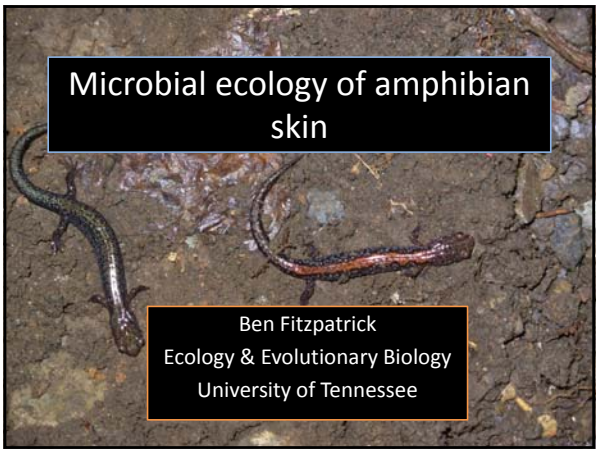

Microbial ecology of amphibian skin
Ben Fitzpatrick
Ecology & Evolutionary Biology
University of Tennessee


Microbial ecology of amphibian skin
• The environment
• Cutaneous biodiversity
• Host defense and selectivity
• Conflict and cooperation


Microbial ecology of amphibian skin
• The environment
– Importance of skin in amphibian ecology
– Climate
– Skin structure
– Skin secretions

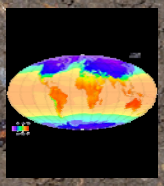
Importance of skin in amphibian ecology

- Respiration
- Sensation
- Absorb/release H_2O
- Heat exchange
- Cocoon in drought
- Chemical defense
- Concealment/warning
- Protection from abrasion & pathogens

(Stebbins and Cohen 1995)

The environment: Climate

- Amphibians have limited capacity to thermoregulate
- Temperature and Humidity
  - Warmer, wetter environments: More bacteria
- pH
  - Might be most important environmental variable in global diversity



The environment: Skin structure

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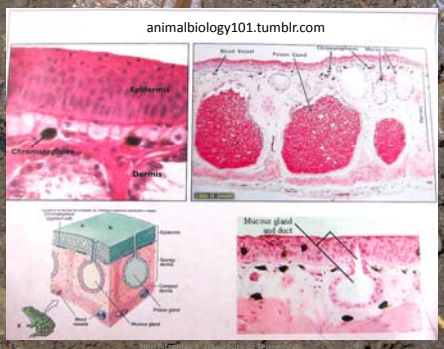
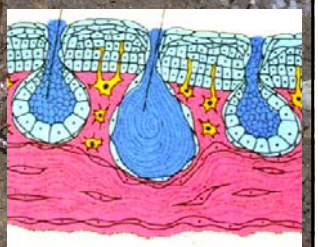


The environment: Skin structure

- Naked, permeable
- Stratum corneum: Outermost layer
- Sloughing continuous or episodic

The environment: Skin secretions

- Mucus glands
  - Moisture, lubrication, mechanical trap
  - Mucins: gel-forming glycoproteins
- Granular glands
  - Pheromones
  - Defensive compounds:
    - Amines
    - Steroids
    - Alkaloids
    - Peptides



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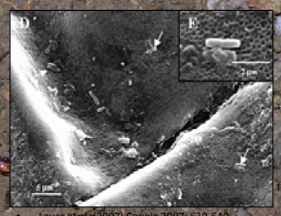
The environment: Review

- Skin a major interface in amphibian ecology
- Constant contact with microbe-rich media
- External environment *probably* influential
  - Abiotic
  - Biotic
- Amphibian skin structurally & chemically complex
- Microbes have to contend with
  - Sloughing (disturbance)
  - Toxic secretions
  - Other microbes
- Microbes might gain
  - Enemy-free space
  - Nutritious secretions
  - Opportunity for infection

Cutaneous biodiversity

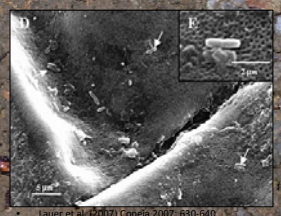
- Resident vs. transient microbes
- Growth forms
- Abundance
- Diversity within and among individuals



