

# Exploring and translating the female genital tract microbiota

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Institute for Genome Sciences  
University of Maryland School of Medicine  
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Durban, South Africa  
April 26, 2024

# Who am I?

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Professor of Microbiology and Immunology, and the Acting Director of the Institute for Genome Sciences at the University of Maryland School of Medicine in Baltimore, Maryland, USA



Lead a laboratory that aims to improve women's health conditions by optimizing the composition and functions of the vaginal microbiome  
Apply genomics approaches to clinical samples, develop bioinformatic tools, test hypothesis and translate findings into innovative interventions

# Who am I?

Master Degree  
*Vibrio cholerae* ecology  
Postdoctoral Fellowship  
Natural Product Chemistry  
Johns Hopkins University

1990

1992

Bachelor Degree  
Microbiology and Molecular Biology  
University Nancy - France

PhD in Microbial Molecular Ecology  
Marine *Streptomyces*  
University of Maryland

Postdoctoral Fellowship  
Natural Product Chemistry  
Johns Hopkins University

1999

2002

2002

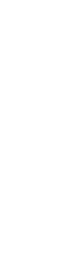
Associate Professor  
Microbiology & Immunology  
University of Maryland  
School of Medicine

2006

NIH grant  
Vaginal microbiome

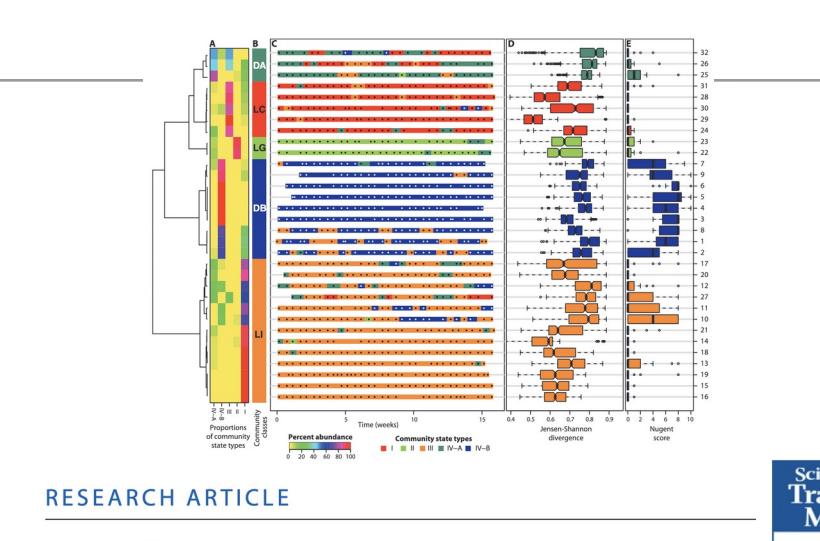
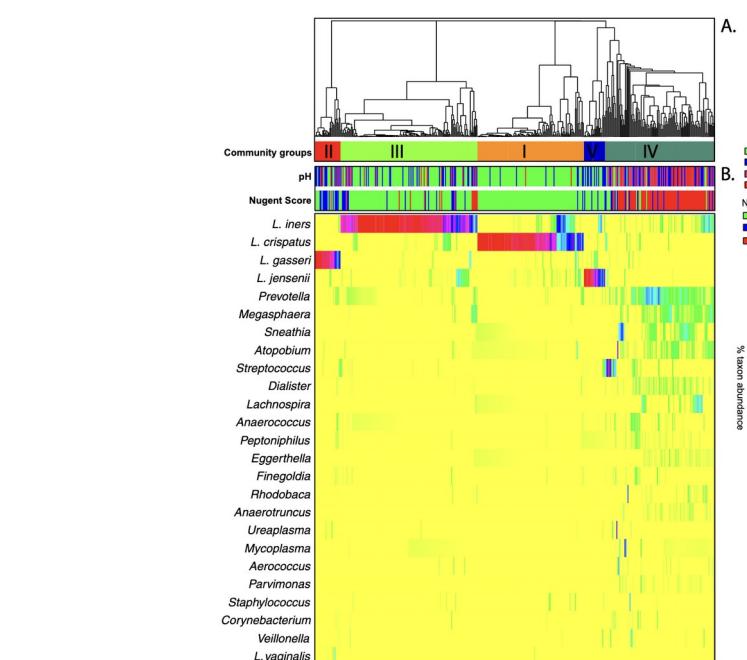


Assistant Investigator  
The Institute for Genomic Research (TIGR)



## Vaginal microbiome of reproductive-age women

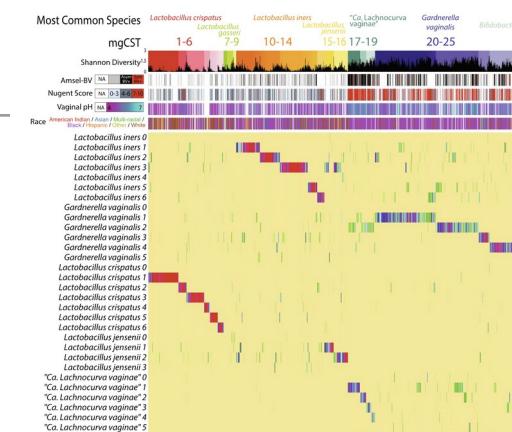
Jacques Ravel<sup>1,2\*</sup>, Pawel Gajer<sup>1</sup>, Zaid Abdo<sup>3</sup>, G. Maria Schneider<sup>4</sup>, Sara S. K. Koenig<sup>5</sup>, Stacey L. McCulle<sup>6</sup>, Shara Karlebach<sup>4</sup>, Reshma Gorle<sup>6</sup>, Jennifer Russell<sup>7</sup>, Carol O. Tacket<sup>8</sup>, Rebecca M. Brotman<sup>9</sup>, Catherine C. Davis<sup>9</sup>, Kevin Aut<sup>10</sup>, Ligia Peralta<sup>11</sup>, and Larry J. Forney<sup>1,2</sup>  
Edited by Jeffrey I. Gordon, Washington University School of Medicine, St. Louis, MO, and approved May 7, 2010 (received for review March 14, 2010)  
Institute of Human Sciences, Moscow, ID 21201; Department of Mathematics and Statistics, and the Initiative for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID 83844; Emory University School of Medicine, Atlanta, GA 30322; Department of Pediatrics Adolescent and Young Adult Medicine, University of Maryland School of Medicine, Baltimore, MD 21201; Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD 21201; and <sup>10</sup>The Procter & Gamble Company, Cincinnati, OH 45224



RESEARCH ARTICLE  
GENOMICS  
Science Translational Medicine  
AAAS

## Temporal Dynamics of the Human Vaginal Microbiota

Pawel Gajer,<sup>1,2\*</sup> Rebecca M. Brotman,<sup>1,3\*</sup> Guoyun Bai,<sup>1,2</sup> Joyce Sakamoto,<sup>1,2†</sup> Ursel M. E. Schütte,<sup>4,5‡</sup> Xue Zhong,<sup>5,6,7§</sup> Sara S. K. Koenig,<sup>1,2</sup> Li Fu,<sup>1,2</sup> Zhanshan (Sam) Ma,<sup>4,5||</sup> Xia Zhou,<sup>4,5</sup> Zaid Abdo,<sup>5,6,7</sup> Larry J. Forney,<sup>4,5§</sup> Jacques Ravel,<sup>1,2§</sup>



Holm et al. Microbiome (2023) 11:239  
<https://doi.org/10.1166/microb.023.01692>

Microbiome

2009

2011

Human  
Microbiome  
Project (HMP)

2012

2022

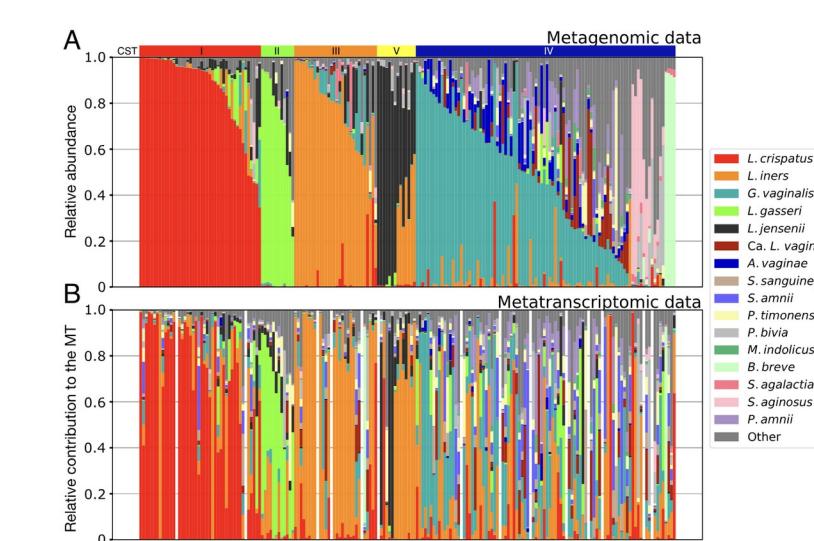
2023

France et al. Genome Biology (2022) 23:66  
<https://doi.org/10.1186/s13059-022-02635-9>

Genome Biology

RESEARCH  
Open Access  
Insight into the ecology of vaginal bacteria through integrative analyses of metagenomic and metatranscriptomic data

Michael T. France<sup>1</sup>, Li Fu<sup>1</sup>, Lindsay Rutt<sup>1</sup>, Hongqiu Yang<sup>1</sup>, Michael S. Humphrys<sup>1</sup>, Shilpa Narina<sup>1</sup>, Pawel M. Gajer<sup>1</sup>, Bing Ma<sup>1</sup>, Larry J. Forney<sup>2</sup> and Jacques Ravel<sup>1,2\*</sup>



Genome Biology

RESEARCH  
Open Access

Check for updates

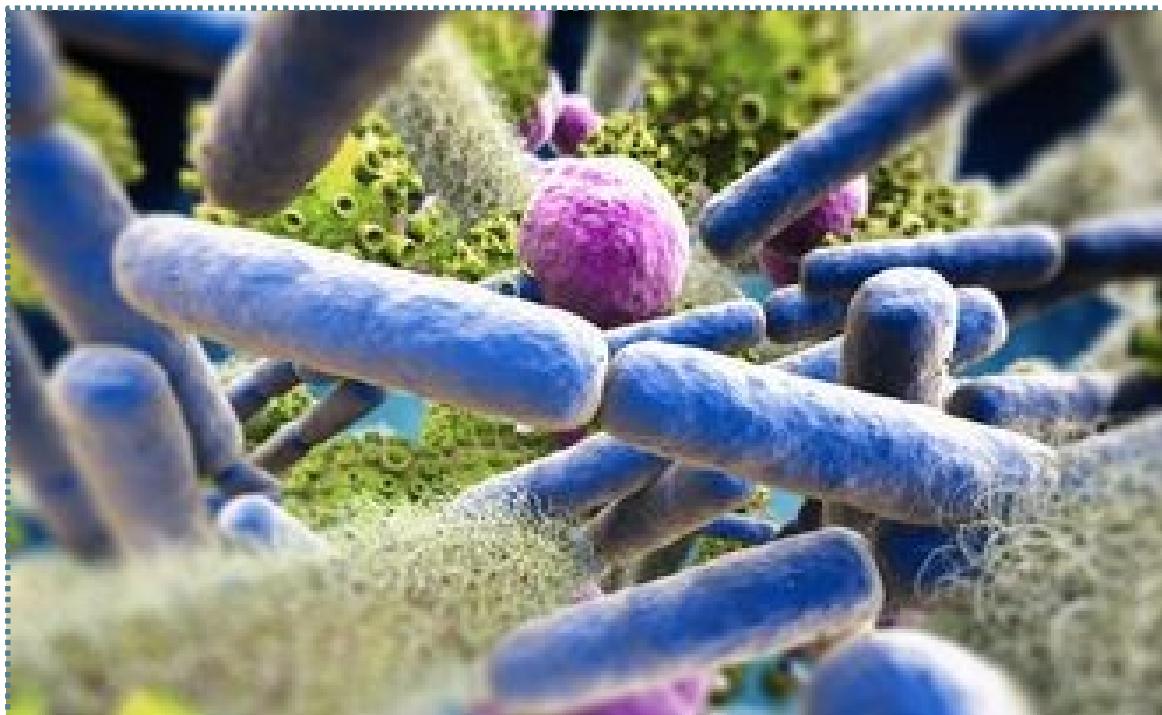
Integrating compositional and functional content to describe vaginal microbiomes in health and disease

Johanna B. Holm<sup>1,2</sup>, Michael T. France<sup>1,2</sup>, Pawel Gajer<sup>1</sup>, Bing Ma<sup>1,2</sup>, Rebecca M. Brotman<sup>1,3</sup>, Michelle Shardell<sup>1,3</sup>, Larry Forney<sup>4</sup> and Jacques Ravel<sup>1,2\*</sup>

Check for updates

# Ravel Lab

## Transformative Bench to Bed-Side Research in Women's Health



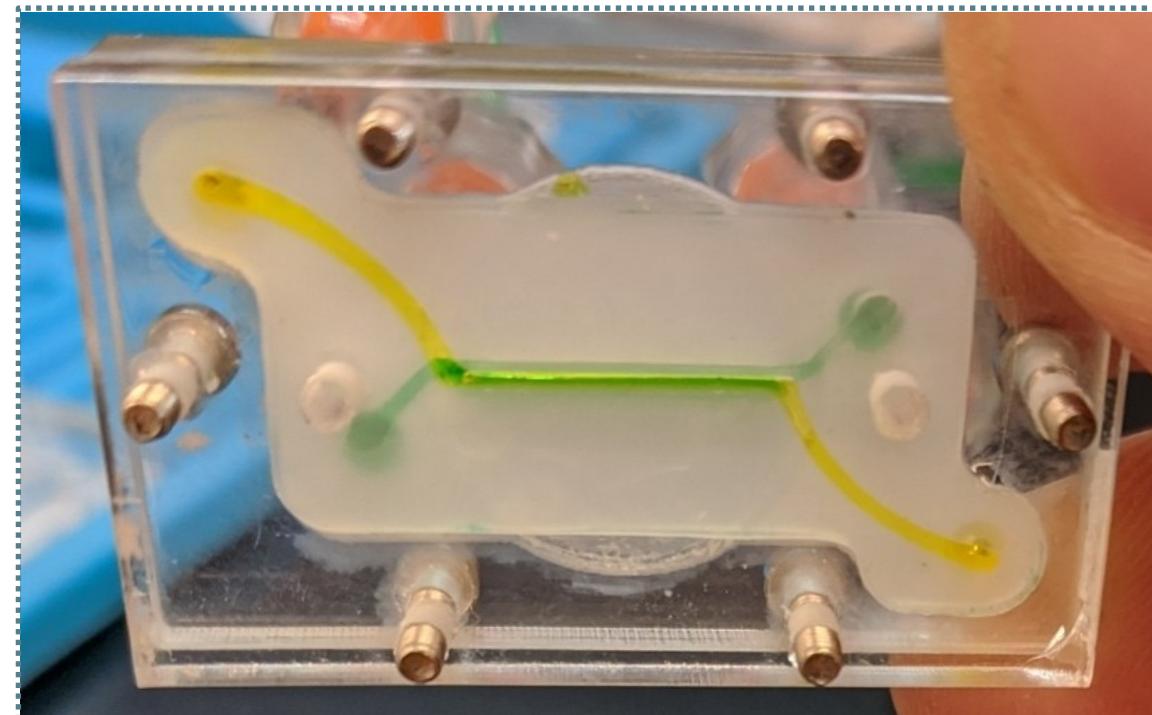
### Microbiome Research

- Multi-omics Analyses
- Laboratory Methods
- Bacterial Vaginosis
- Preterm Birth
- STIs
- Menopause
- Transgender



### Data Analytical Tools

- SpeciateIT
- VALENCIA
- VIRGO
- RLLM



### 3D Biomimetic Models

- Modeling vaginal and cervical epithelia
- Include Microbiome
- Study host-microbiome interactions and STIs



### Live Biotherapeutics Products

- Innovative Formulation
- In-human clinical trial to prevent bacterial vaginosis recurrence

