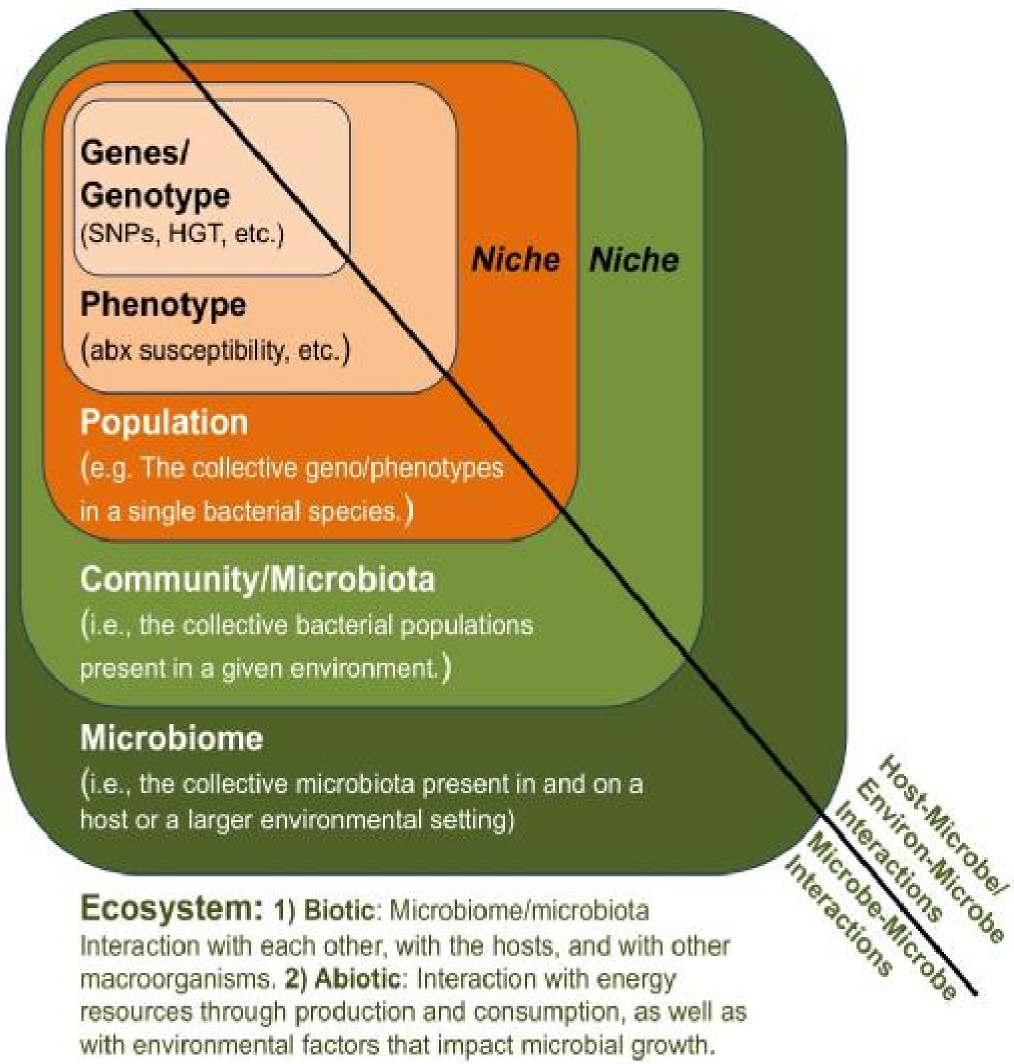
Mutuality of Environmental Agents and the Microbiome



Many interesting new perspectives

- It’s evolution, stupid
- The 11th organ
- “mutualism with a cost”
- Developmental microbiomics

We didn't discuss as much: The ecological perspective: not just for the landscape

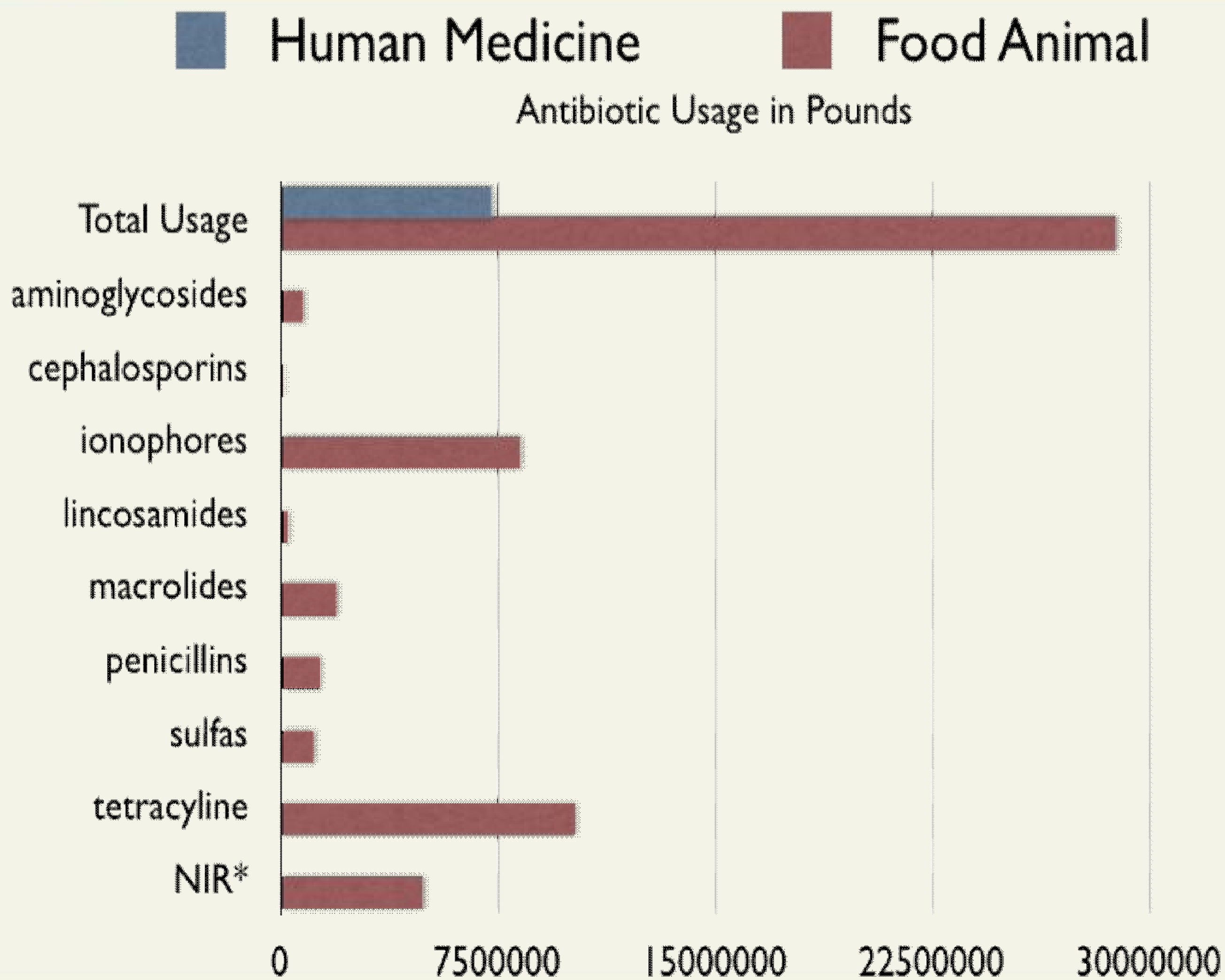


Language and definitions: we choose words by where we start

- The problem of antimicrobial resistance
  - Not just a problem of good drugs/bad bugs
  - Not even just a problem of the microbiome
- *Maybe studying the microbiome (as organisms) is not enough?*
- Locating the resistome
  - The microbiome and cloud computing
- Evolution
  - The long view
  - The crisis view

Influential experiences: the hospital, evolutionary biology – or, the modern livestock farm

Willie Sutton and the laboratory of microbial evolution: where are the antimicrobials?