- Lauer et al. (2007) Copeia 2007: 630-640

Cutaneous biodiversity

- Resident:
  - Grow/multiply on skin
  - Consistent abundance
  - Attached
- Transient
  - Non-reproducing
  - Exogenous
  - Lie free
- Composition of samples in rinse water significantly different from samples of remaining (attached) bacteria



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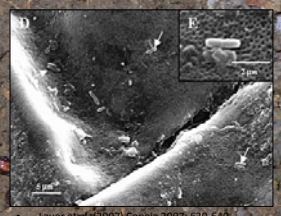
Cutaneous biodiversity

- Growth forms
  - Distinct rods, cocci
  - *Small* microcolonies?
  - No evidence of biofilms



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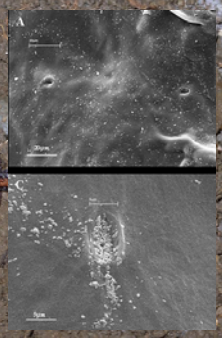
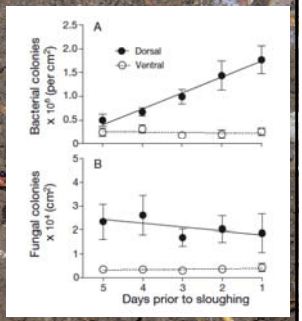
Cutaneous biodiversity

- Abundance
- *Plethodon cinereus*
  - Based on SEM (Lauer et al. 2007):
  - Average 17 million per cm^2
  - Fairly even distribution
  - Sometimes aggregated in/near glands



Cutaneous biodiversity

- Abundance
- *Rhinella marina*
  - Based on cultivable plate counts (Meyer et al. 2012):
  - Average 0.5 to 2 million per cm^2
  - Depends on location (dorsal vs. ventral) *and* time since moult



Cutaneous biodiversity

- Composition (16s RNA)

Pond-dwelling larvae (McKenzie et al. 2011)

- 100's of OTU's
- Mostly β-proteobacteria
- Differences between salamanders (*Ambystoma*) and frogs (*Rana, Pseudacris*

Cutaneous biodiversity

- Composition (16s RNA)

Pond-dwelling larvae (McKenzie et al. 2011)
- 100's of OTU's
- Mostly β-proteobacteria
- Differences between salamanders (*Ambystoma*) and frogs (*Rana, Pseudacris*)

Terrestrial plethodontids(Allison 2012)
- 100's of OTU's
- Mostly γ-proteobacteria
- No consistent differences among *Plethon jordani* and *Desmognathus ocoee*

Cutaneous biodiversity

- Plethodontids in GSMNP
  - *Nevskia* (γ)
  - *Hydrocarboniphaga* (γ)
  - *Koribacter* (Acidobacteria)
  - *Burkholderia* (β)
  - *Pseudomonas* (γ)
- *Pseudacris* tadpoles in AZ
  - *Curvibacter* (β)
  - Actinomycetales (Actinobacteria)
  - *Sphingomonas* (α)
  - Pseudomonadaceae (γ)
  - *Nevskia* (γ)

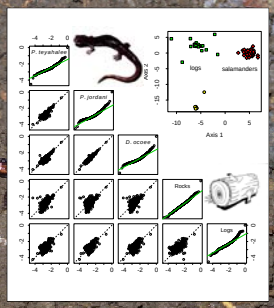
- Human (Roth & James 1988)
  - Microccoci (Actinobacteria)
  - Staphylococci (Firmicutes)
  - *Corynebacterium*(Actinobacteria)
  - *Propionibacteria*(Actinobacteria)
  - *Acinetobacter* (γ)

Cutaneous biodiversity

- Terrestrial plethodontids in GSMNP

Similar "species" to other moist surfaces in the habitat

Different variants within "species"



Cutaneous biodiversity

- Terrestrial plethodontids in GSMNP
- Similarity of microbial communities on salamanders depends more on proximity than species
- I.e., interactions are *diffuse* rather than *specific* or *pairwise*

Cutaneous biodiversity

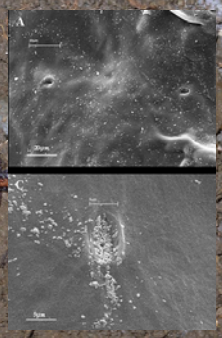
- Plethodontids in GSMNP
- Salamander skin and cover objects have very similar bacterial community structure.
- Specific strains or species differ between salamander skin and cover objects.