# The vaginal microbiome

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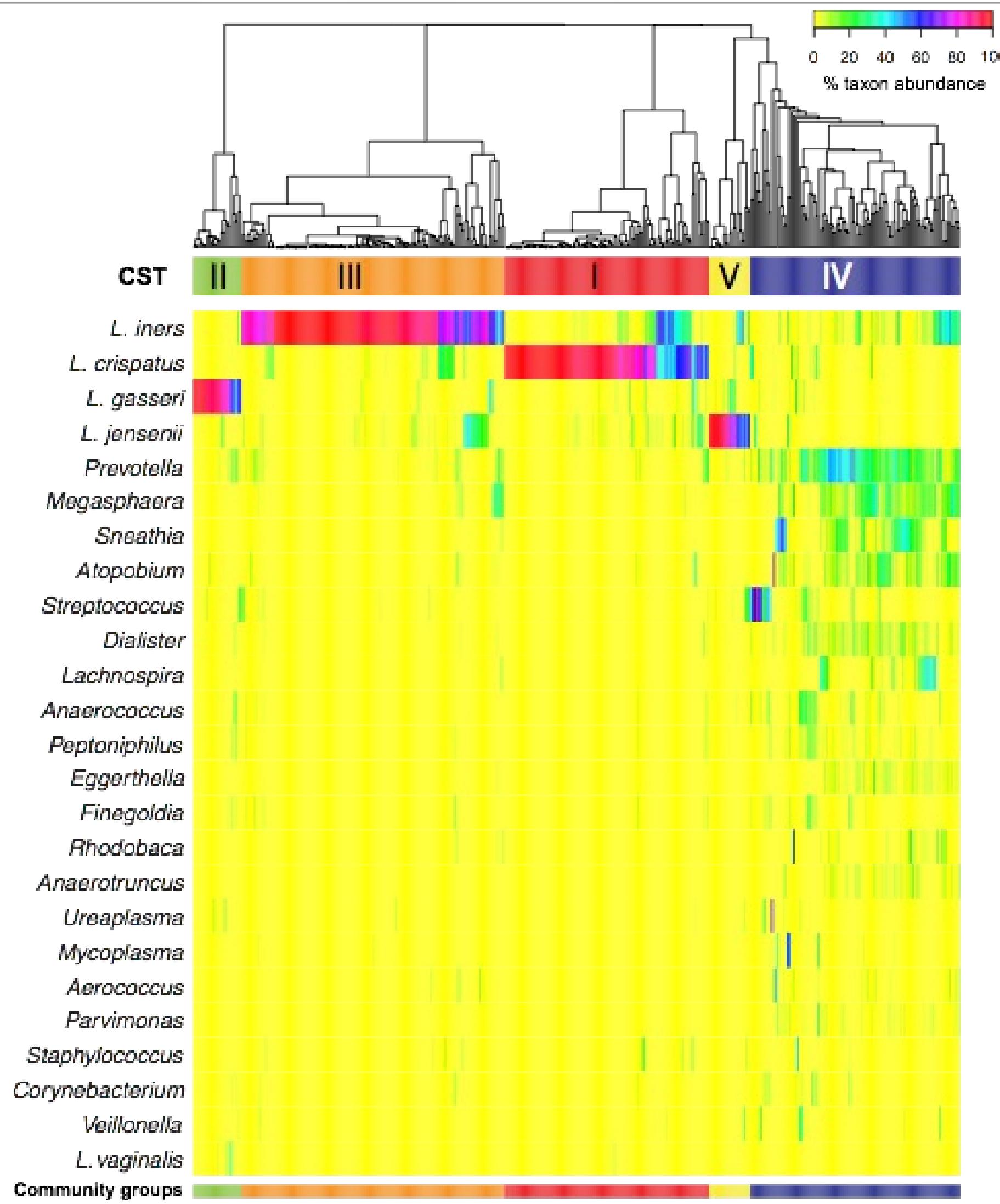
In reproductive age women, high abundance of *Lactobacillus* spp. are often characteristic of an optimal vaginal microbiota. A feature unique to human as no other mammals carry *Lactobacillus* spp.

*Lactobacillus* spp. are lactic acid producers and acidify the vagina to pH < 4

An acidic environment is thought to restrict the growth of non-indigenous microbes

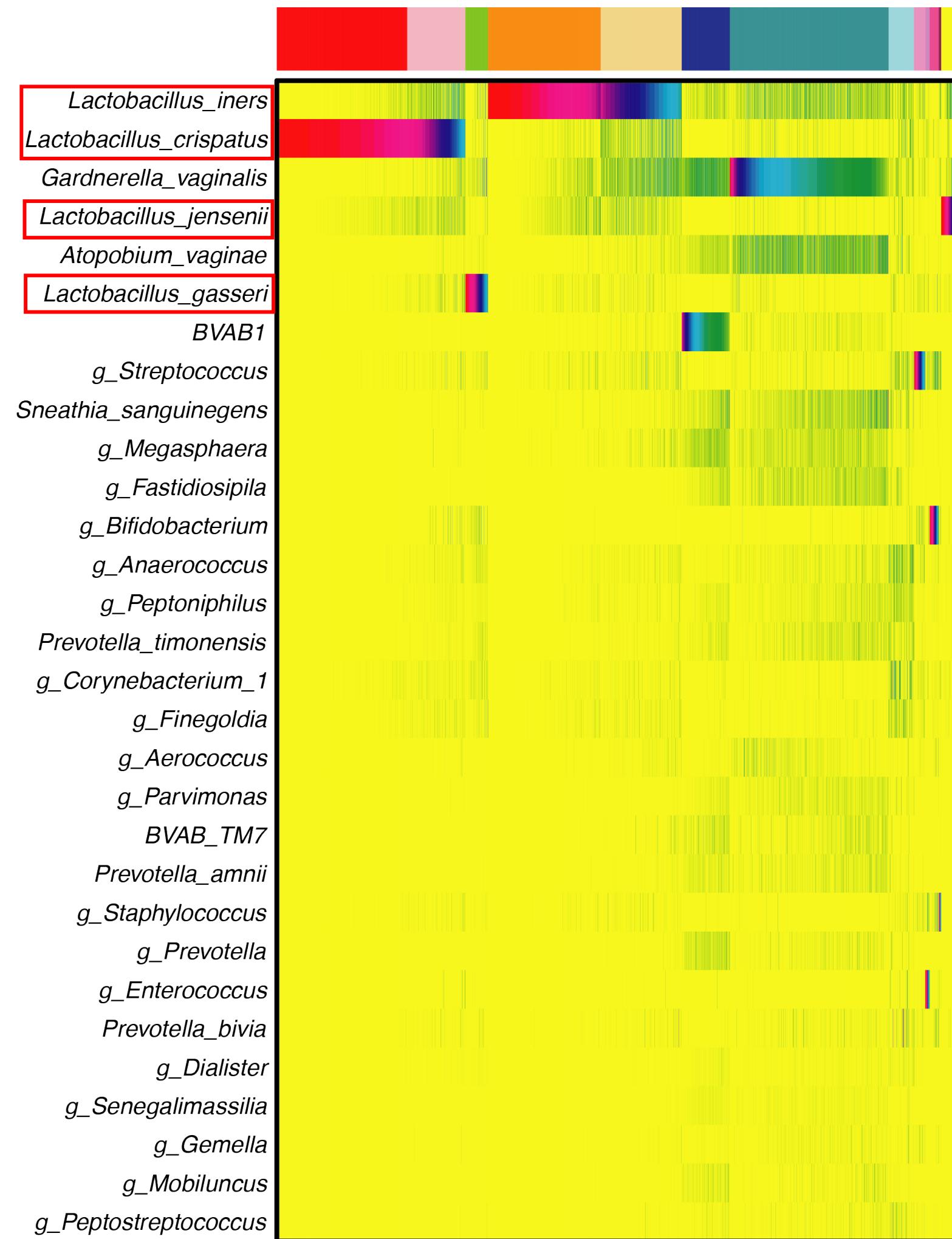
Changing environment throughout a women's lifespan - microbiota changes accordingly

# The vaginal microbiota composition and structure

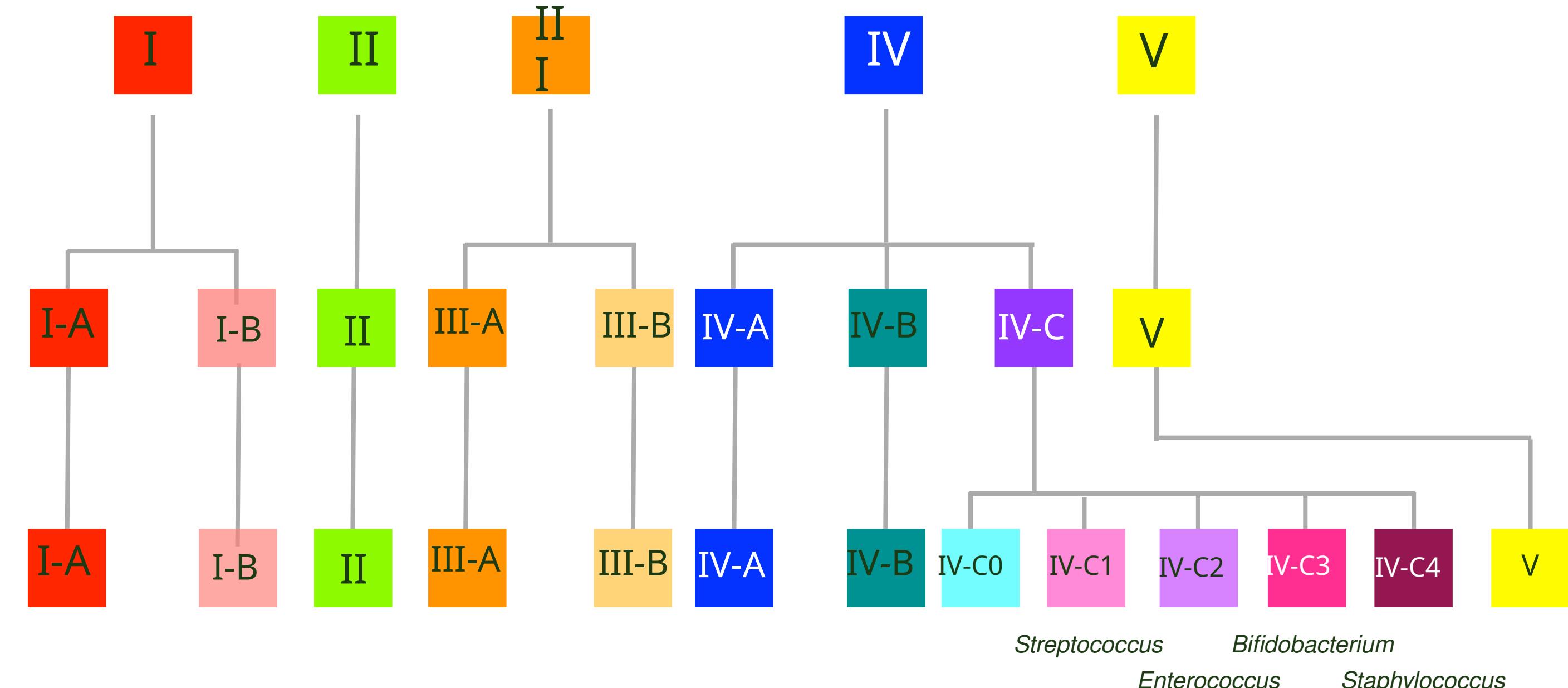


- A study of 400 women of reproductive age representing 4 ethnic background – one sample per women
- Five broad community state types (CST) that differ in their microbial composition and abundance, some of them dominated by *Lactobacillus* spp. and considered optimal.
- CST IV lack significant number of *Lactobacillus* - higher diversity. Non-optimal and associated with adverse outcomes.

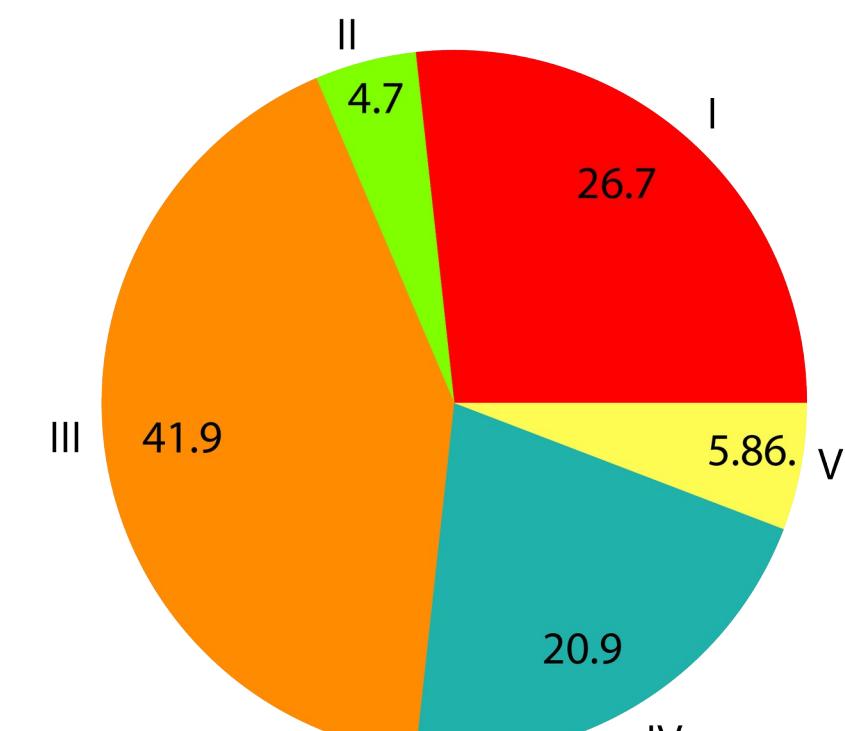
# Vaginal community composition and structure



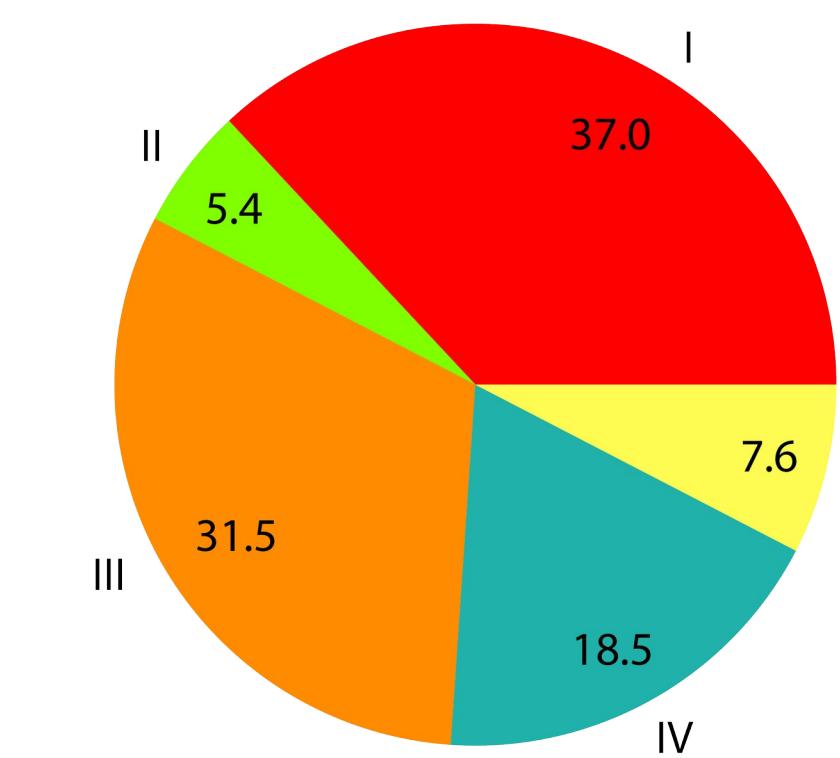
Composition of the vaginal microbiota from 13,000 samples (16S rRNA gene V3-V4 regions)



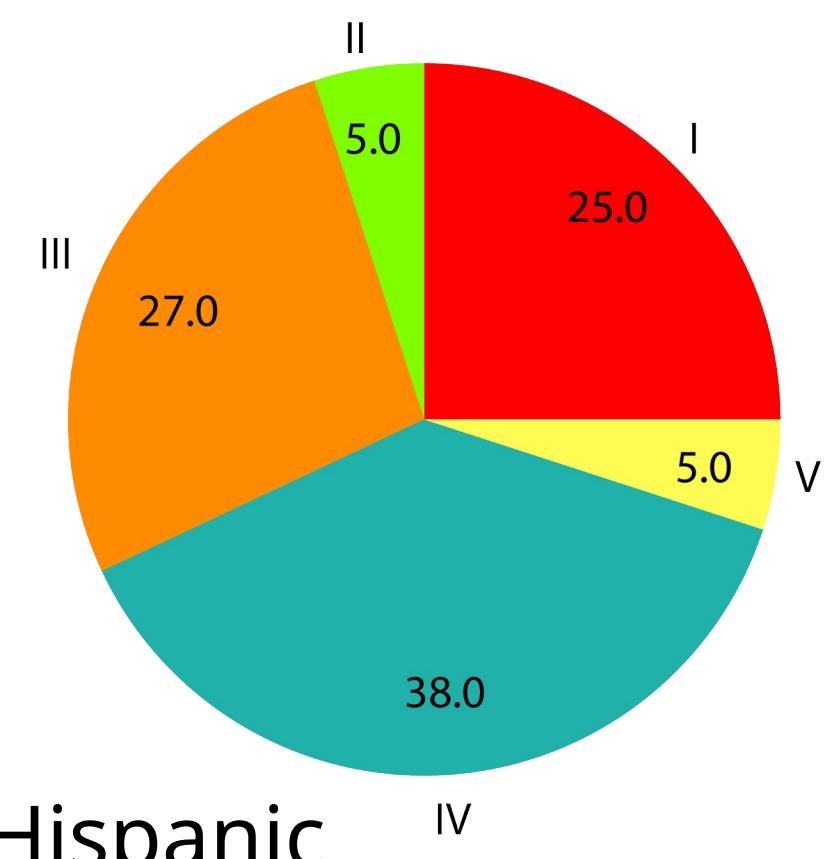
# Frequency of vaginal community state types in ethnic groups