Multidrug resistant *E coli* from domestic animals (Lindsay et al MDR 2011)

2006	Chicken	Aug Amp Fox Gen Str Smx Tet
2005	Swine	Aug Amp Fox Tio Tet
2005	Chicken	Aug Amp Fox Tio Smx Tet
2007	Chicken	Aug Amp Fox Tio Str Tet
2005	Chicken	Amp Fox Tio Chl Str Smx Tet
2005	Swine	Aug Amp Fox Tio Str Tet
2005	Cat	Aug Amp Fox Tio Cip Nal Tet
2005	Dog	Aug Amp Fox Tio Cip Nal Tet
2007	Dairy cattle	Aug Amp Fox Chl Kan Str Smx Tet
2005	Swine	Aug Amp Fox Tio Smx Tet
2005	Chicken	Aug Amp Fox Tio Gen Str Smx Tet
2007	Dairy cattle	Aug Amp Fox Tio Chl Gen Str Smx Tet
2006	Dog	Aug Amp Fox Tio Chl Nal Smx Tet Cot
2005	Dog	Aug Amp Fox Tio Axo Cip Nal Str Smx Tet
2007	Dairy cattle	Aug Amp Fox Tio Chl Kan Str Smx Tet Cot
2005	Dog	Aug Amp Fox Tio Cip Gen Nal Str Smx Tet
2005	Cattle	Aug Amp Fox Tio Chl Cip Gen Nal Str Smx Tet
2007	Chicken	Aug Amp Fox Tio Chl Gen Kan Nal Str Smx Tet

Dreaming about the AR microbiome of the chicken gut

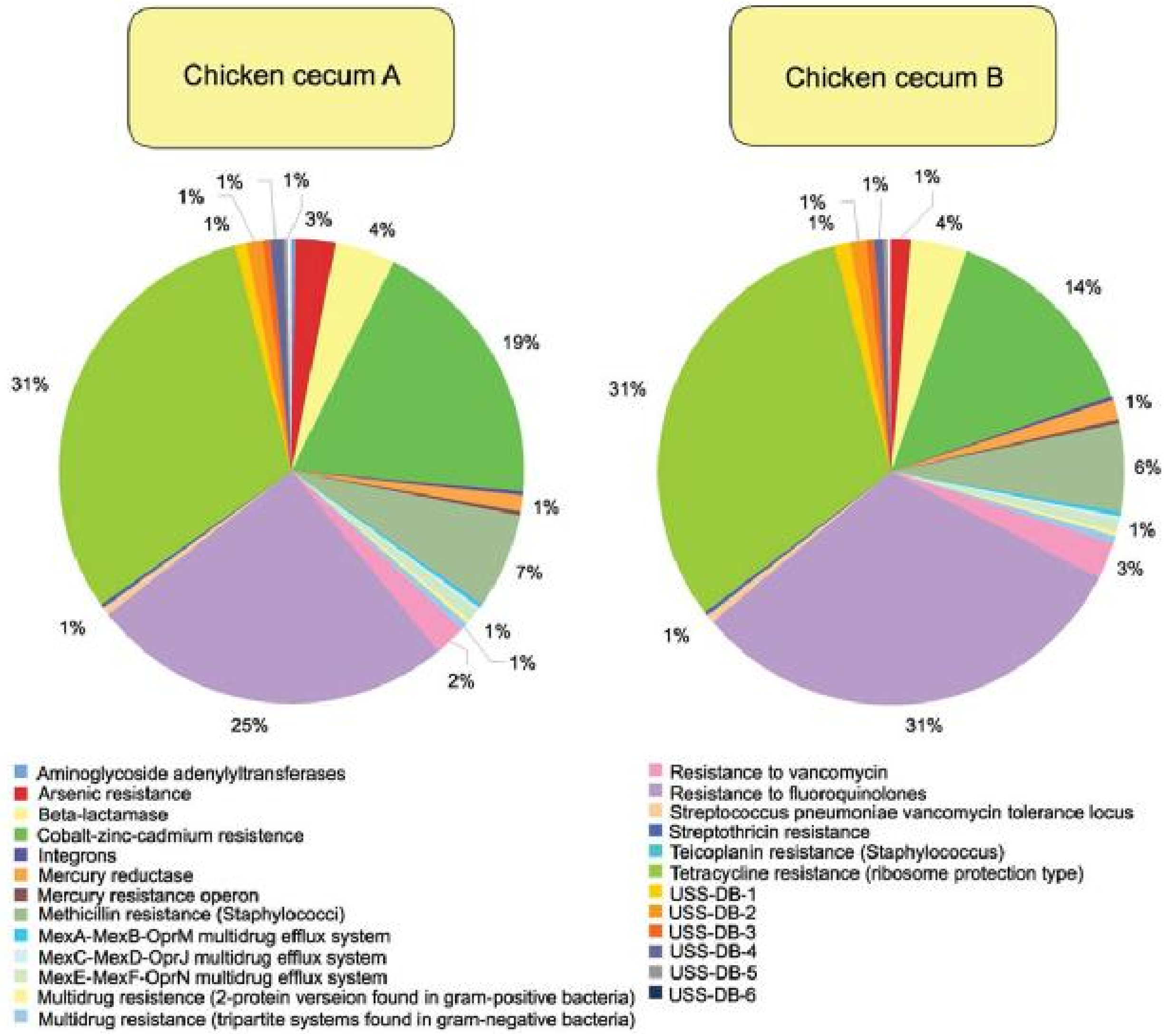
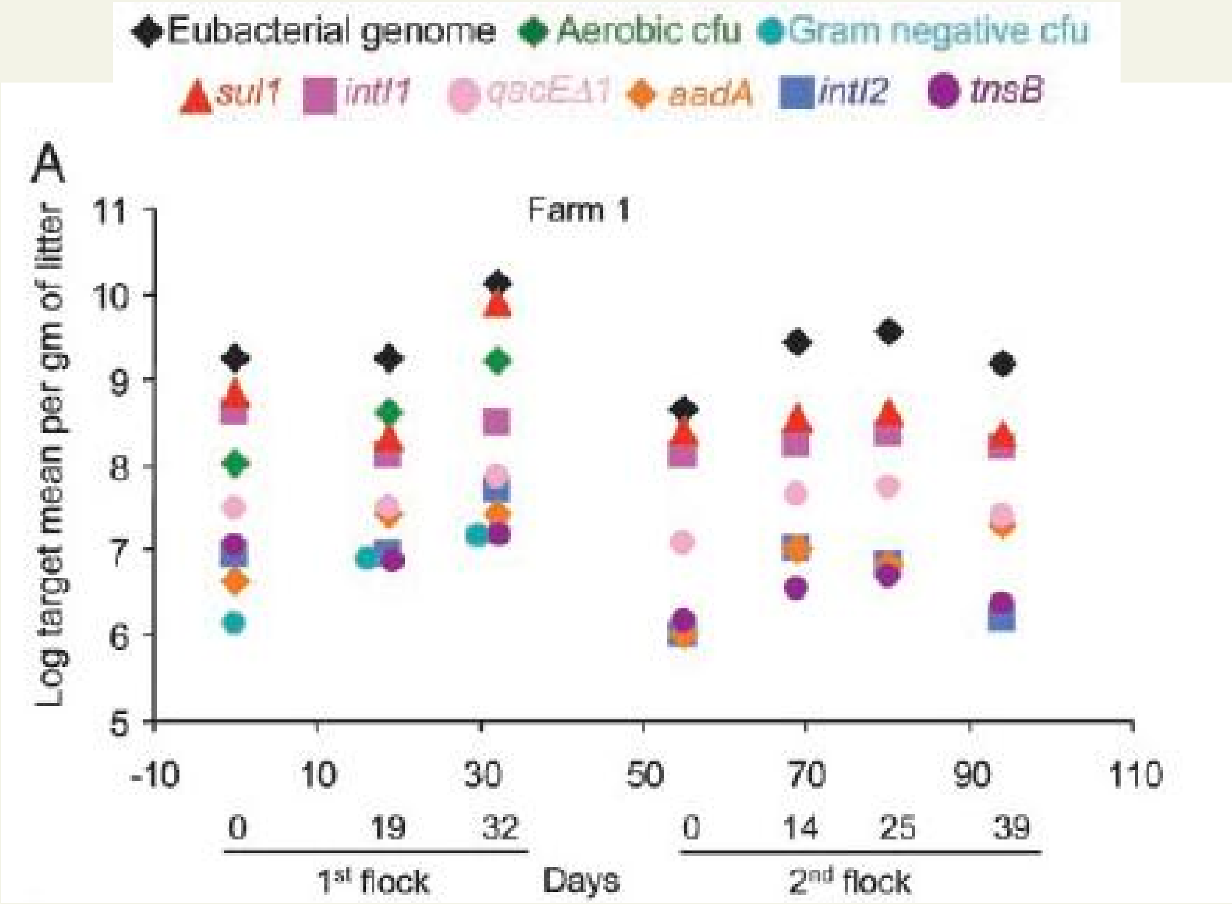


