



The Dynamics of the Microbiome:  
The Human Vagina

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## Definitions

### *Dynamics*

The dynamics of microbial communities can be defined as temporal and spatial changes in community structure or function



## Definitions

### Stability/Instability



- In 1997 Grimm and Wissel catalogued 167 definitions of community stability in the field of ecology
  - **resistance** (or constancy) – staying essentially unchanged over time
  - **resilience** – returning to the reference state after a disturbance
  - **persistence** – persistence of an ecological state through time.
- Stability quantifies the extent to which a community “stays the same” over a long period of time (depending on the time scale) or in the face of some disturbances.



V. Grimm and C. Wissel. Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. *Oecologia*, 109:323–334, 1997.

## Definitions

### Measures used to study stability/instability



- Culture/physical measurement (i.e., pH), microscopy
- Microbiota compositional change (16S rRNA gene survey) - Relative abundance
- Microbiota compositional change (species specific qPCR - pan-bacterial qPCR)
- Estimates of diversity (evenness and richness, relative entropy)
- Metagenomic characterization (microbiome: genes/genomes content, SNP)
- Functional analysis of metagenomic data (metabolic potential)
- Metatranscriptomics/metaproteomics (function of a community)
- Metabolomics (functional output of a community)

Communities can appear stable given one metrics and unstable given another one.



McNulty, N. P., Yatsunenkov, T., Hsiao, A., Faith, J. J., Muegge, B. D., Goodman, A. L., et al. (2011). The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Science translational medicine*, 3(106), 106ra106.

# Definitions

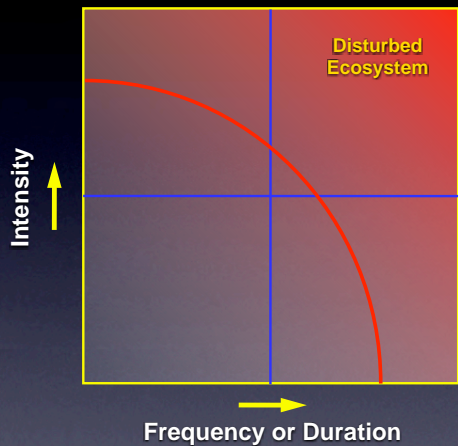
## Disturbance/Resilience

- A disturbance may be defined as an event that disrupts community structure and/or simultaneously changes resources, substrate availability, or the physical environment.
- A disturbance can also be defined as a killing, displacement, or damage to one or more populations in a community that directly or indirectly creates opportunities for new individuals to become established.
- Resilience is the amount of disturbance that an ecosystem can withstand without changing its self-organizing processes.
- Communities with fundamental differences in species composition and structure will differ in resilience.



Putman, R.J. (1994). Community Ecology (Chapman and Hall, New York).  
Ma, Z. S., Geng, J.W., Abdo, Z., & Forney, L. J. (2012). A Bird's Eye View of Microbial Community Dynamics. Microbial Ecology Theory: Current Perspectives. Norwich, UK: Horizon Scientific Press, 57–70.

# Ecosystem disturbances/resilience



Increased Risk to Disease?  
Disease itself? Cause/effects?

Disturbances to the microbiome are often imposed by human actions:

- hygiene
- diet
- behaviors
- antibiotics

But also include:


- natural states (menstruation)
- hormonal variations
- immunological changes

What are the drivers of change and resilience in a microbial community?



## Issues with current study

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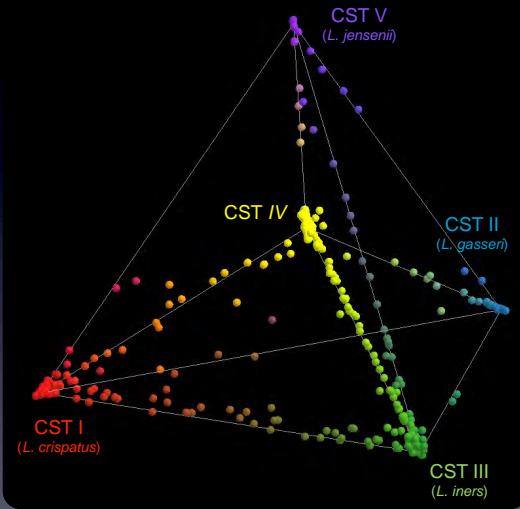


- While key studies, most looked at either low number of time points (short sampling period, long period between samples) or very few subjects (sampled frequently) - Difficulties in generalizing the findings/conclusions
  - Feasibility and cost have limited these studies
- Principles of community dynamics are certainly body site specific and not applicable across the spectrum of community types on the human body
- Compositional analysis often done at low taxonomic resolution (Phylum, Class, Order)



The dynamics of the vaginal microbiota  
in reproductive age women

## The Vaginal Community Space



- Cross sectional study of 410 asymptomatic healthy women
- Five community state types (CST) that differ in their microbial composition and abundance
- Community state type IV lacks significant number of *Lactobacillus* - higher diversity - (IV-A and IV-B)

A plot of principle component analysis (PCA) shows distribution of community states in 3-D space.