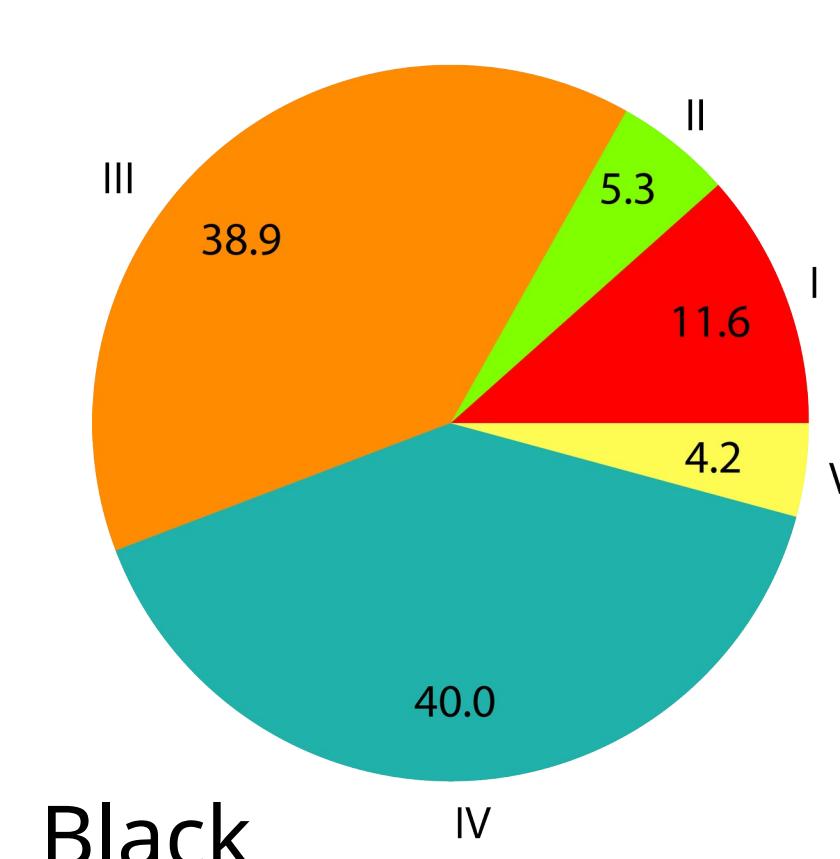
Asian



Caucasian

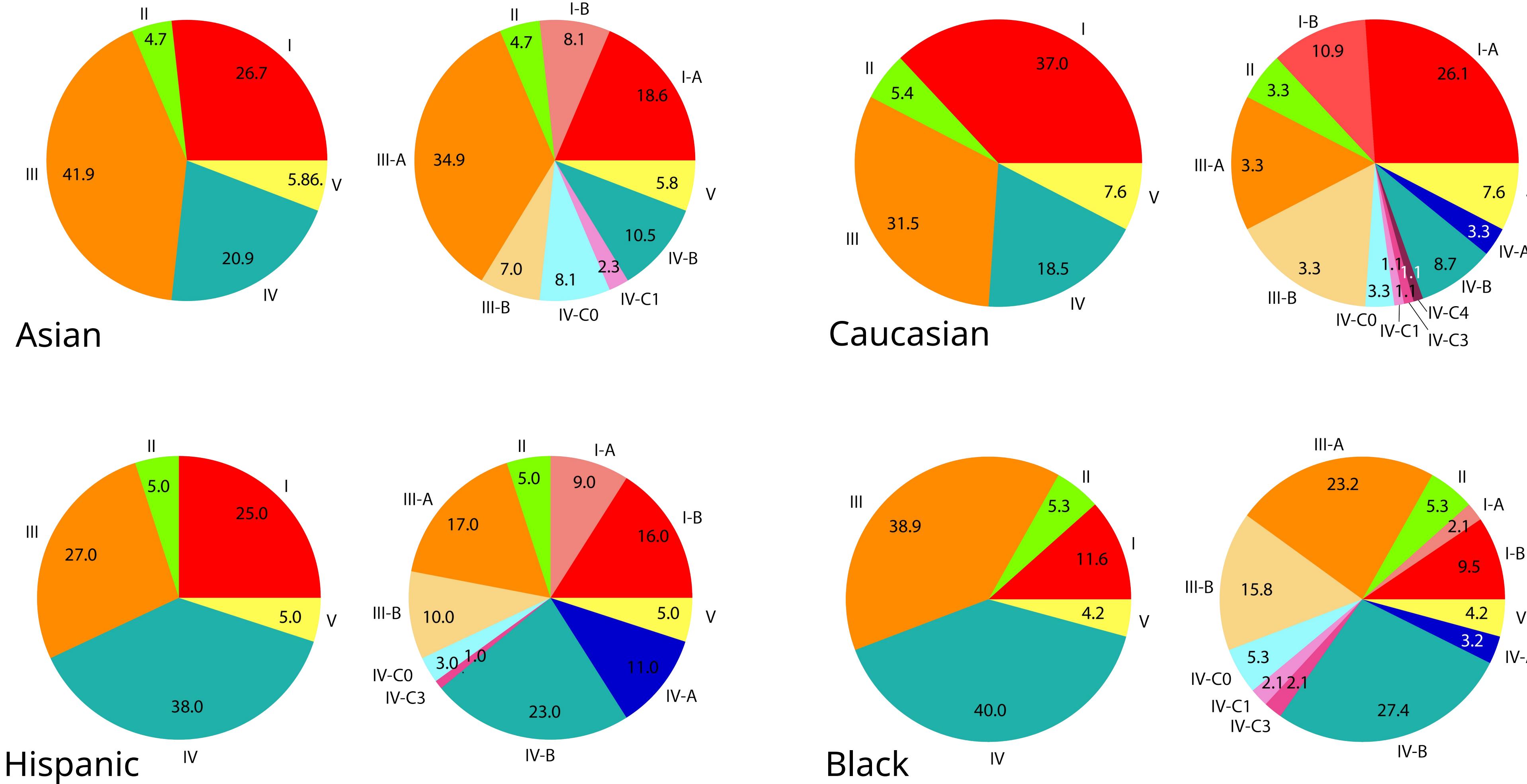


Hispanic



Black

# Frequency of vaginal community state types in ethnic groups

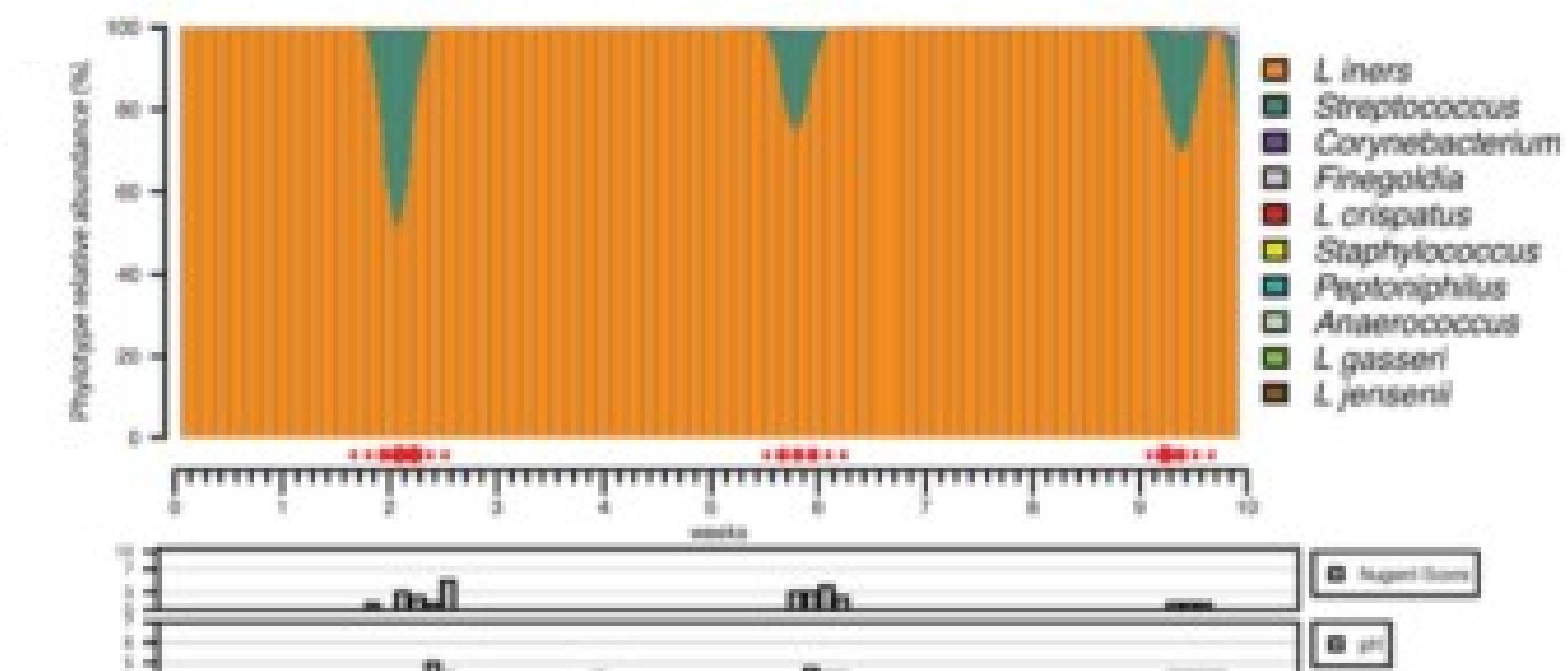
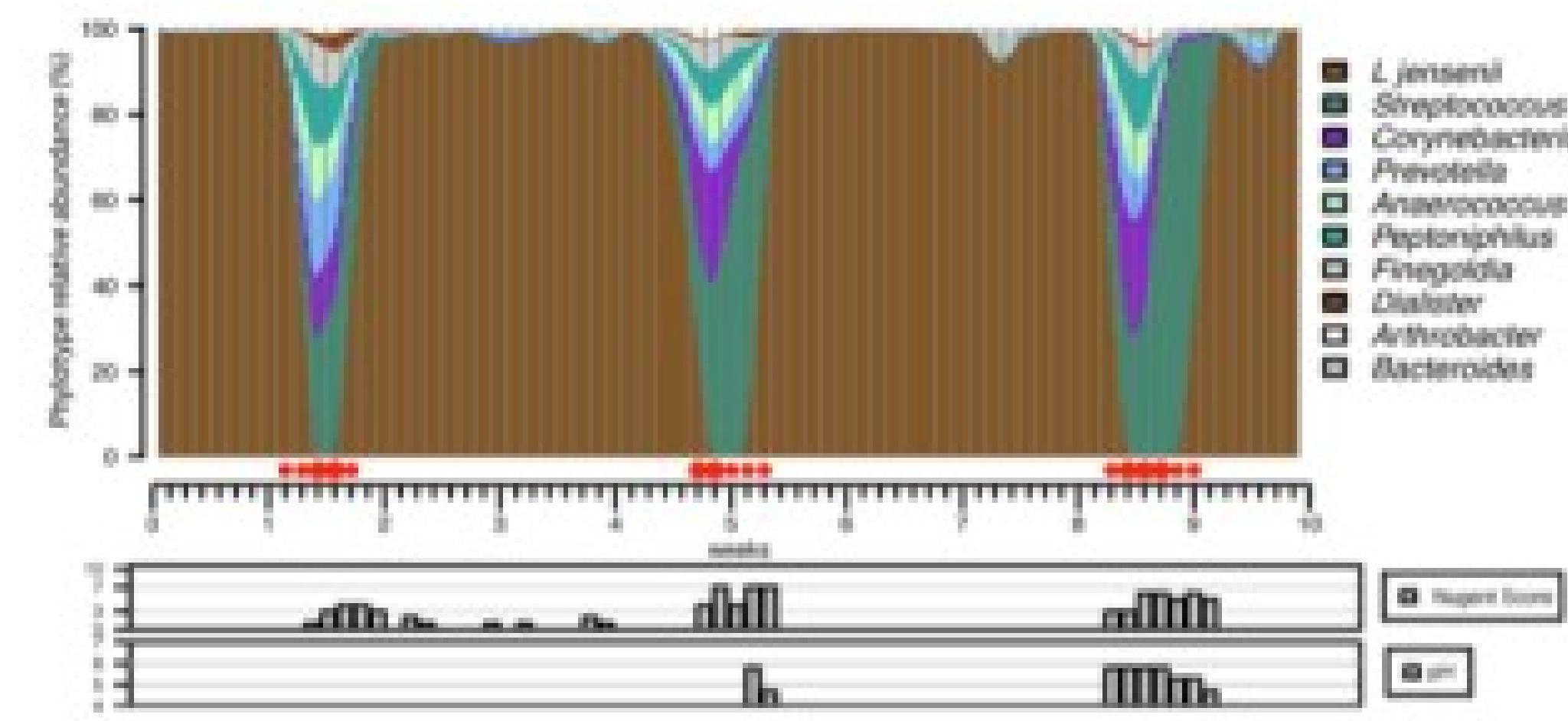
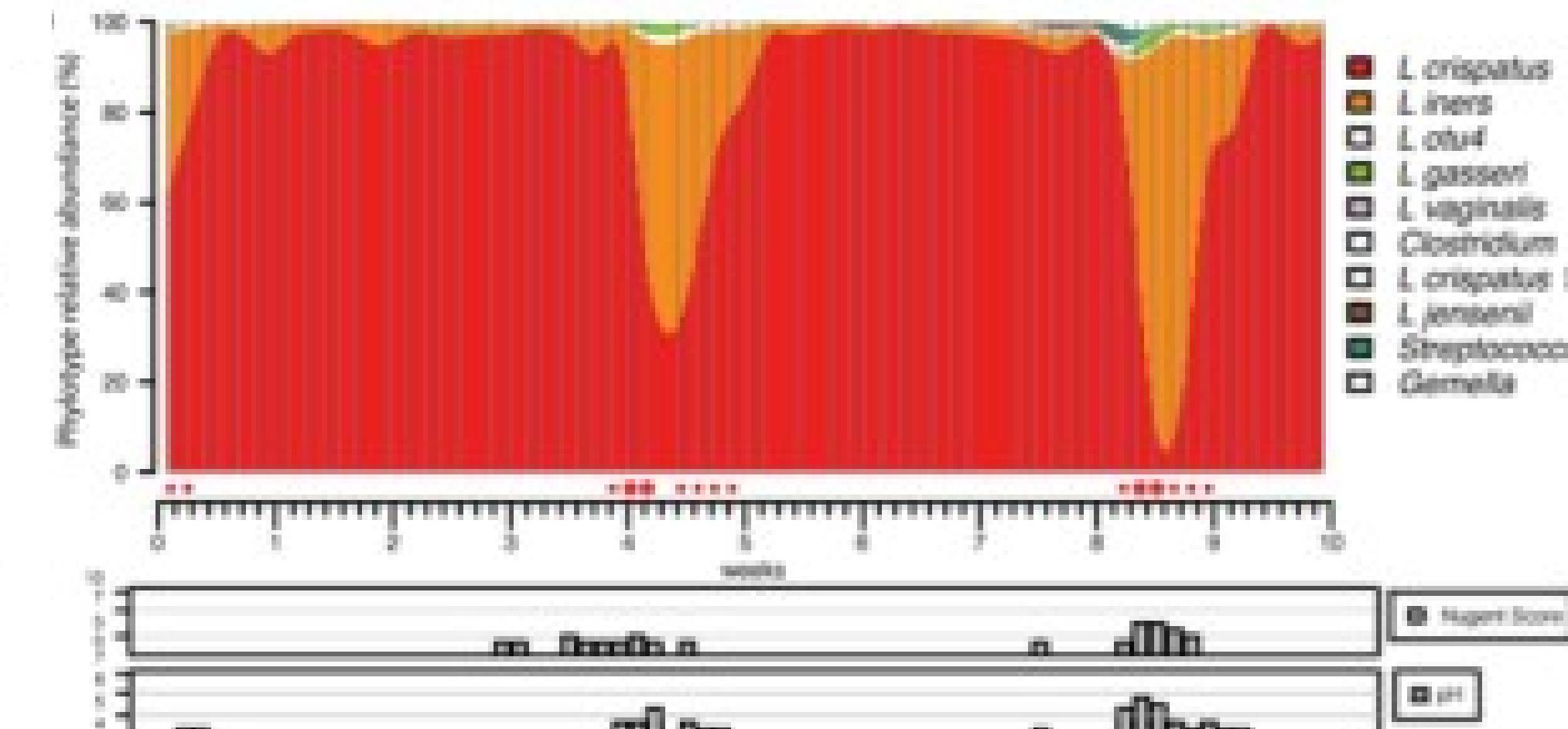
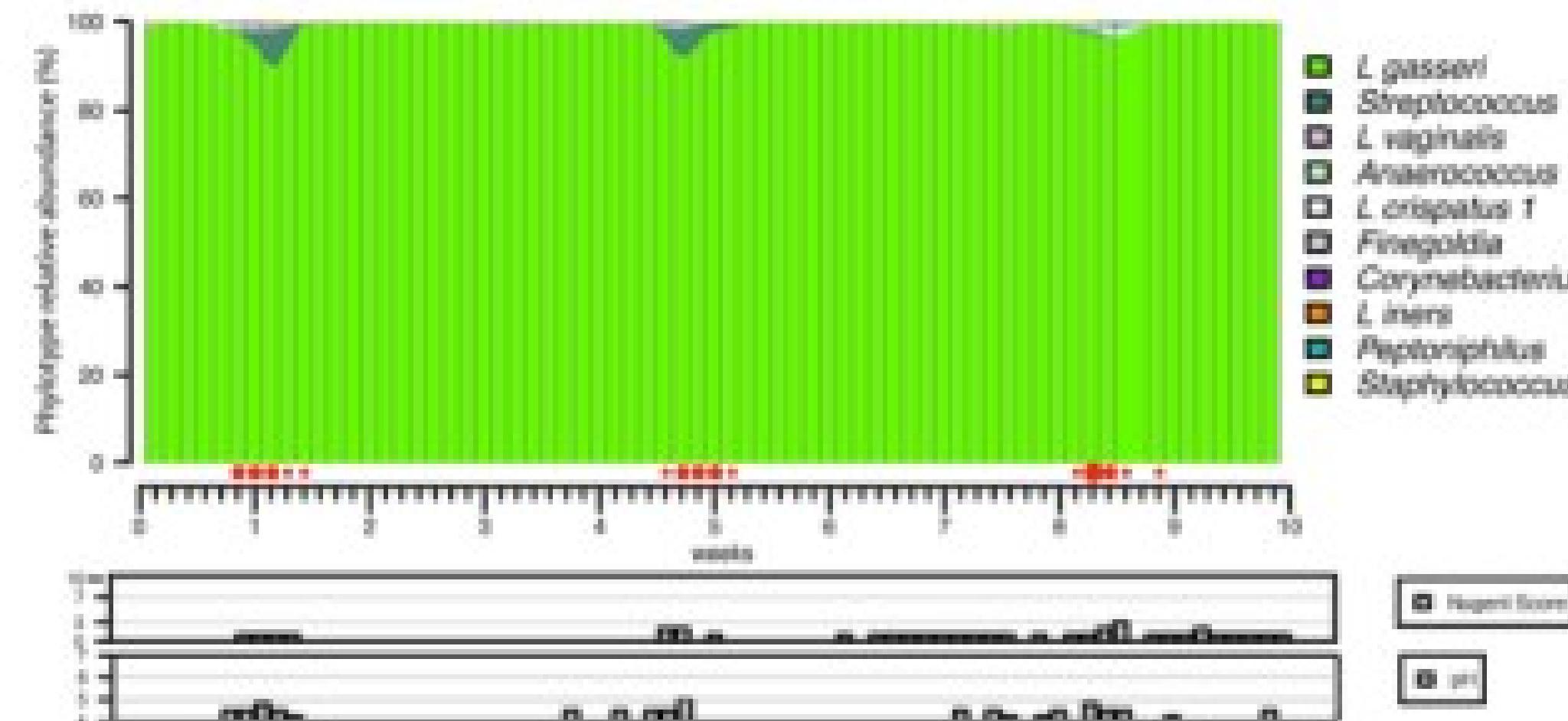