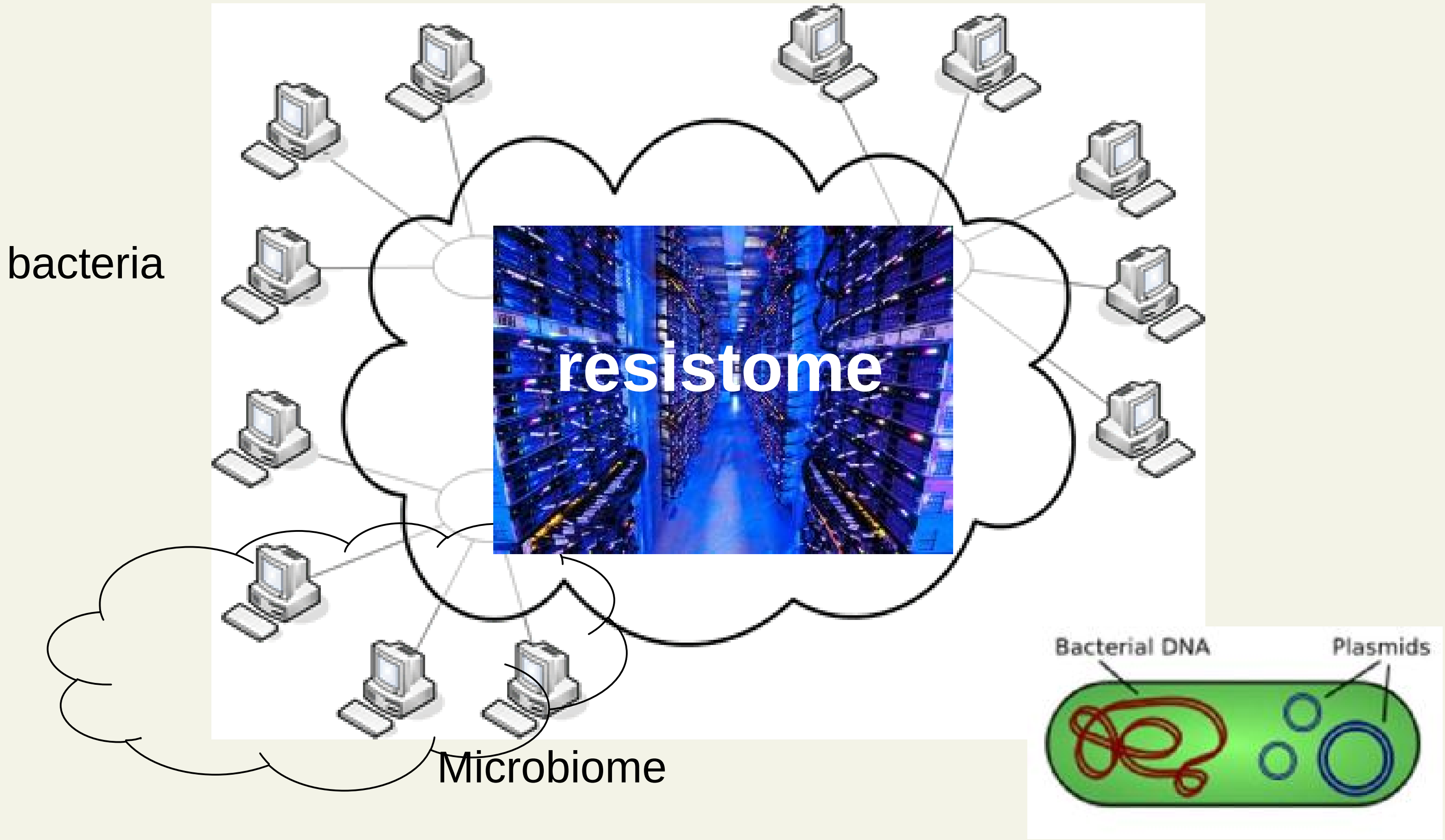
Figure 6. Resistance to antibiotics and toxic compounds subsystem composition of chicken cecum A and B microbiomes is shown. The percent of environmental gene tags (EGTs) in each of the Resistance to antibiotics and toxic compounds subsystems from the chicken cecum A and B microbiomes is shown. The BLASTX cutoff for EGTs is 1×10^{-5}.
doi:10.1371/journal.pone.0002945.g006