Cutaneous Biodiversity: Review

- Distinct resident flora on amphibian skin
- Millions per cm^2: 10x to 100x that of human skin
- Single cells or microcolonies
- 100's of OTUs
- Composition very different from human skin
- Composition different between pond-dwelling and terrestrial amphibians
- Diffuse rather then specific interactions
  - Subtle differences between co-occurring tadpoles and salamander larvae
  - No differences between co-occurring *Plethodon*, *Desmognathus*
- Community structure similar between salamanders and cover objects.
- Genetic differentiation between host-associated and free-living populations

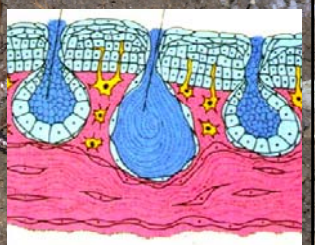
Host defense and selectivity

- Resistance and Containment
- Antimicrobial Secretions
- Sloughing
- Selectivity



Host defense and selectivity

- Resistance and Containment
  - Stratum corneum
  - Mucus



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Host defense and selectivity

- Resistance and Containment
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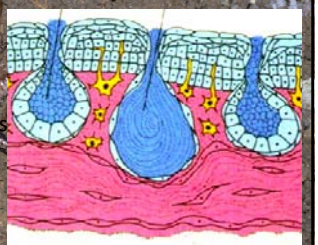

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Host defense and selectivity

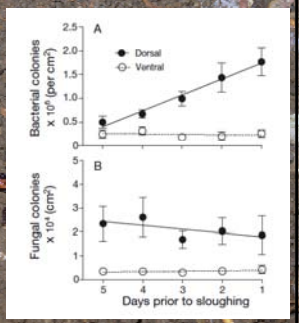
Antimicrobial Secretions

- Amines
  - Histamines, epinephrine, adrenalin, bufotenins
- Steroids
  - bufadenolides – anesthetics, cardiac stimulants
- Alkaloids
  - 100 s of compounds in poison dart frogs, sequestered from prey
- Peptides
  - Likely most important antimicrobial compounds

Host defense and selectivity

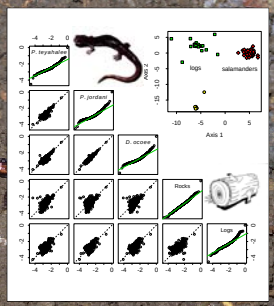
Sloughing (Meyer et al. 2012)

- Disturbance/recolonization dynamics
- May prevent "fouling" via biofilm formation
- May increase vulnerability to opportunistic pathogens
- Sloughed skin often eaten



Host defense and selectivity

- Evidence of selectivity
  - Resident vs. transient flora
  - Salamanders vs. cover objects



Host defense and selectivity

- Salamanders vs. cover objects
  - Work in progress:
  - Swabbed zigzag salamanders and cover objects
  - Genotyped *Pseudomonas* isolates
  - Only one strain found on cover objects: *P. putida*
  - 11 distinct strains found co-occurring on salamanders
  - 7 related to a pathogen of fish

Host defense and selectivity

- Salamanders vs. cover objects
  - Work in progress:
  - Salamanders harbor a *distinct* and *more diverse* assemblage of *Pseudomonas*
  - Do salamanders select a beneficial flora, or are they under attack?

Host Defense and Selectivity: Review

- Barrier: Stratum corneum
- Trap: Mucus
- Poison: Antibiotic secretions
- Removal: Sloughing

Selection for certain microbial genotypes over others

Prevention of competitive dominance?