# The dynamics of the vaginal microbiota

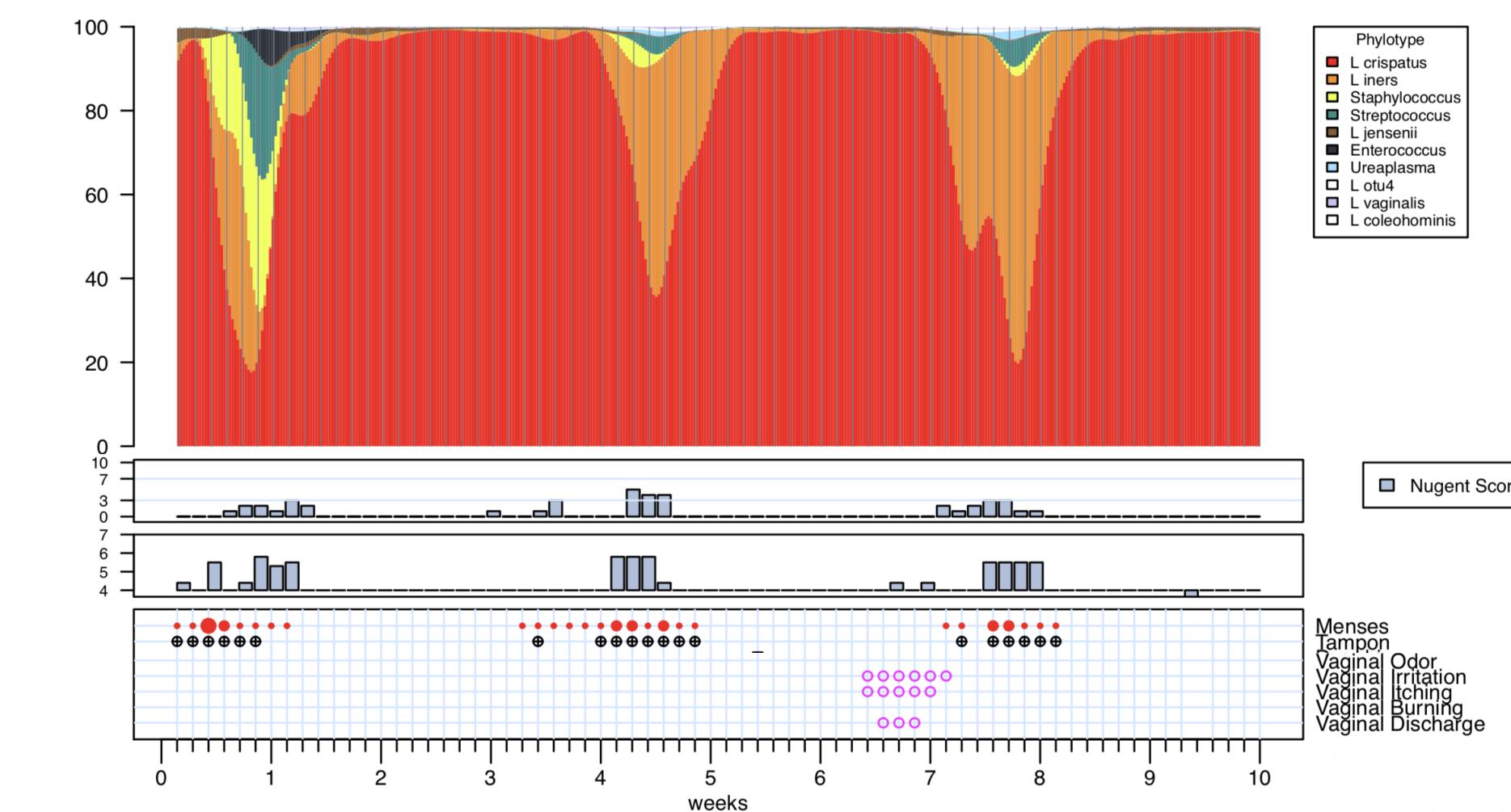
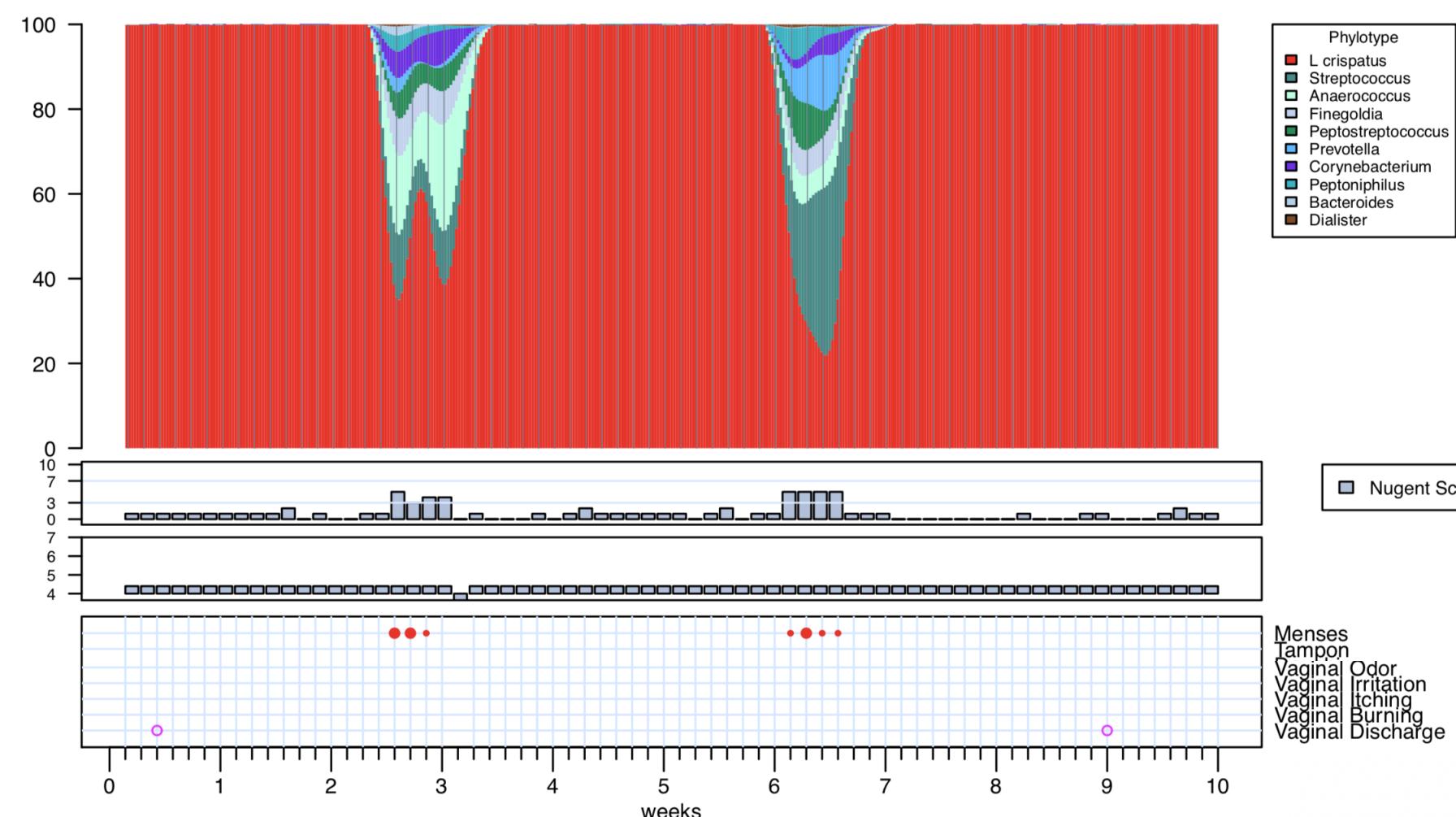
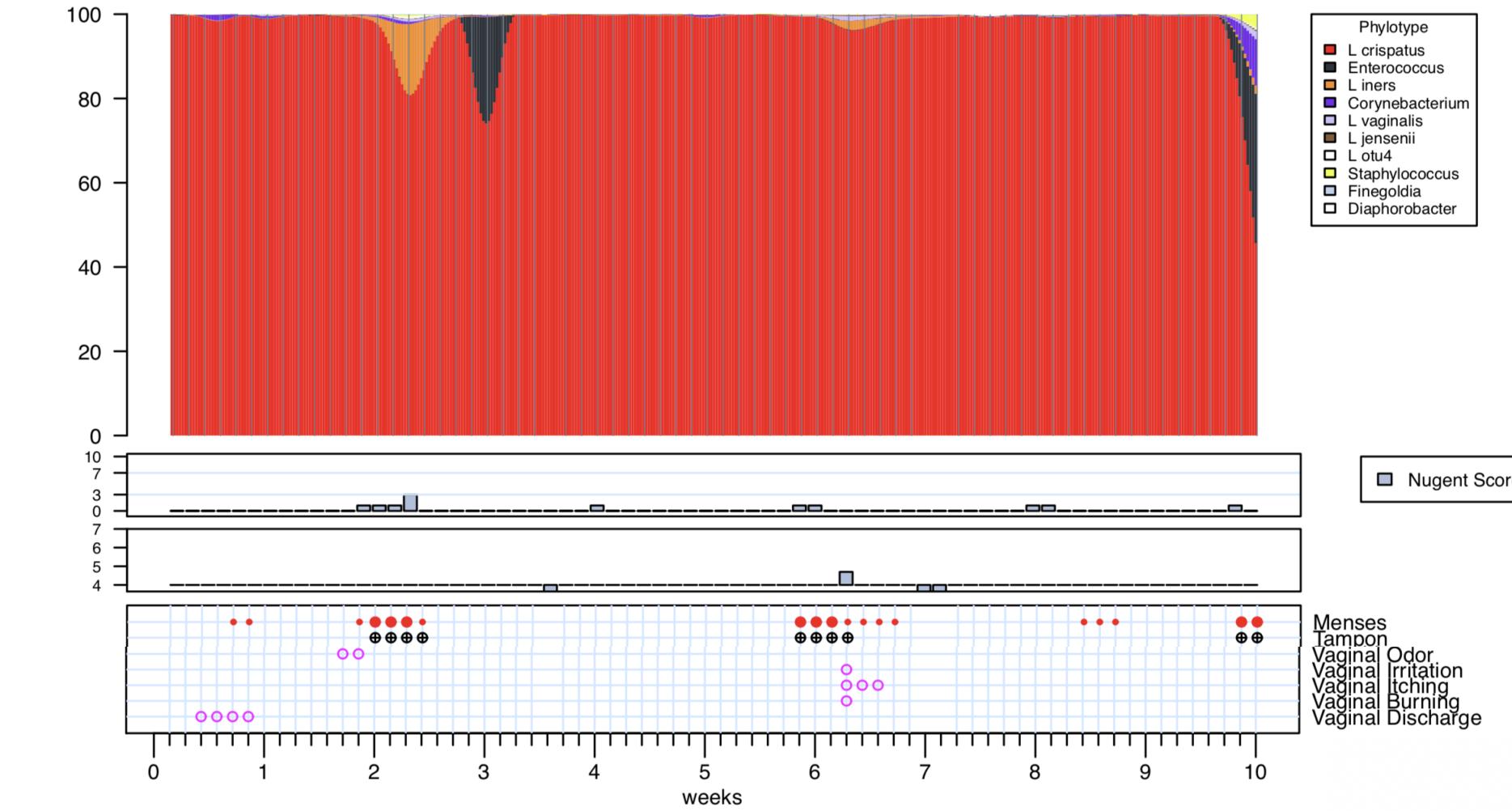
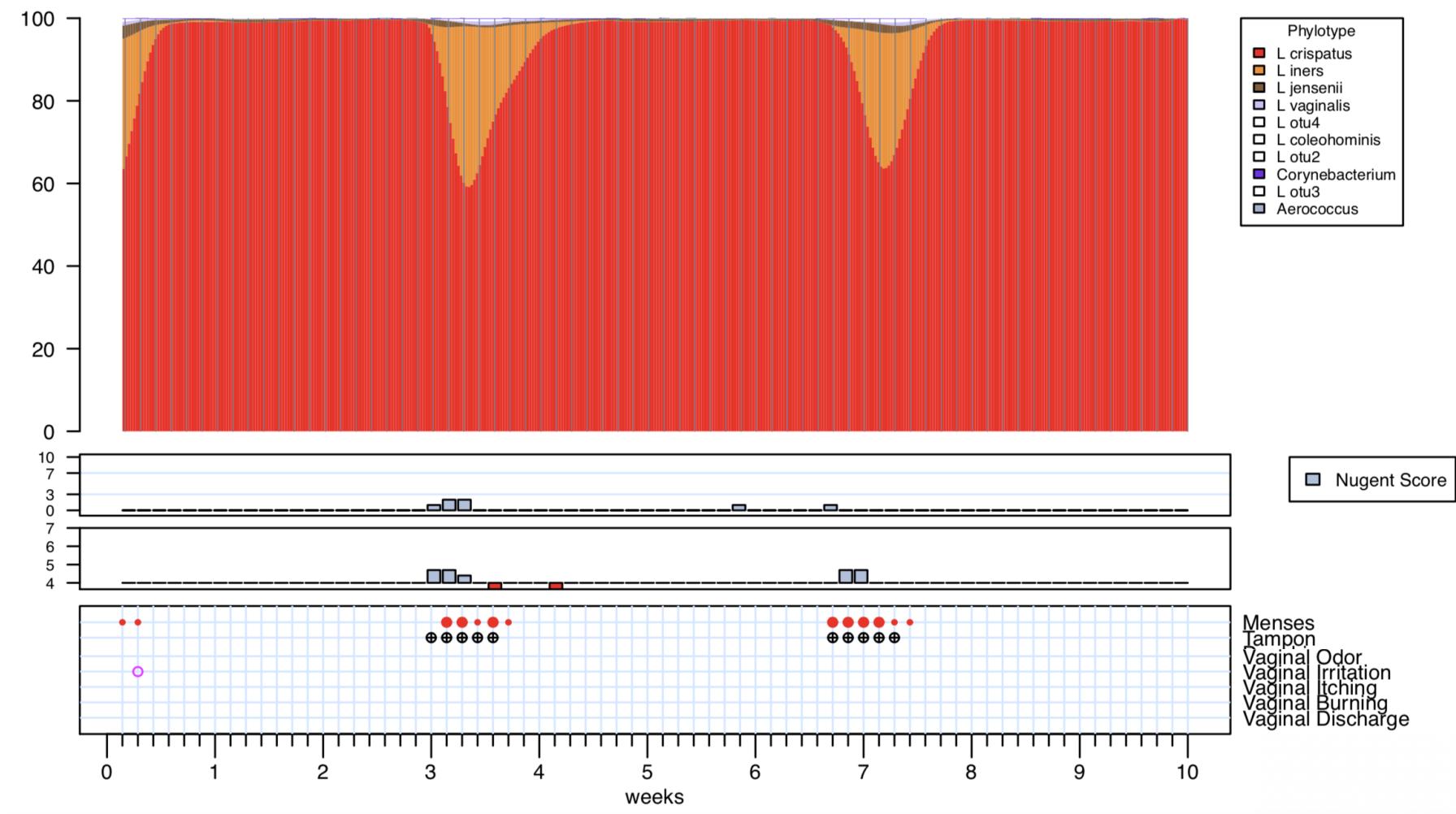
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- Clinical study of 160 women who collected daily samples for 10 weeks
- Daily dairies used to collect relevant metadata, including hygiene, sexual practices among others
- Characterize the composition of the vaginal microbiota using 16S rRNA gene sequencing
- Several types of longitudinal profiles were obtained