Building the resistome: resistance genes move from poultry litter into soil

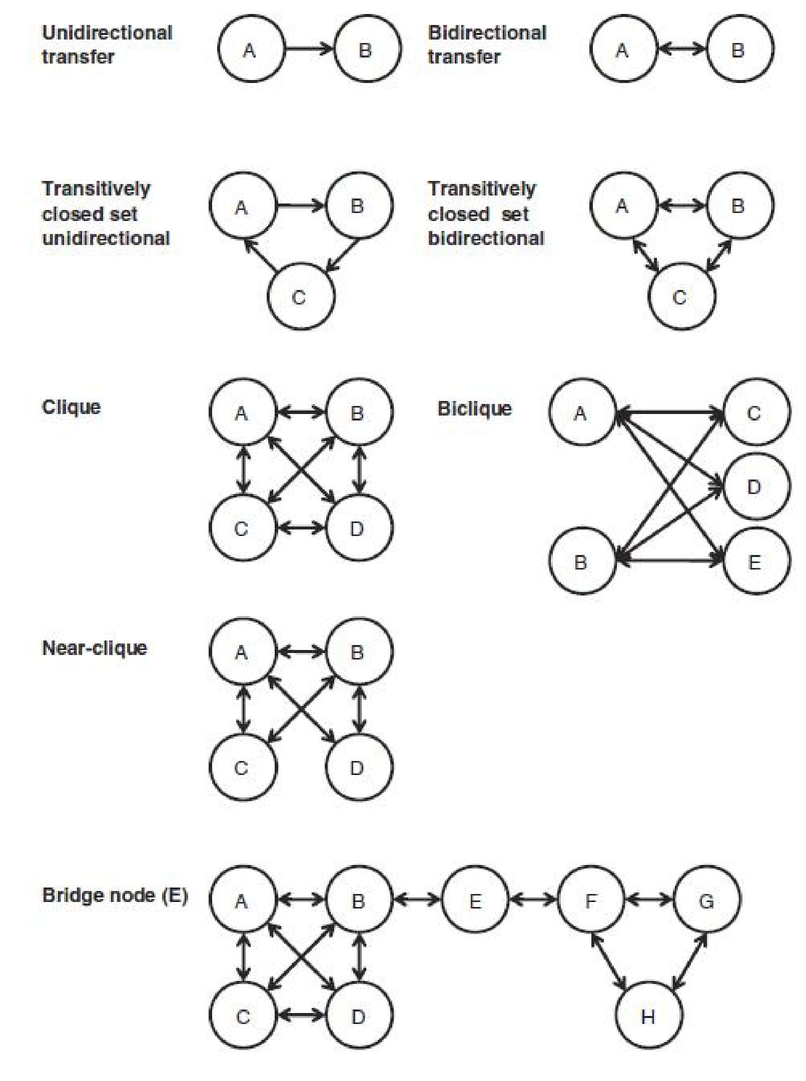


Nandi et al (2004) PNAS 101:7108

The resistome as cloud computing

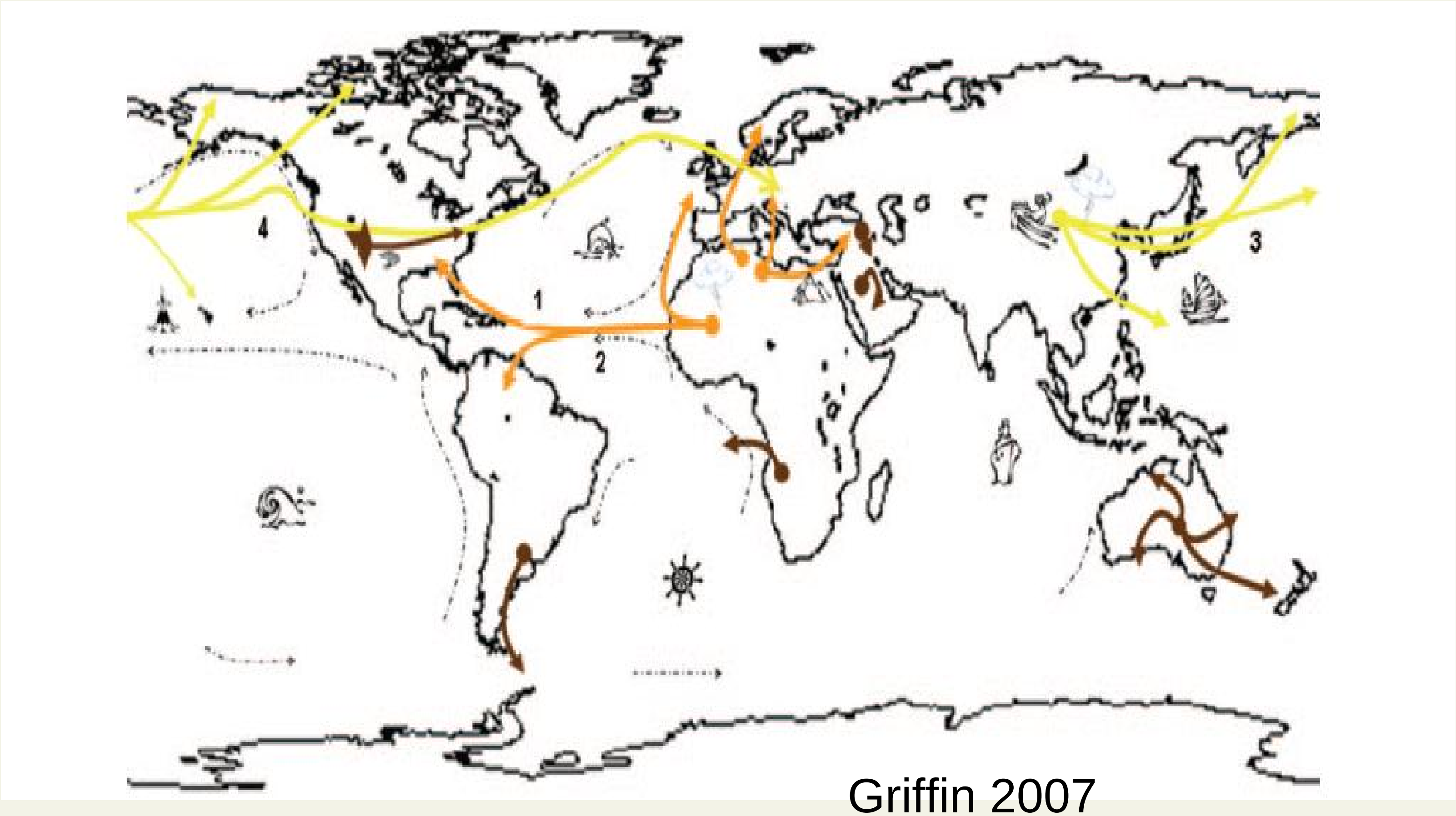


“Genetic exchange communities” (Skippington and Ragan FEMS Micro Reviews 2011



- A collection of organisms that can share genes by HGT
- Not necessarily in close physical proximity (shared ecologies?)
- Not all microbes are equal – cliques and frenemies

Global movement of dusts (and bacteria)



Griffin 2007

Are we living in a new stage of animal:human:microbial evolution?

- Massive deployment of natural products as antimicrobial agents
  - Concentration
  - Inappropriate uses
- Rapid increases in plasmids
  - Complex multigene cassettes
  - Expansion of the resistome
- Increased importance of horizontal gene transfer as a driver of microbial evolution

The interactive roles of the microbiome in environmental health

- Ecosystem microbiomes mediate fate and transport of toxicants in the environment
- Host microbiomes participate in host metabolism and uptake of toxicants
- Both ecosystem and host microbiomes are targets for some environmental toxicants
- Horizontal gene transfer among microbes is responsive to environmental toxicants
- Host microbiomes are involved in acute and chronic diseases

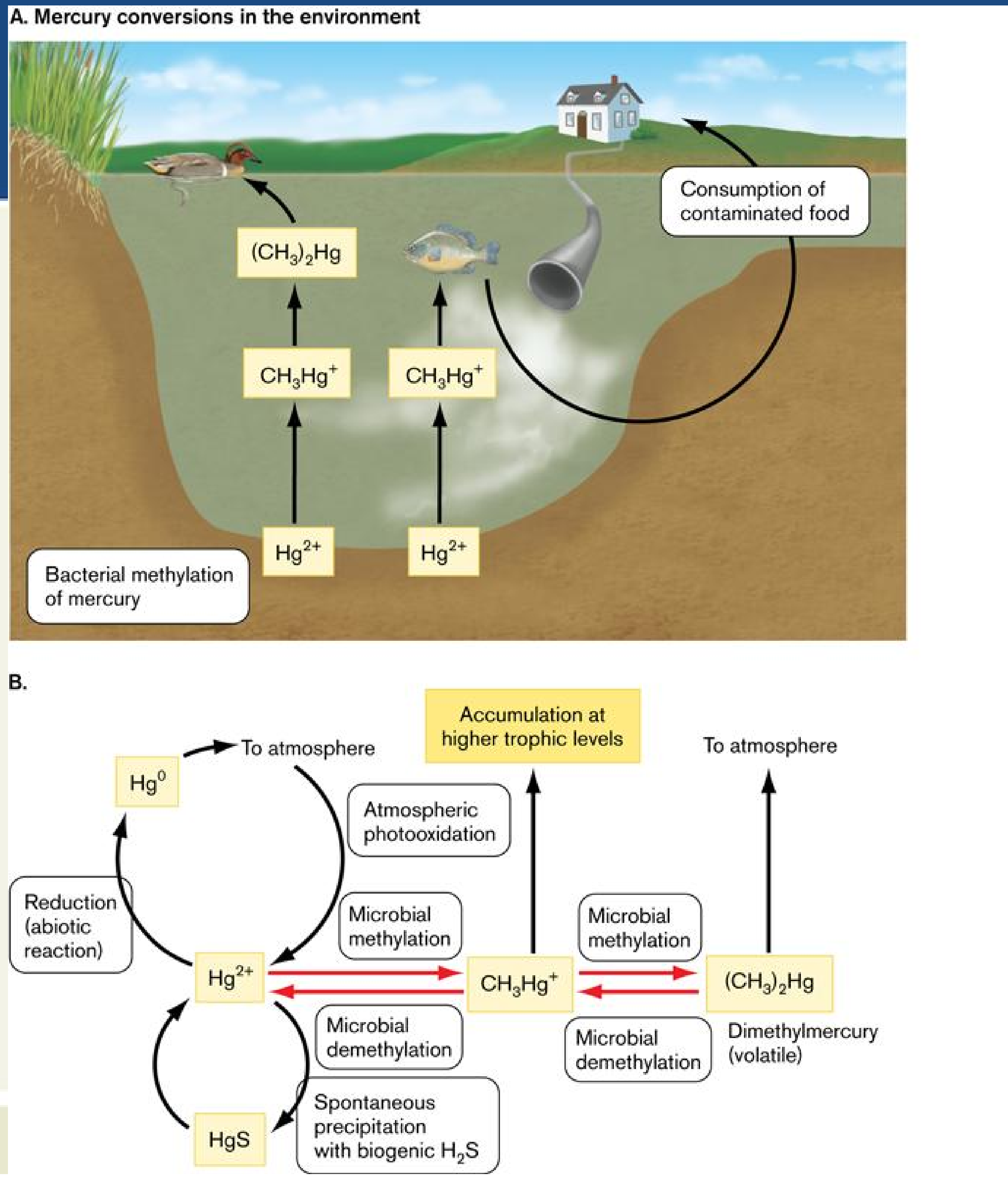
Putting the microbiome into toxicology: the example of mercury

- Mercury is a major environmental health risk
- Environmental fate and transport of mercury is regulated by microbial metabolism
- Ecological food web transfers are dependent on microbial transformation
- Human toxicity varies with mercury species