Conflict and Cooperation
• Mutualism-parasitism continuum
• Exchangeable goods/services
• Importance of transmission dynamics
• Disease and defense in amphibian conservation
3/11/2013
Barry Fitzpatrick, University of Tennessee


Conflict and Cooperation
• Mutualism-parasitism continuum
– Defensive mutualism
• Good microbes may help protect skin/eggs from bad microbes
– Costs
• Any microbial load might be costly
• Any microbes might be opportunistic pathogens
• Amphibian skin might be a harsh environment
• Sloughed skin (+ microbes) might be eaten
– Bet-hedging
• Benefits of a costly partner might be infrequent, but life-saving
3/11/2013
Barry Fitzpatrick, University of Tennessee


Conflict and Cooperation
• Exchangeable goods and services
– Mutual exploitation?
– Benefits to bacteria self evident?
• Hosts provide a habitat free of certain enemies
• Hosts might provide nutrition
• Amphibian skin might be a harsh environment
• Sloughed skin (+ microbes) might be eaten
– Benefits to host?
• Additional defensive compounds
– Are benefits provided at a cost, or as byproducts?
• When goods are costly in any sense, *cheating* is possible!
3/11/2013
Barry Fitzpatrick, University of Tennessee

Conflict and Cooperation

- Exchangeable goods and services
  - Mutual exploitation?
  - Red Queen dynamics
    - Arms race: mechanisms to manipulate and combat cheating continually evolve
    - Faster evolving party can gain advantage
  - Red King dynamics
    - "Bargaining": within mutualism, amount of cost/benefit can evolve in response to other
    - Party with slower dynamics can gain advantage

*** Probably much more complicated when populations of many species are involved!

Conflict and Cooperation

- Importance of transmission dynamics
  - Feedback against cheating stabilizes mutualism
    - Partner choice/sanctions
      - Sloughing
      - Secretions
    - Partner fidelity
      - Egg brooding: mother-offspring transmission?
      - Transovarian passage?
      - Biphasic life cycle a barrier to effective inheritance of skin flora

Conflict and Cooperation

- Disease and defense in amphibian conservation
  - Amphibians unlikely to harbor human pathogens
  - Defensive mutualism might be disrupted by habitat modification or captivity
    - Presence/absence of important microbes
    - Changes to cost/benefit structure
  - Symbionts might protect amphibians from Bd
    - Experiments …

Conflict and Cooperation

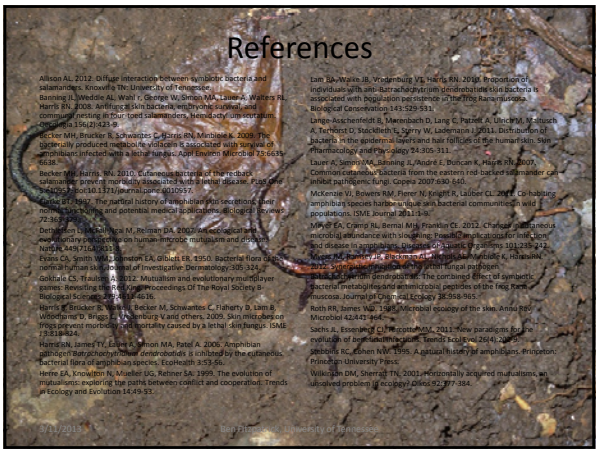
- Disease and defense in amphibian conservation
  - *Janthinobacterium*
    - Reduced mortality of frogs (Harris et al. 2009)
    - Violacein increased survival of infected salamanders (Becker et al. 2009)
    - Prevalence associated with population persistence (Lam et al. 2010)
  - *Pseudomonas*
    - Synergistic inhibition of Bd by Host secretion and *Pseudomonas* metabolite (Myers et al. 2012)

Conflict and Cooperation: Review

- Symbiosis ≠ mutualism
- Defensive mutualism hypothesis
- Specific *costs* and *risks* not well known
- Interactions are diffuse
- Interactions among microbes have not been studied
- Amphibian "choice" or "sanctions" via secretions, sloughing
- Maternal transmission possible in egg tenders
- Candidate probiotics against Bd

Overall Review

- Skin is a key way amphibians interact with the world
- Amphibians live in microbe-rich habitats
- Structure, chemistry, sloughing probably affect skin micro-flora
- Millions of bacteria live on each cm^2 of amphibian skin
- 100's of OTUs are normally found
- Host associated bacteria are distinct from related free-living bacteria
- Some host-associated bacteria produce antifungal compounds
- Some host-associated bacteria may help against Bd
- Extent of mutualism vs. parasitism unknown
- Normal functions of skin symbiotes unknown
- Costs and benefits of symbiotes in nature unknown
- Mechanisms of partner choice unknown
- Transmission dynamics unknown



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