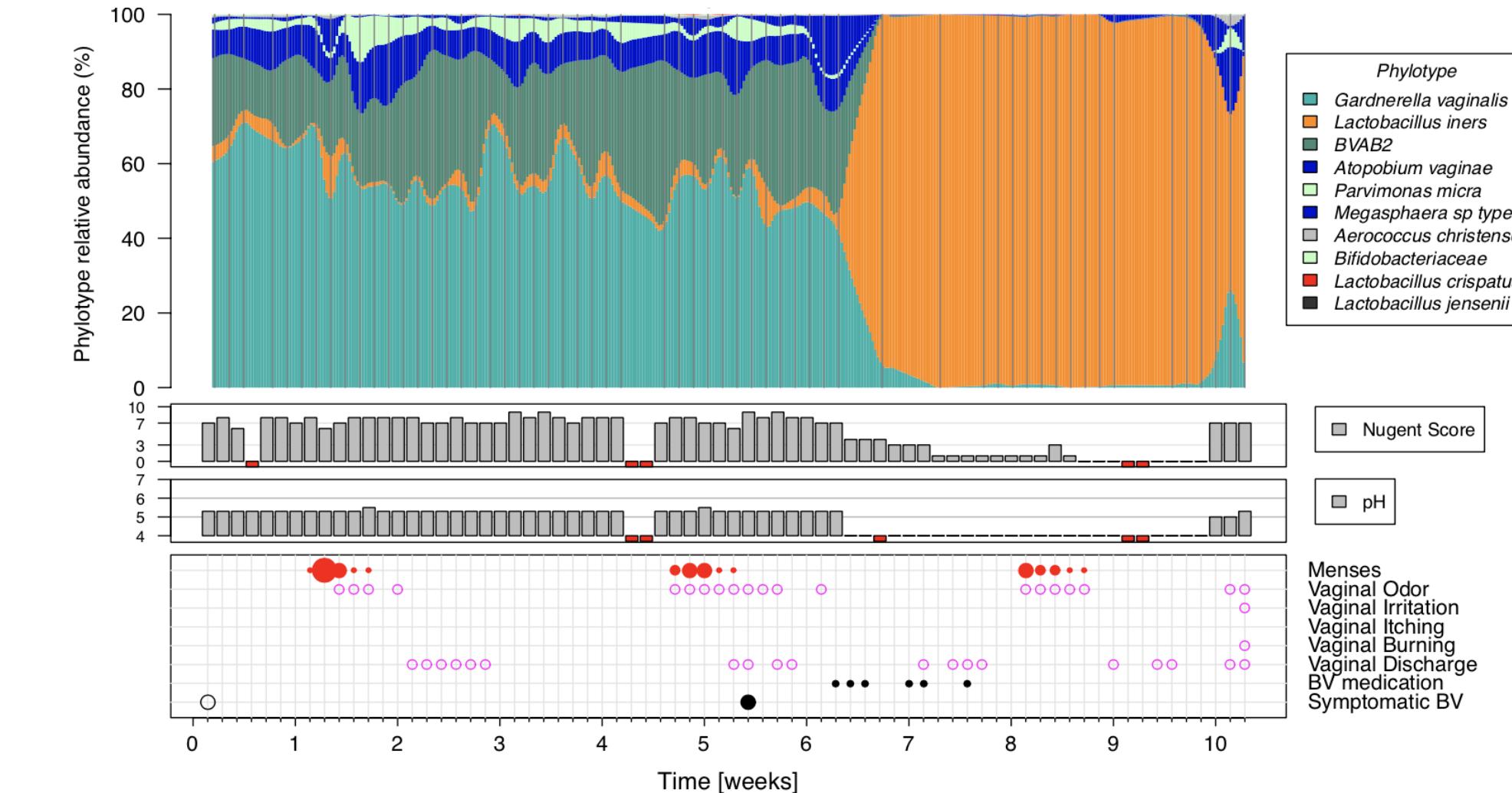
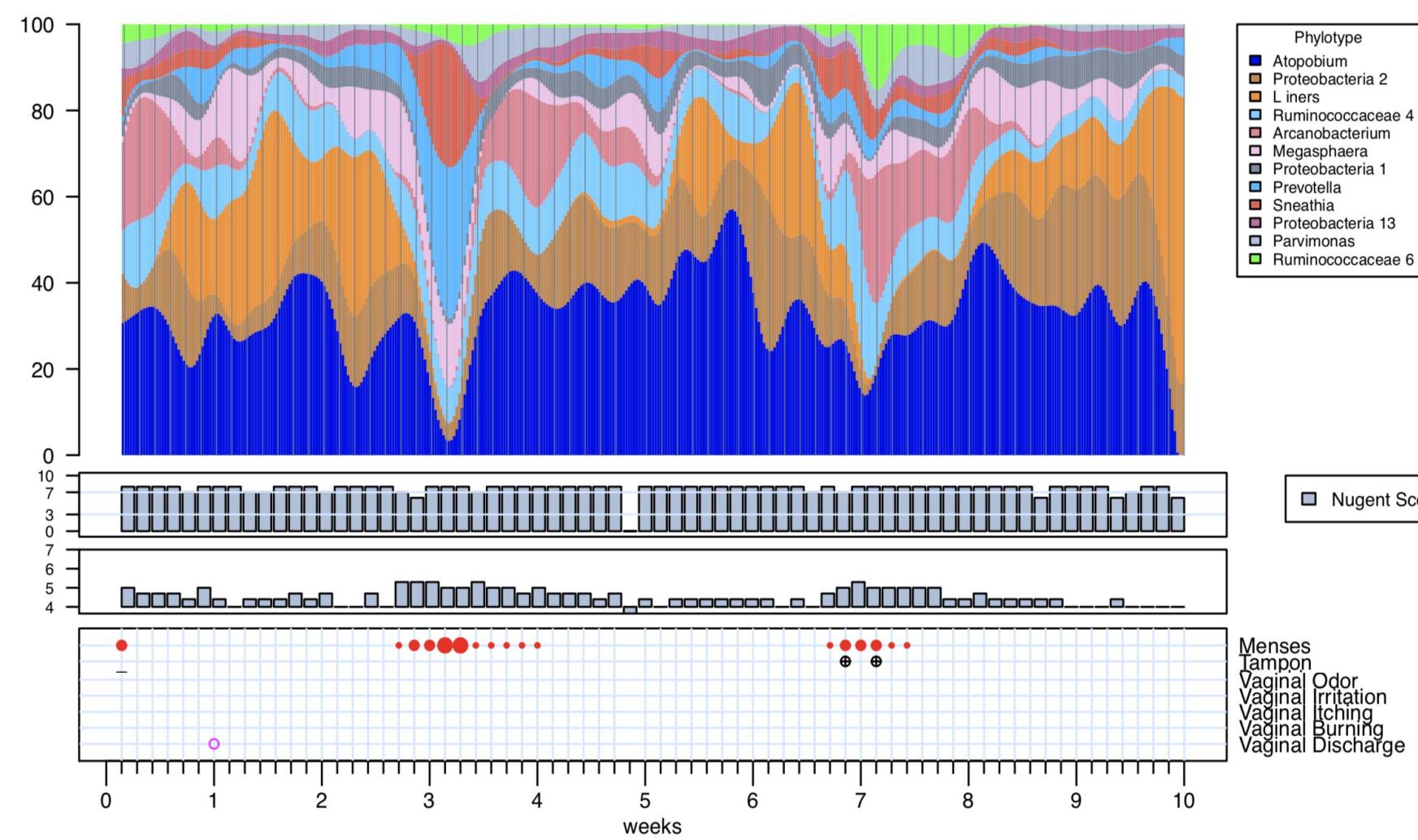
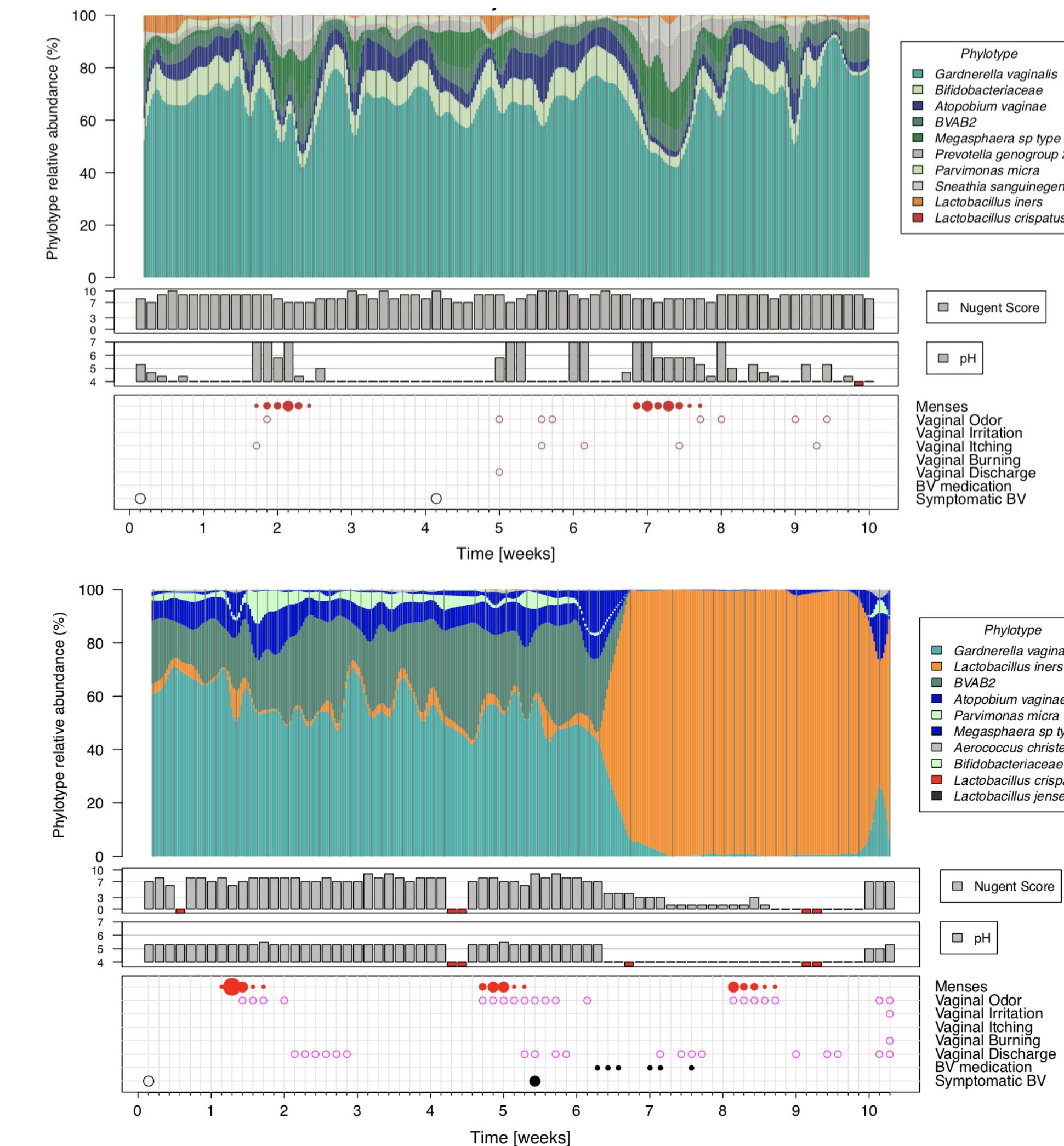
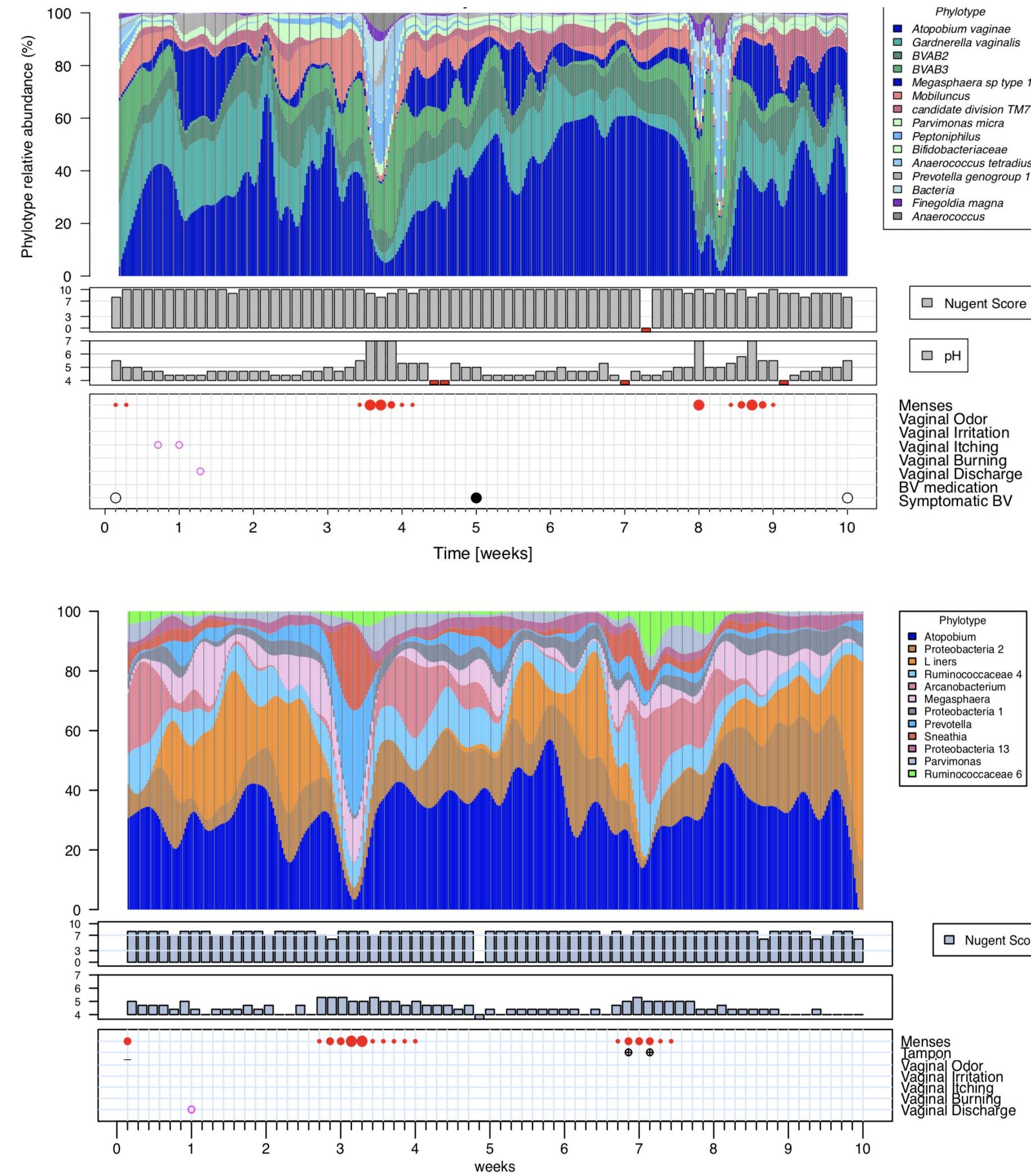
# The dynamics of the vaginal microbiota