Mercury and the microbiome

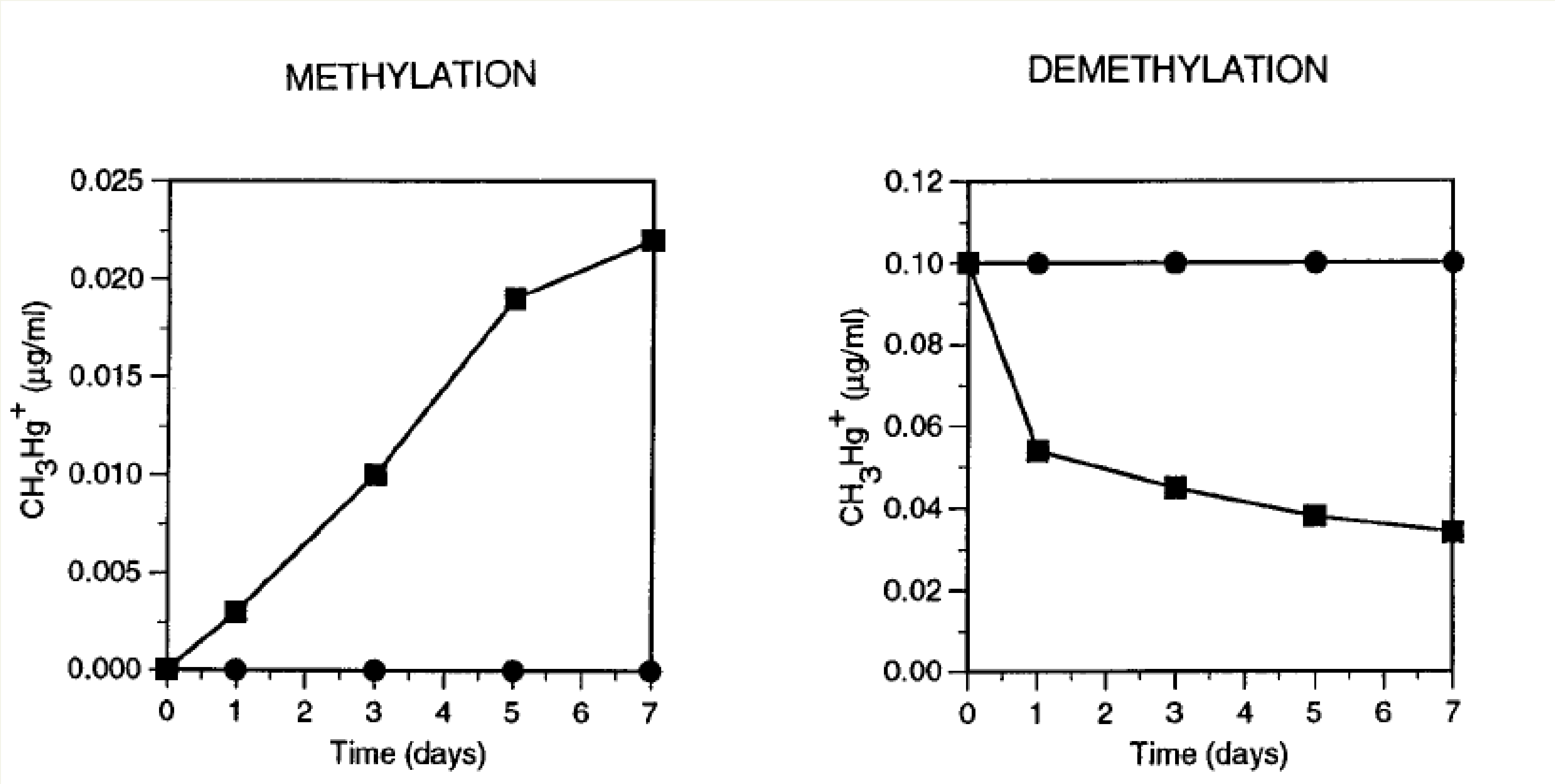
- What you probably know:
  - Mercury is biotransformed in ecosystems by bacteria
  - Methylated mercury is more readily bioaccumulated and bioconcentration within ecological food webs
  - Methylmercury is one of the most widespread environmental toxicants
    - Exposures
    - Health impacts

The mercury cycle



Slonczewski and Foster Microbiology

Mercury methylation and demethylation by *Desulfovibrio desulfuricans* (and at least 8 other bacteria)



Macrocosm studies --Tan and Bartha (1998) AEM

Mercury and the microbiome

- What you probably didn’t know:
  - Mercury is biotransformed by the gut microbiome
    - iHg **à** MeHg **à** iHg
  - Inorganic mercury as also highly toxic
  - Inorganic mercury affects the gut microbiome through mucosal immune system (mast cells)
  - Mercury affects the environmental microbiome by driving antimicrobial resistance through horizontal gene transfer

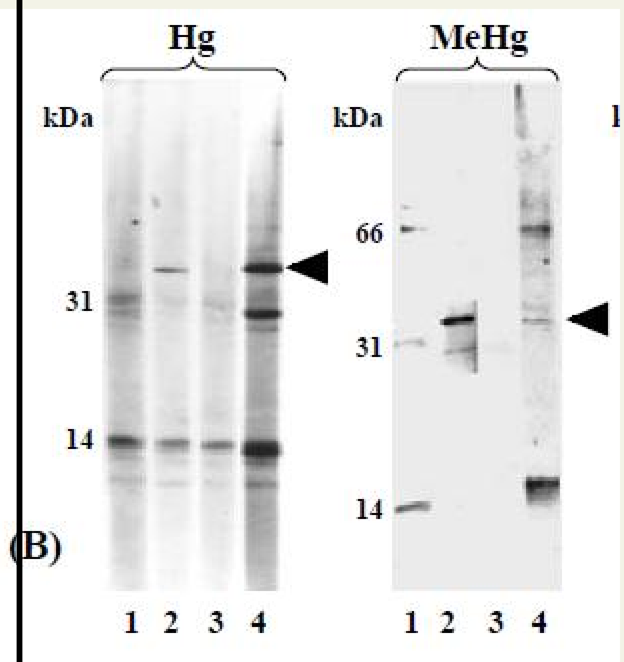
Mercury species and immunotoxicity

- iHg is more potent than MeHg in
  - Inducing autoimmune biomarkers and phenotypes (murine)
  - Inhibiting host response to malaria (murine)
  - Increasing circulating autoantibodies (human)
  - Altering cytokine production by PBMCs *in vitro* (human)

(Silbergeld et al 2000; Silva et al 2004; Gardner et al 2010). Havaranisab et al 2007)

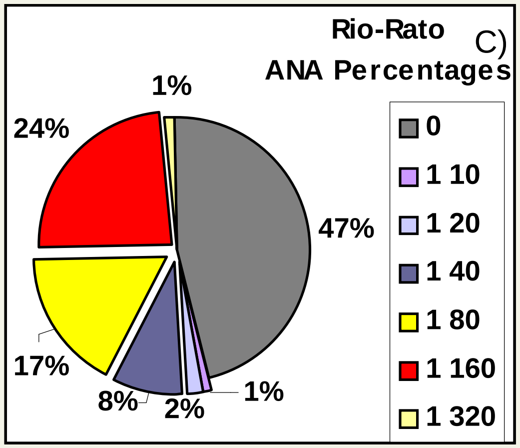
Relative potency of iHg and MeHg in A.SW mice (Havarinasab et al 2005)

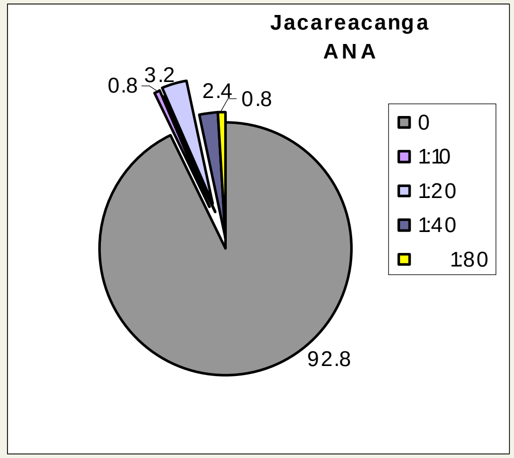
Immune parameter	$HgCl_2$	MeHg
Immunosuppression	±0[a]	+[b]
Serum antinucleolar/antifibrillarin antibodies	+++[a]	+[b]
Polyclonal B-cell activation	+++[a]	±0[b]
Splenic B-lymphocytes	++[a]	+[b]
Splenic T-lymphocytes	+++[a]	+[b]
Total serum IgG1 concentration	+++[a]	+[b]
Total serum IgG2a concentration	+++[a]	±0[b]
Total serum IgE concentration	+++[a]	+[b]
Immune-complex deposits		
Glomerular deposits	++[a]	±0[b]
Systemic vessel walls deposits	++[a]	±0[b]

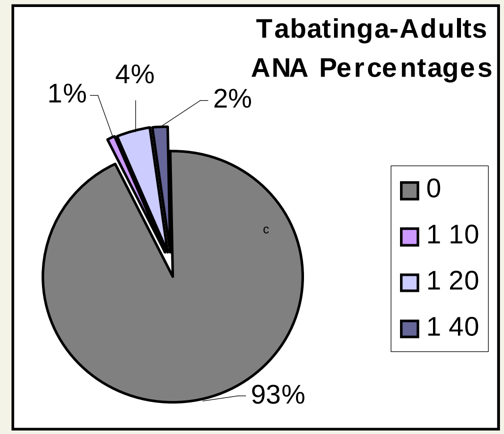


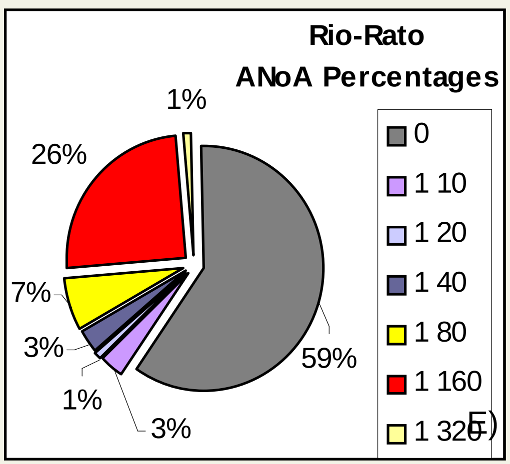
IFA analysis of intranuclear autoantibodies: Sera from mice exposed to iHg or MeHg. Arrow denotes antibrillarin