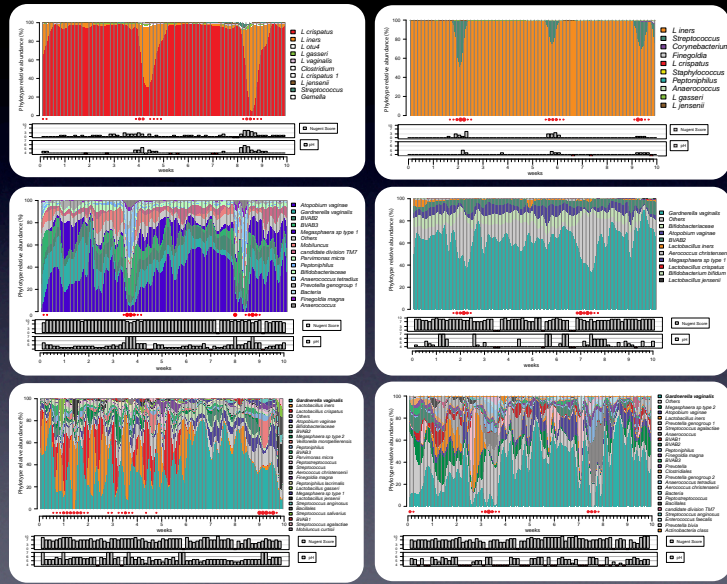
## Longitudinal studies of the vaginal microbiota



### Study design

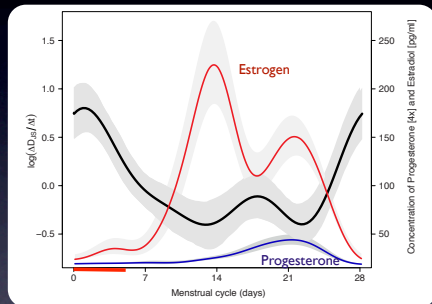
- 160 women cohort - prospective longitudinal study
  - Self-collect vaginal smears, pH and swabs daily for 10 weeks
  - Three swabs a day: Amies, RNAlater and dry
  - Daily diary and weekly clinic visits
  - Clinical assessment at enrollment, week 5 and 10
  - Determine bacterial community composition by 454 pyrosequencing of barcoded V1-V3 16S rRNA gene of 50 women

# Longitudinal profiles - stability/instability



# Drivers of instability

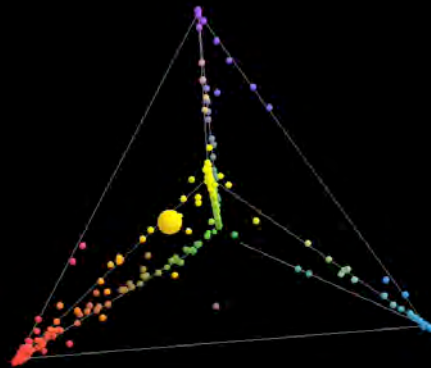
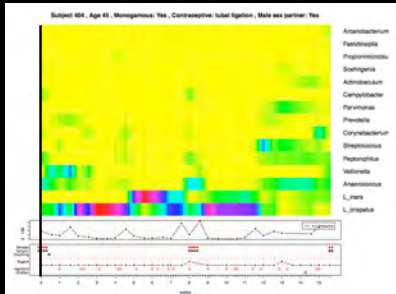
Modeling the dependence of the log of Jensen-Shannon divergence rate of change (estimate of stability) on the menstrual time (normalized time) - Bayesian Markov Chain Monte Carlo methods using linear mixed effect models.



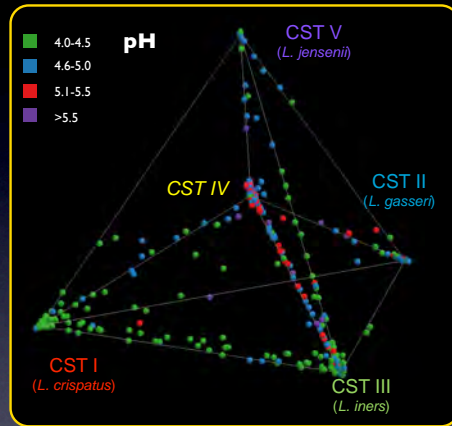
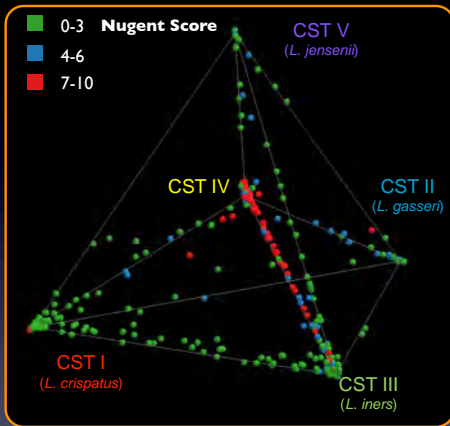
The rate of community change is affected by time in the menstrual cycle (natural change) and sexual activities to some extent