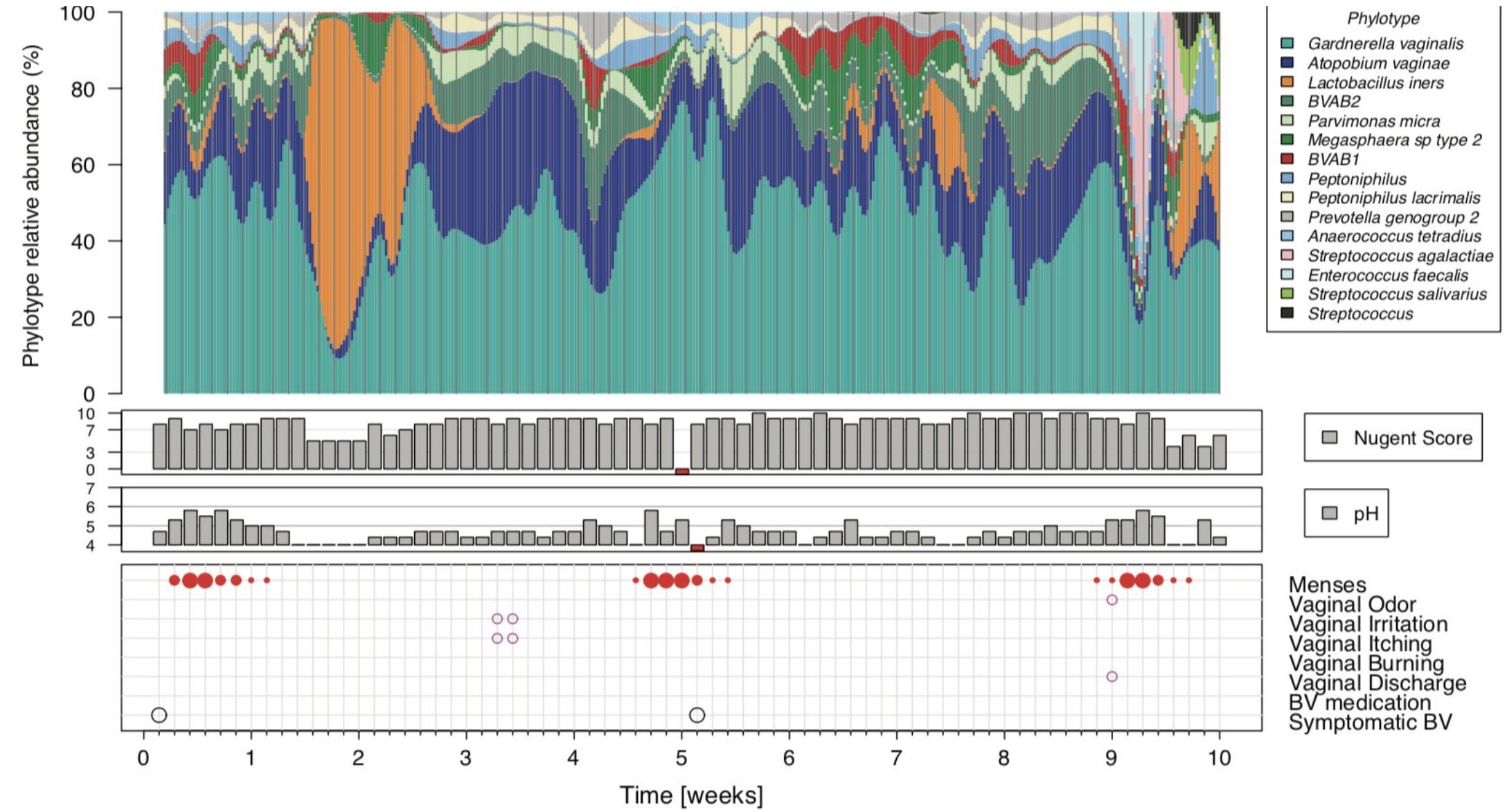
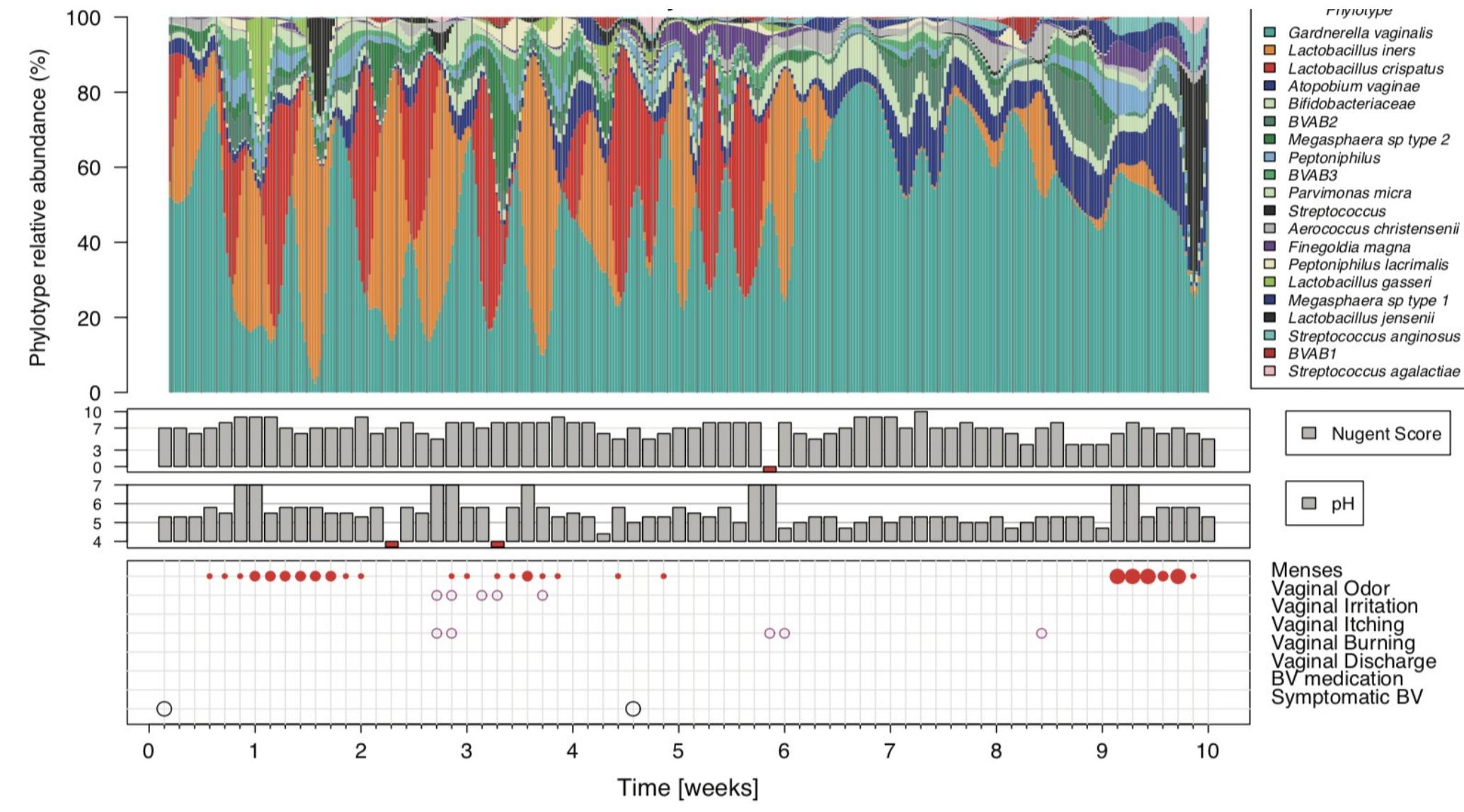
# The dynamics of the vaginal microbiota



# The dynamics of the vaginal microbiota

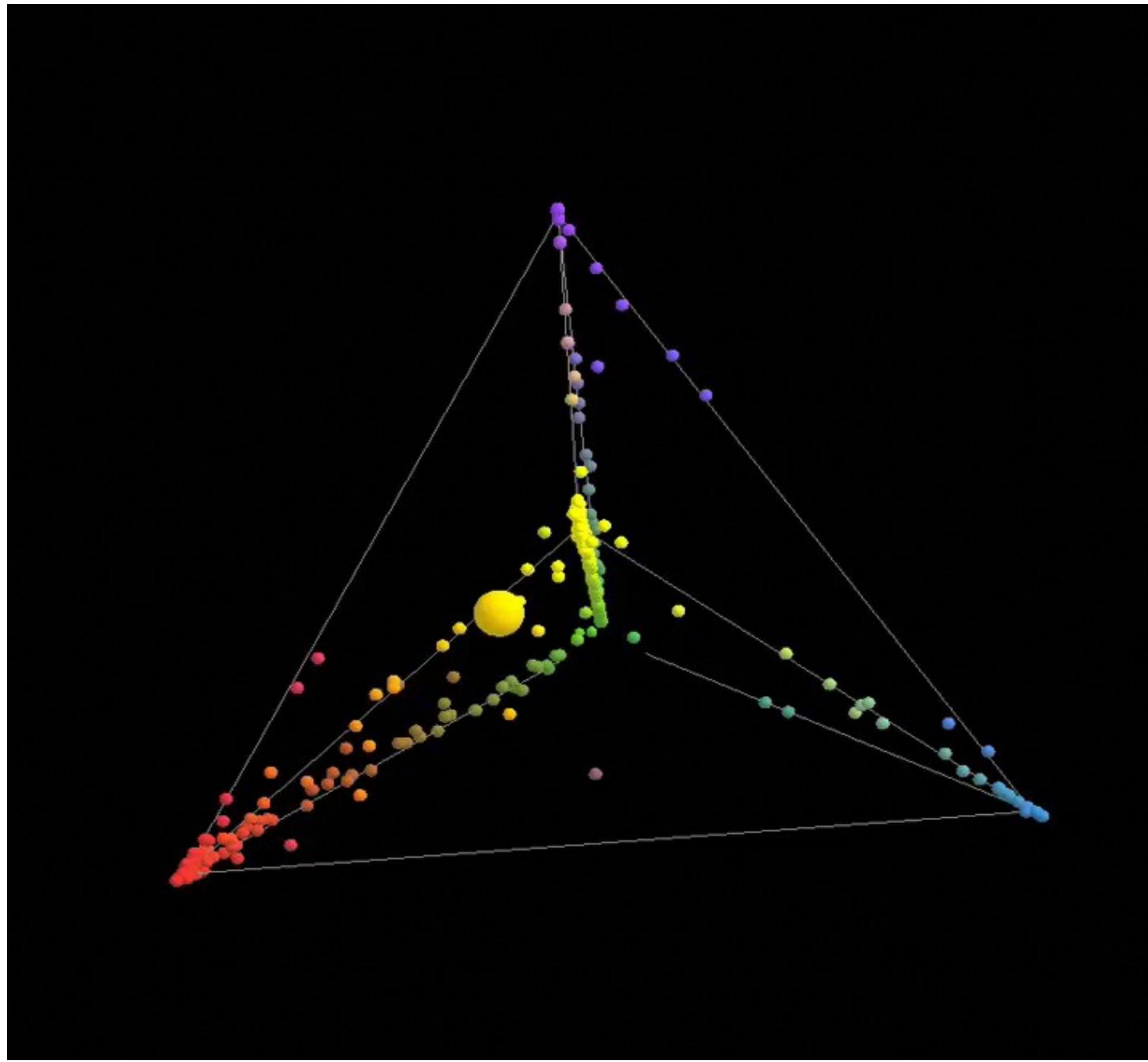


# The dynamics of the vaginal microbiota



# The dynamics of the vaginal microbiota

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# Adverse outcomes associated with CST IV types microbiota

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Whether associated with symptoms or not, non-optimal vaginal microbiota carry many risks:

## Gynecologic

Pelvic inflammatory disease (PID)  
Acquisition of STIs – HSV-2, HIV, *Chlamydia*, UTI...  
Cervical cancer  
Morbidity –vaginal discharge and malodor

## Reproductive

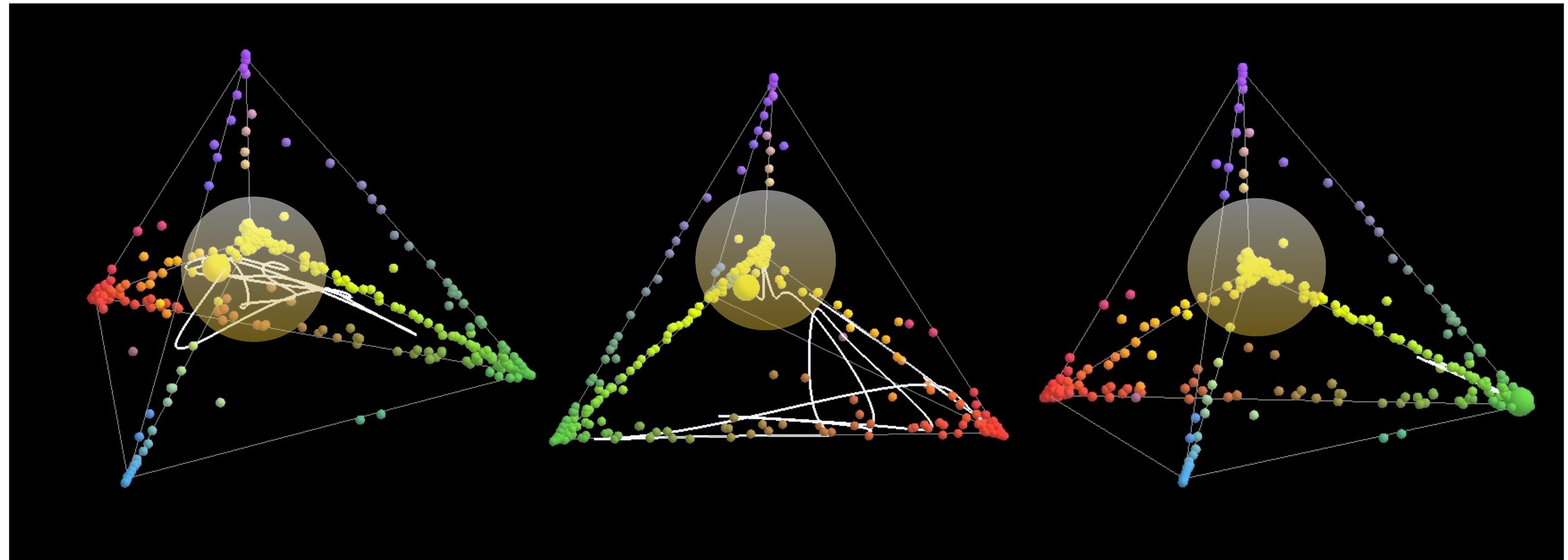
Endometriosis, Myomatosis, Hyperplasia,  
Polyposis...  
Endocervical cancer  
Difficulty to conceive  
ART failure

## Obstetric

Preterm delivery and low birth weight  
Postpartum endometritis  
Amniotic fluid infection  
Chorioamnionitis