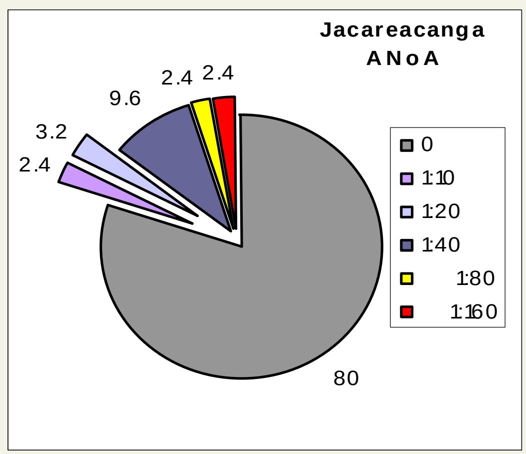
Biomarkers of autoimmuity (ANA, ANOA) in persons exposed to inorganic Hg compared to MeHg (Silva et al 2004)

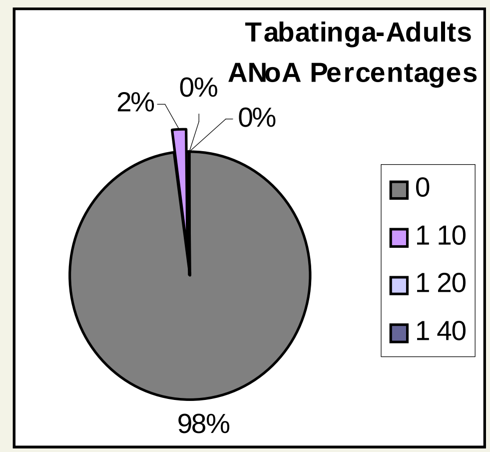










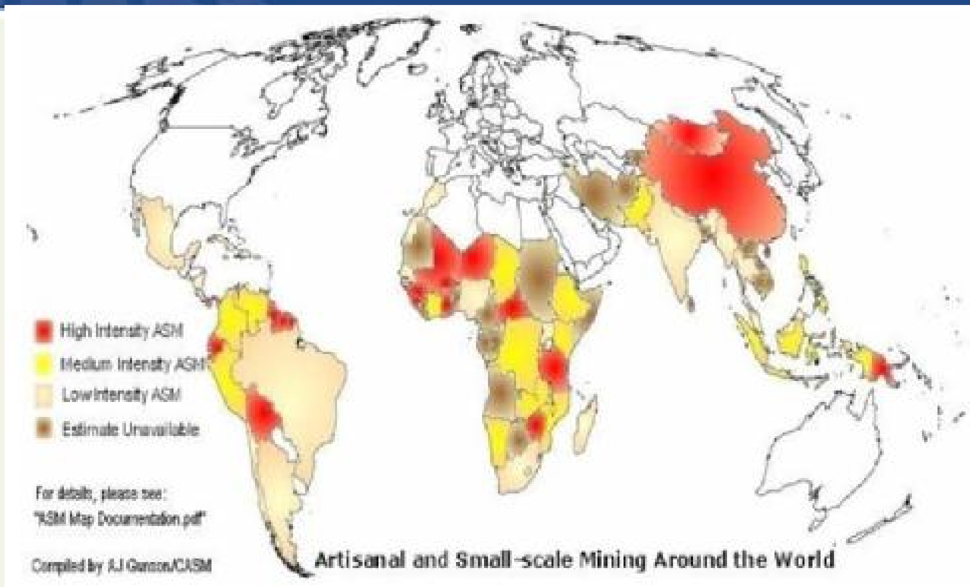


iHg – miners

MeHg – fish eaters

reference

Millions are exposed to inorganic mercury in small scale gold mining (WHO 2011)

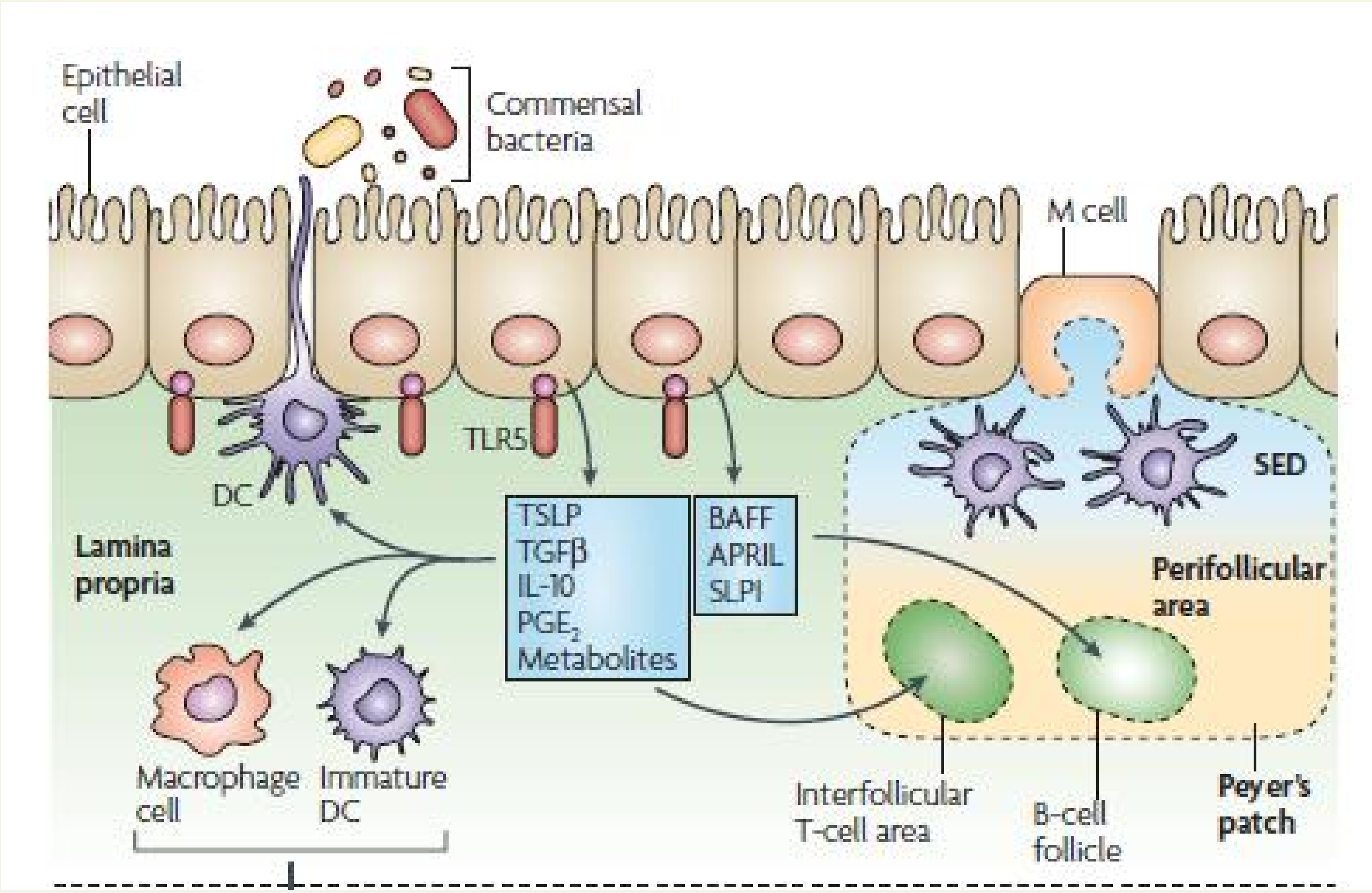


And maybe the rest of us are internally exposed after gut demethylation?

Mercury exposures in children living near or working in small scale gold mining

Urine Hg (µg/l)###		***	***
N	50	36	80
Mean ± S.D.	0.58 ± 0.54	10.20 ± 12.40	47.35 ± 146.35
Median	0.40	6.49	10.05
Minimum	<0.20	0.35	0.29
Maximum	2.15	70.53	941.89
95% percentile	1.88	29.87	203.54

Mercury and the gut microbiome – impacts on bacteria (Summers 1999) and on the mucosal immune system (TLR4 and 5 signaling; macrophages, microglia)