# Vaginal community dynamics



# pH, Nugent score, microbial community state



In these healthy, asymptomatic women, CST IV is associated with higher Nugent scores and higher pH



## CST IV: an normal state that carry risks?



- At any given time, >25% of women are in a non-*Lactobacillus* dominated state.
  - This state is associated with higher Nugent scores and higher pH
  - High Nugent score is strongly associated with increased risk of sexually transmitted infection acquisition and transmission, including HIV, as well as preterm birth
  - These women are asymptomatic and apparently healthy, but potentially at increased risk of STI or other adverse outcomes

Wiesenfeld HC, et al. : Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2003, 36(5):663-668.  
Taha TE, et al. : Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV/AIDS (London, England) 1998, 12(13):1699-1706.  
Cohen, C. R. et al. (2012). Bacterial Vaginosis Associated with Increased Risk of Female-to-Male HIV-1 Transmission: A Prospective Cohort Analysis among African Couples. (H. Watts, Ed.) PLoS Medicine, 9(6), e1001251.



## CST IV: an healthy state that carry risks?



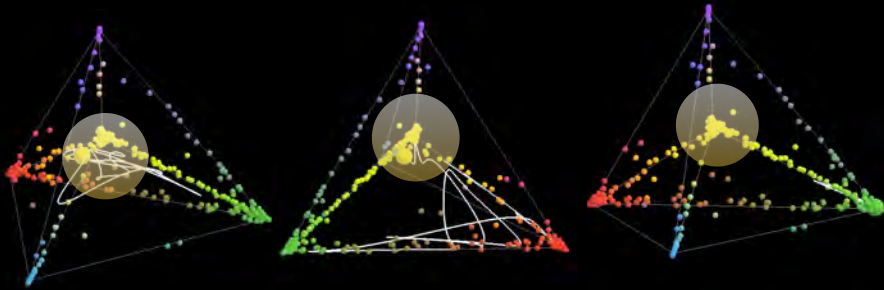
There are windows of higher risk that open and close on a temporal scale





## Vaginal community dynamics

*Windows of higher risk that open and close on a temporal scale*



Community stability/dynamics (frequency and duration of CST IV) might better represent risks to disease

Low resilience = increased risk

## Vaginal community dynamics

*Windows of higher risk that open and close on a temporal scale*

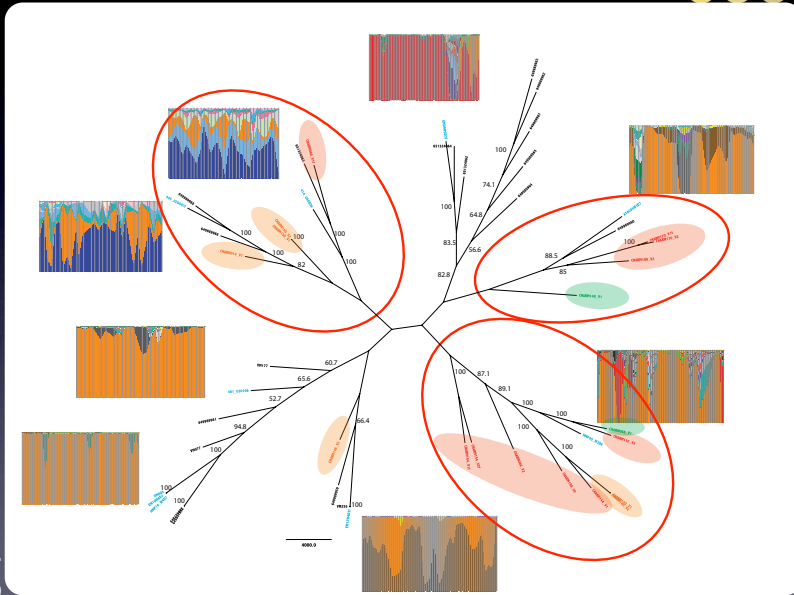


Still, we do not understand the underlying causes for stability/dynamics (frequency and duration of CST IV)

Understand the molecular basis of the association between stability and susceptibility



## *L. iners* and community stability



## Drivers community stability



- Certain kinds of *L. iners* genomes appear associated with community resilience to enter CST IV
- Does the “fragility” of a single keystone species drive the community into dysbiotic states that carry risk to disease?
- We hypothesize that vaginal microbiome stability/dynamics (frequency and duration of CST IV) might better represent estimates of risks to infection and that stability/instability can be driven by the lack of resilience of certain keystone *Lactobacillus* sp.

## Gaps and Challenges

- The issue of cause or effects - Need for prospective longitudinal studies
- Better understand the drivers of dynamics/stability or instability in health (risk to disease) over a women's lifespan
- Understand what a shifting microbiome means functionally (role of microbiota and host).
- Need to evaluate the dynamics using different measures (metabolomics, metatranscriptomics [host and microbes] and immunological)
- Expand the studies to dynamics of phages, viruses and fungi
- Develop predictive models of stability/instability/susceptibility that accounts for health practices and behaviors.
- Translate this information in better diagnostics, prognostics, and treatments - moving toward personalized medicine



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