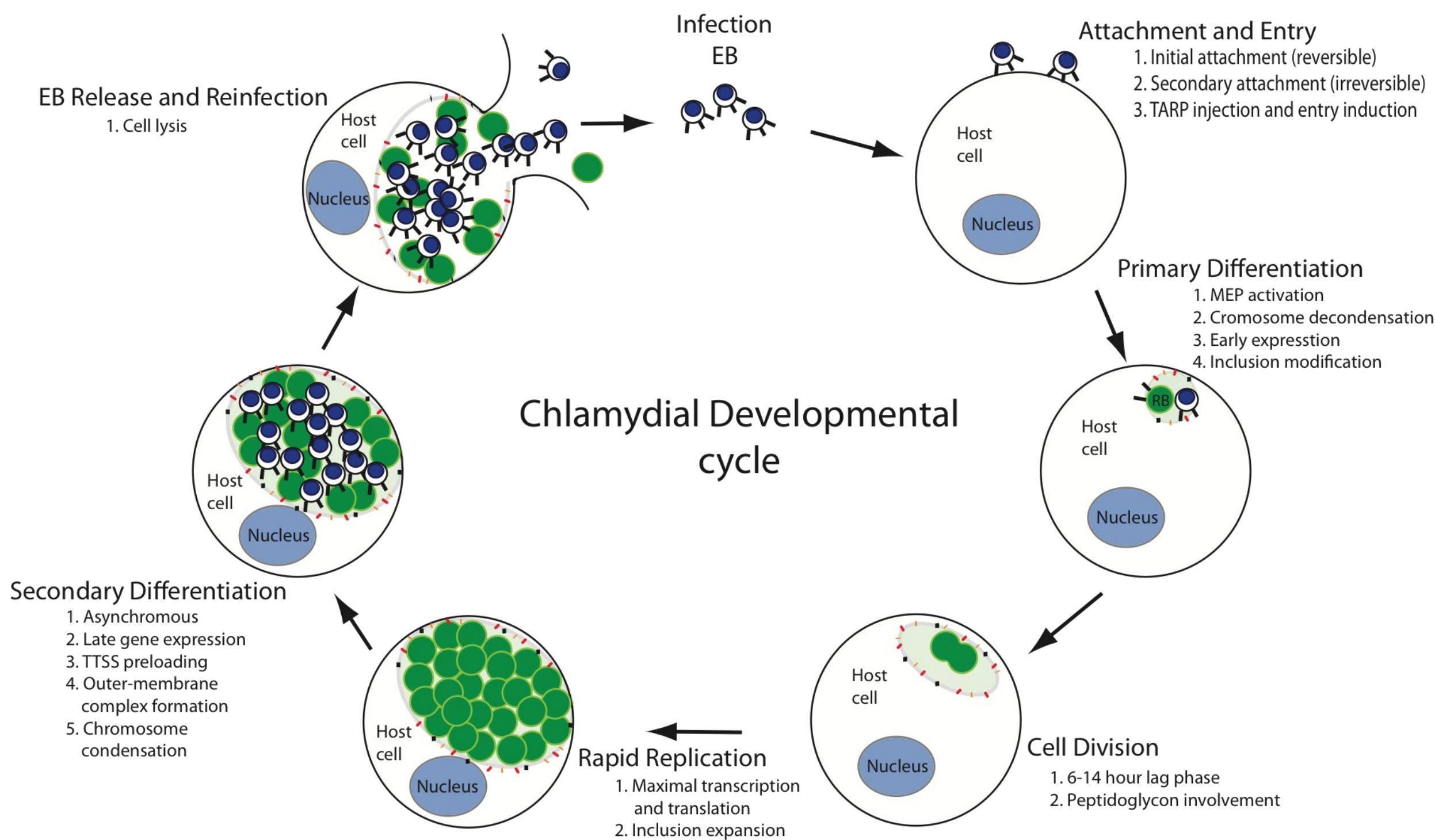
# The dynamics of the vaginal microbiota and risk of adverse outcomes

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



The dynamics of the vaginal microbiota (frequency and duration of CST IV events) better represents the risk of adverse outcomes.

# *Chlamydia trachomatis*

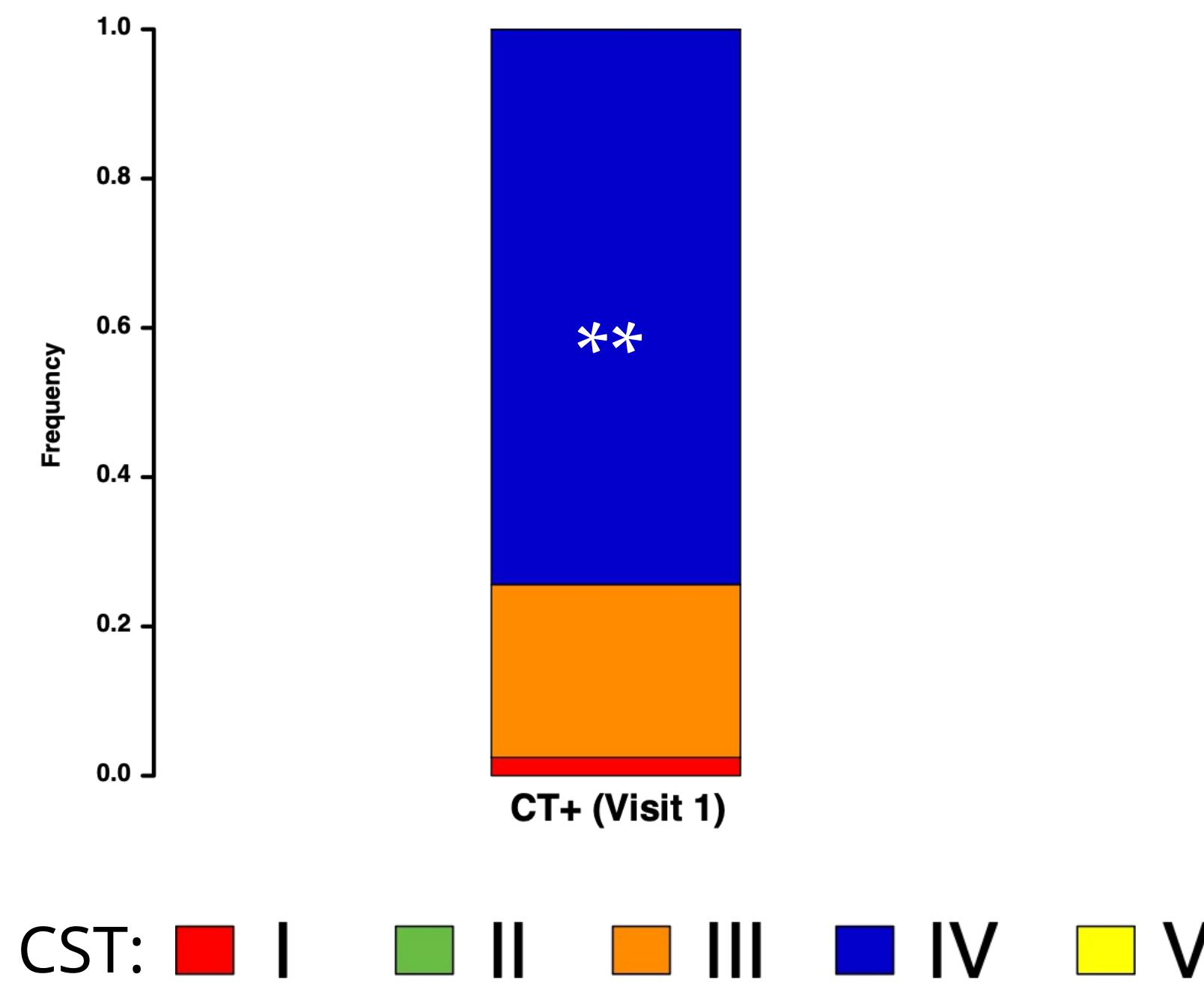


- The rate of infection after a sexual contact with an infected partner by *C. trachomatis* is estimated at 25-40%

# The vaginal microbiota and *Chlamydia trachomatis* infections

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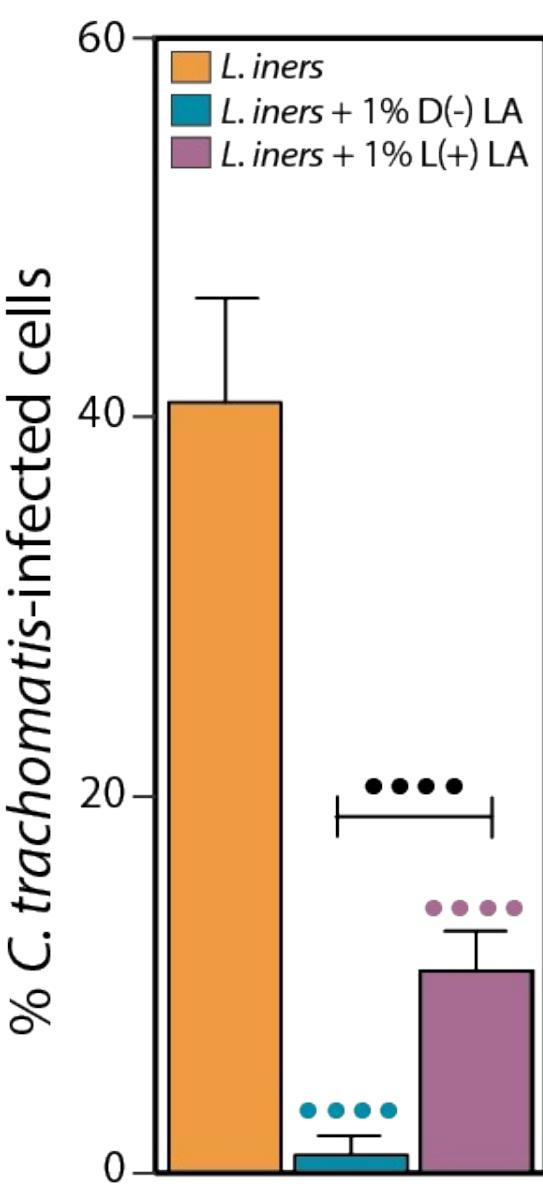
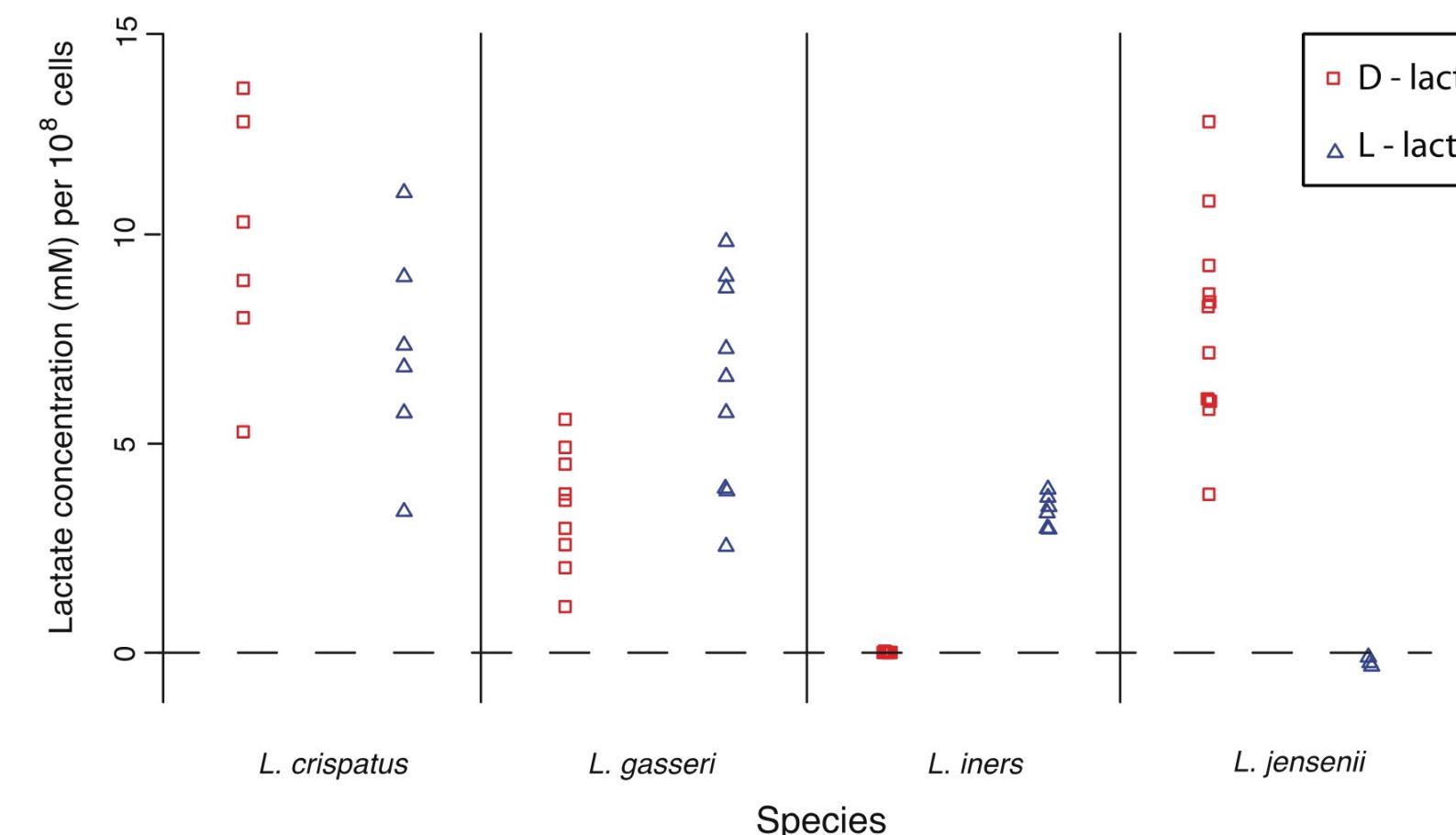
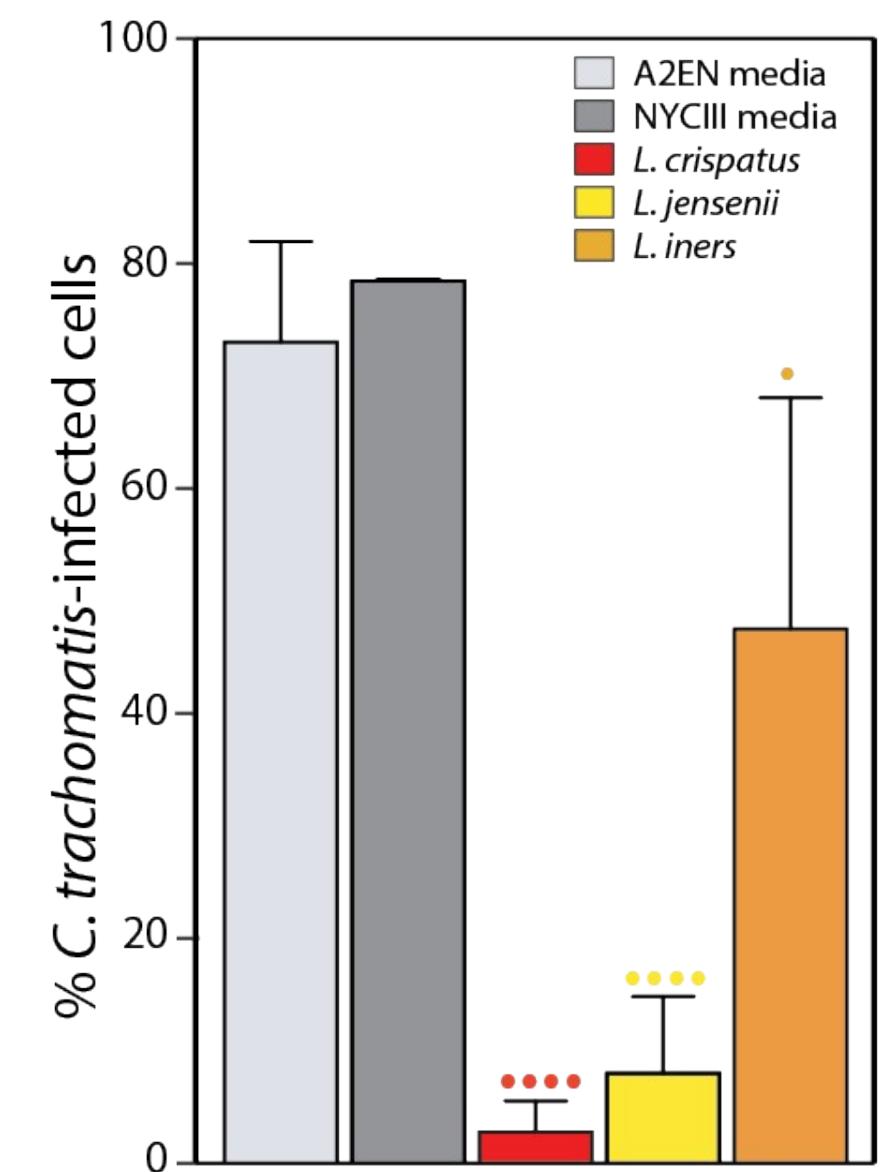
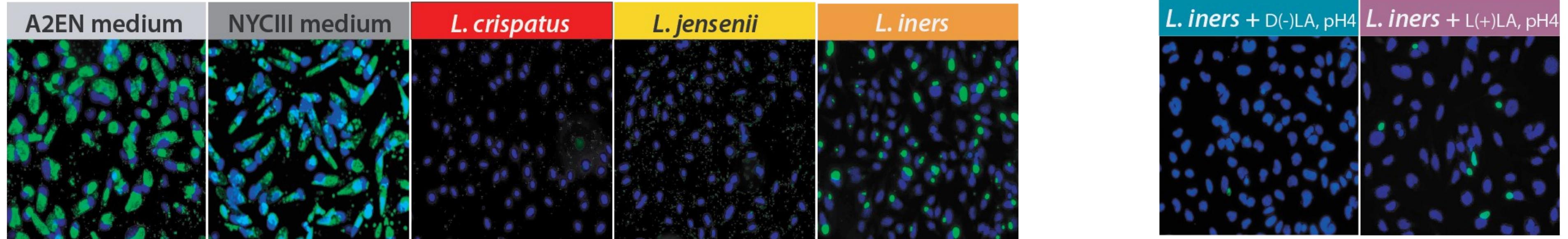
- 150 CT+ women
- Azithromycin treatment
- Resampled 3 months later