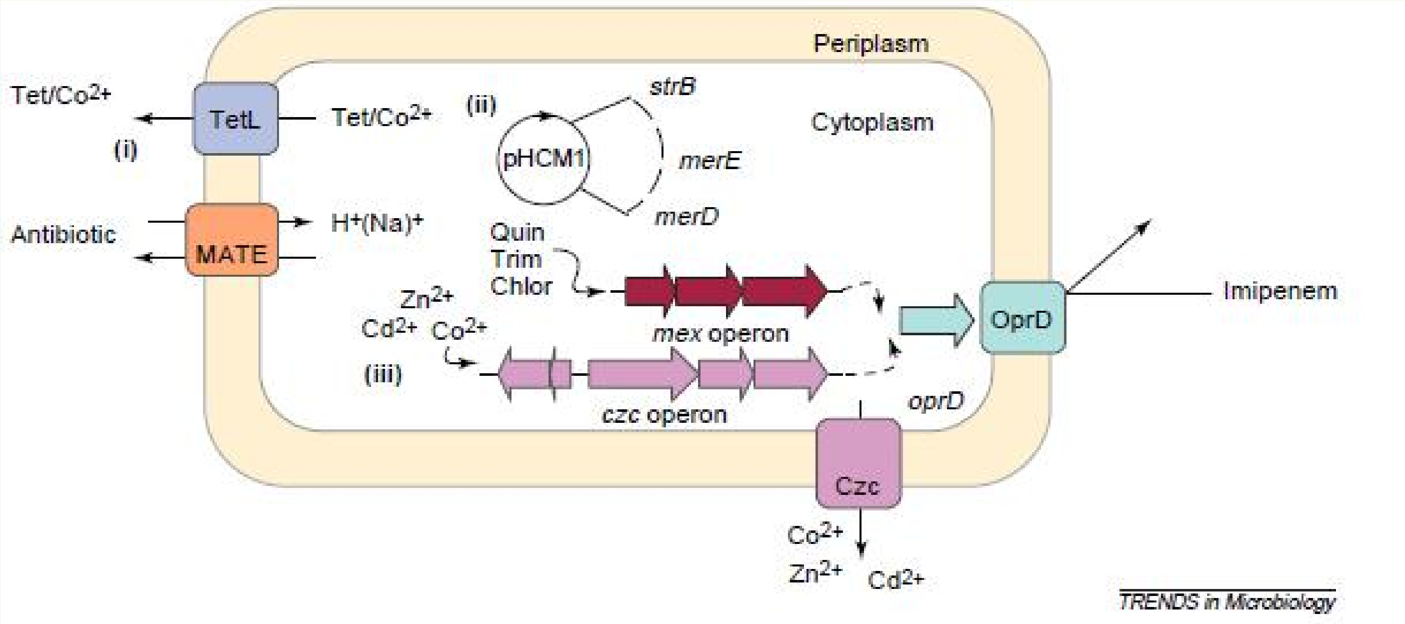


Also effects on gut microbiome will affect Hg kinetics

Mercury as a driver of antimicrobial resistance in bacteria and in the resistome

- Cross – resistance
- Co-selection

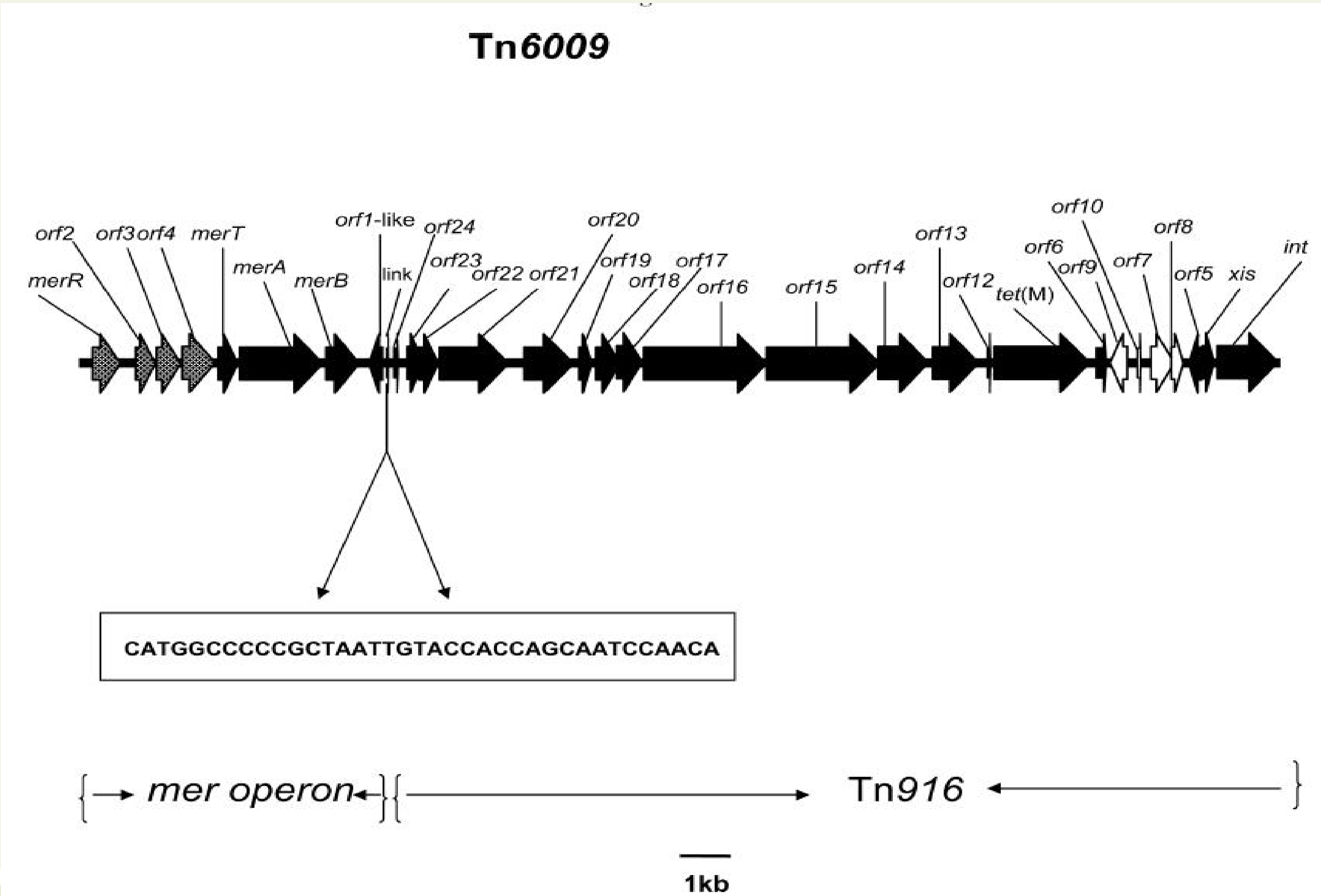
Metal and antimicrobial co-resistance by shared mechanism



Resistance mechanism	Metal ions	Antibiotics
Reduction in permeability[b]	As, Cu, Zn, Mn, Co, Ag	Cip, Tet, Chlor, ß-lactams
Drug and metal alteration[c]	As, Hg	ß-lactams, Chlor
Drug and metal efflux[d]	Cu, Co, Zn, Cd, Ni, As	Tet, Chlor, ß-lactams
Alteration of cellular target(s)[e]	Hg, Zn, Cu	Cip, ß-lactams, Trim, Rif
Drug and metal sequestration[f]	Zn, Cd, Cu	CouA

Shared mechanisms of resistance Baker-Austin et al 2006

Bacterial resistance by co-selection: mercury resistance genes (iHg and MeHg) along with tet(M) in transposon from *S aureus* (Soge et al 2008)

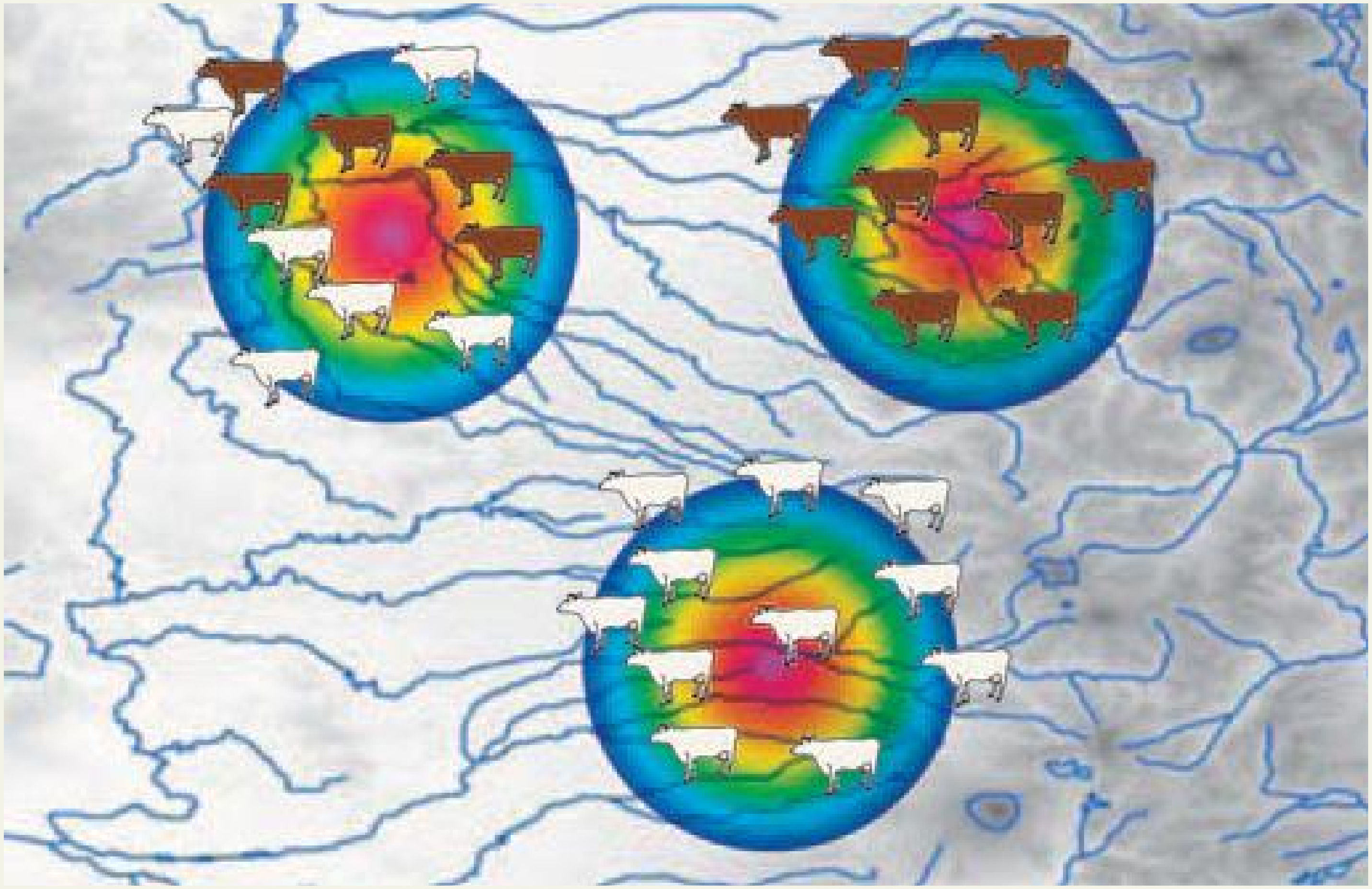


Mercury exposure increases risks of carrying drug resistant *E coli* in French Guyana (Skurnik et al 2010)

Population	Exposure	carriage of *mer* or AMR genes
• Wayampi (indigenous)	low AB high Hg	high *merA* and AMR
• Europeans	high AB low Hg	lower *merA* and lower AMR

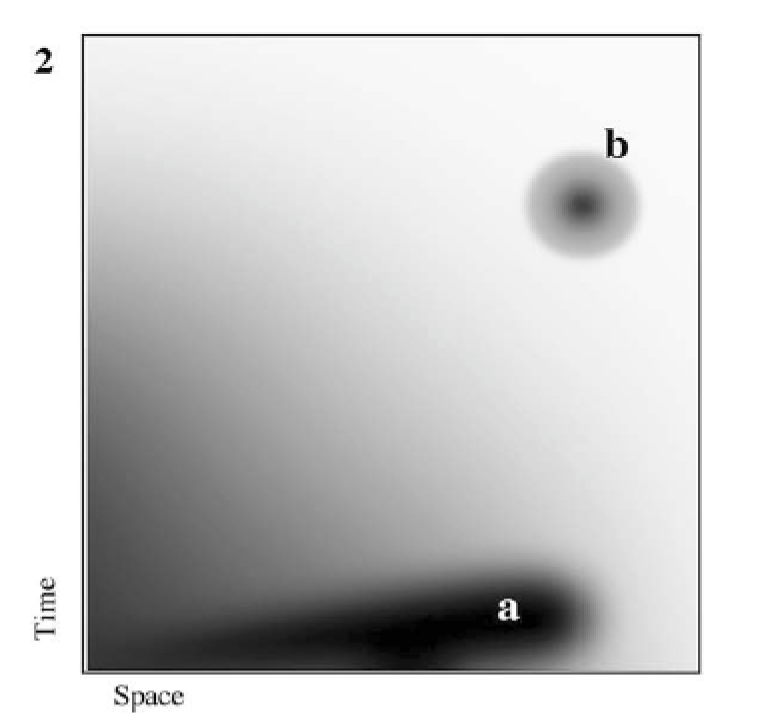
- **Wayampi and Europeans with *merA* more likely to carry transposons with multidrug AMR gene cassettes**

Do we need to map metal contamination and antibiotic pressure to understand resistance? (Singer et al 2006)



Antibiotics and antibiotic resistance genes as environmental pollutants (Martinez 2009)

- Antibiotics are mostly short lived in the environment but may be continuously added
- Antibiotic resistance genes are more stable
- AR genes may be “bioaccumulated” by microbiomes and persistent within the microbiome
  - The “cost of resistance” can be compensated for
  - Maintaining the resistome is prudent in a (human-made) sea of antimicrobial pressures



Does this make a difference?

- Understanding environmental fate and transformation
- Rethinking toxicokinetics and biomarkers
- Adding a dimension to individual susceptibility
- Restoring immunology and evolution to environmental health sciences

Can we unite the disciplines?

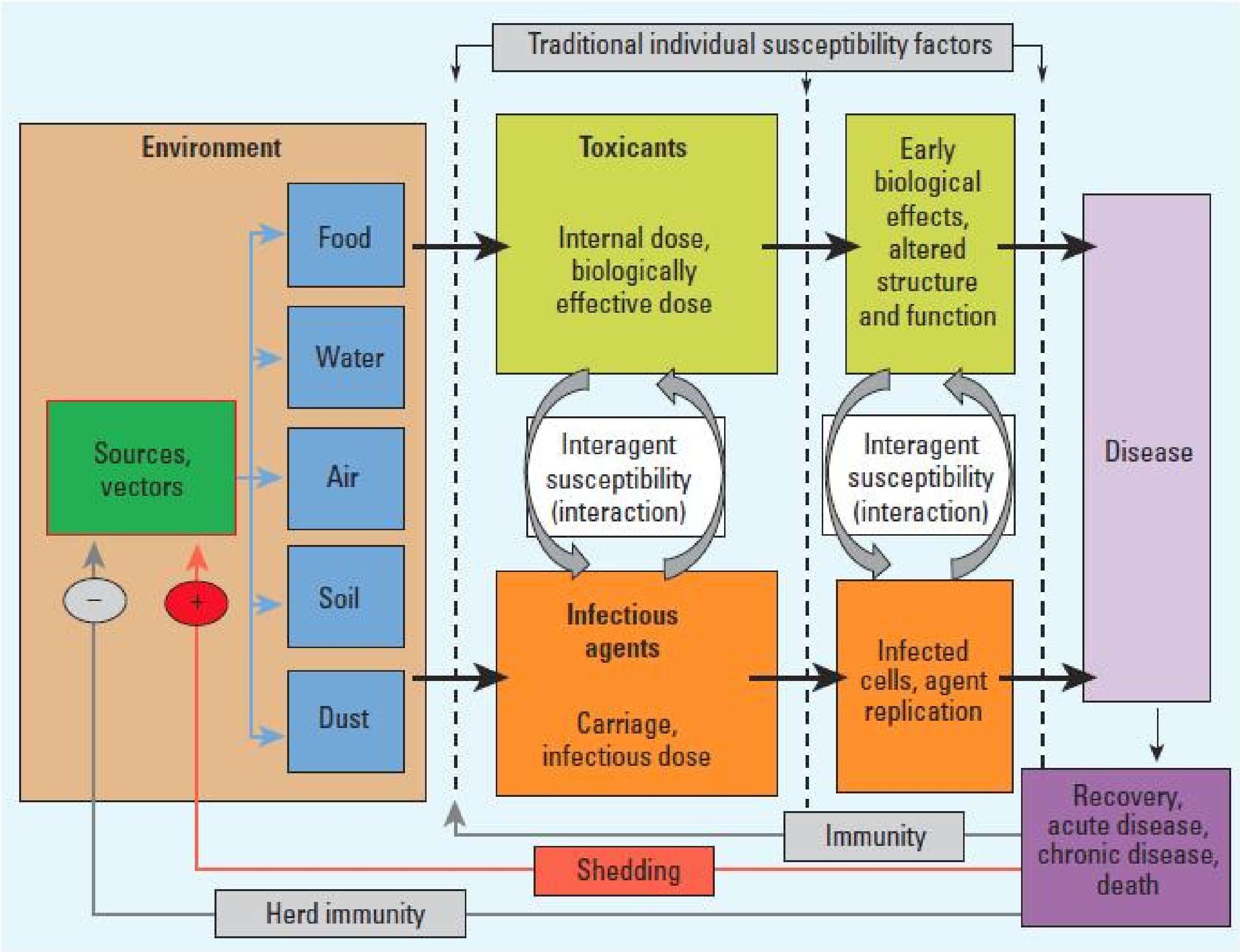


Figure 1. Integrated toxicological–pathogen conceptual paradigm for disease etiology.

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