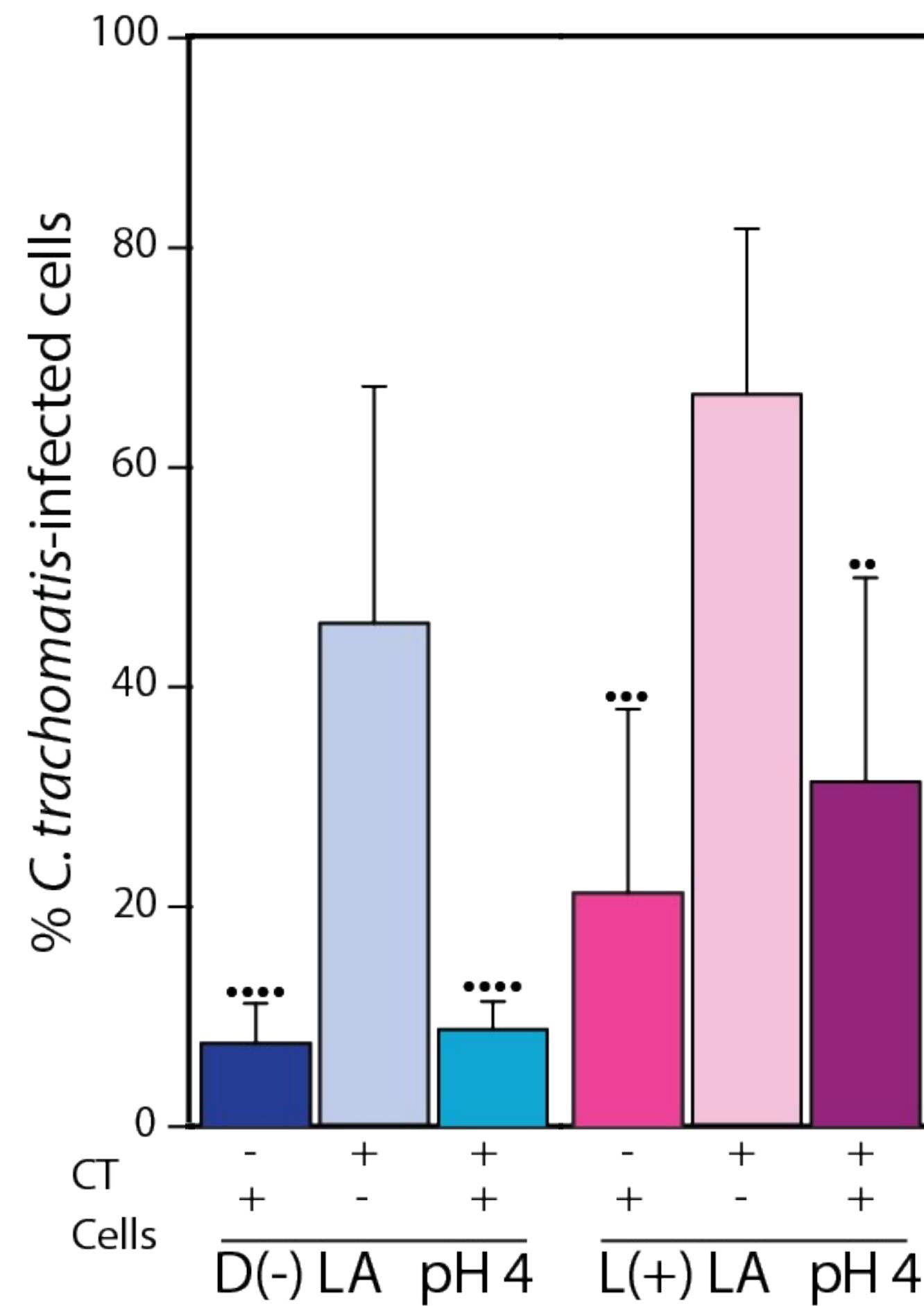
# *Lactobacillus* spp. differentially affect *C. trachomatis* *in vitro* infection



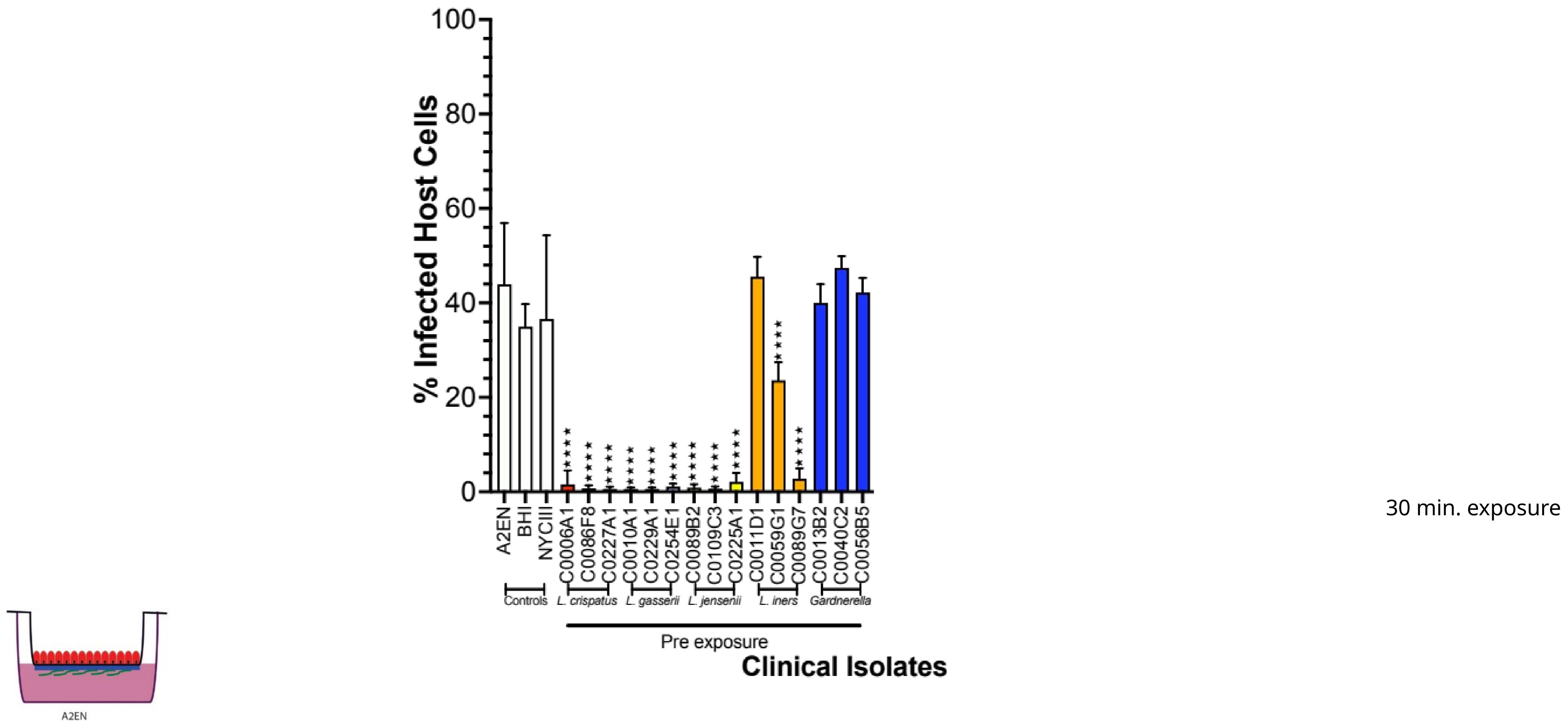
Vonetta  
Edwards



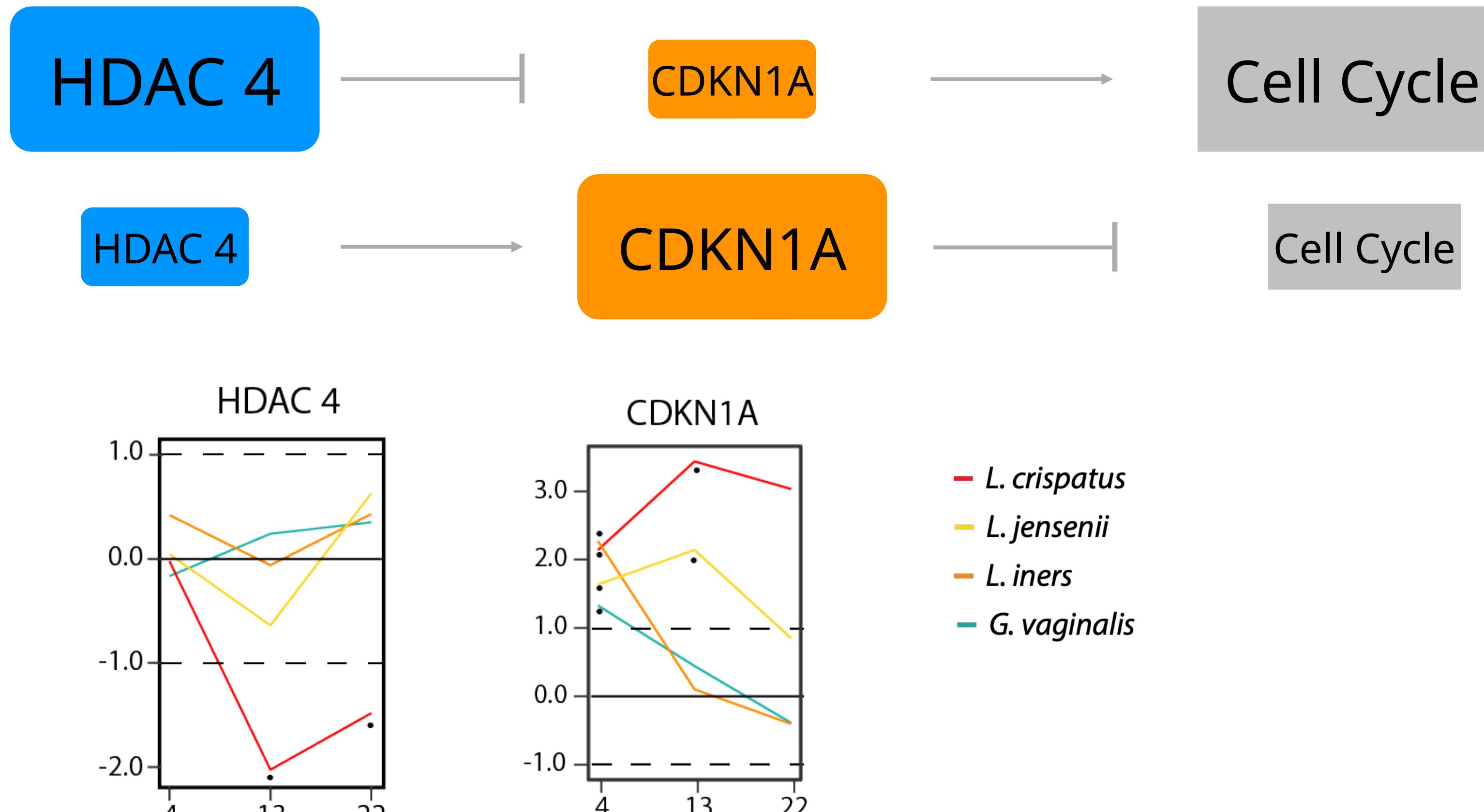
# Lactic acid does not directly affect *C. trachomatis*



# *Lactobacillus* spp. differentially affect *C. trachomatis* *in vitro* infection



# Epigenetic modulation of the cell cycle by the vaginal bacteria

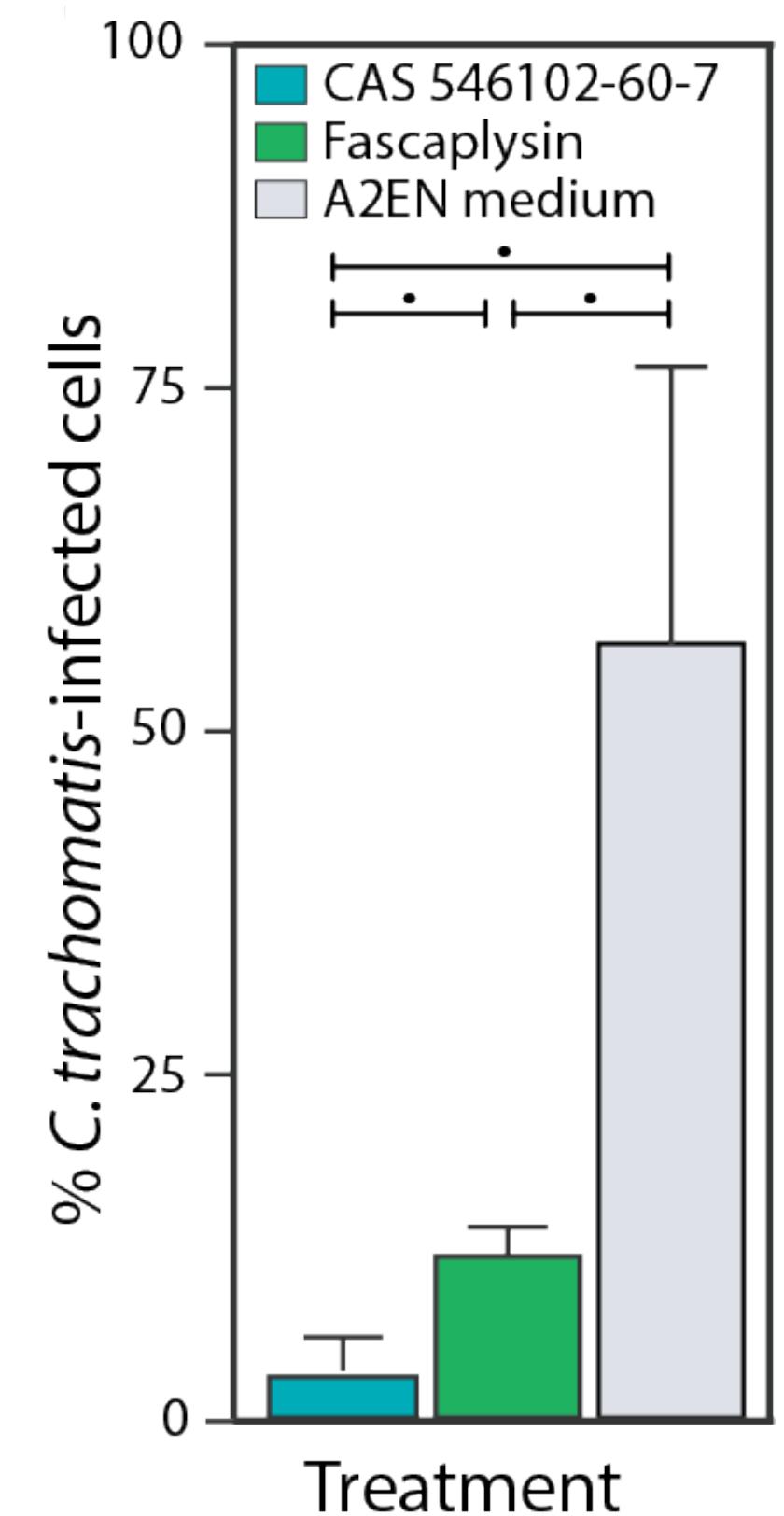


RESEARCH ARTICLE

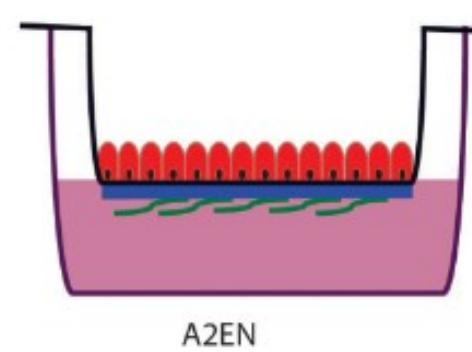
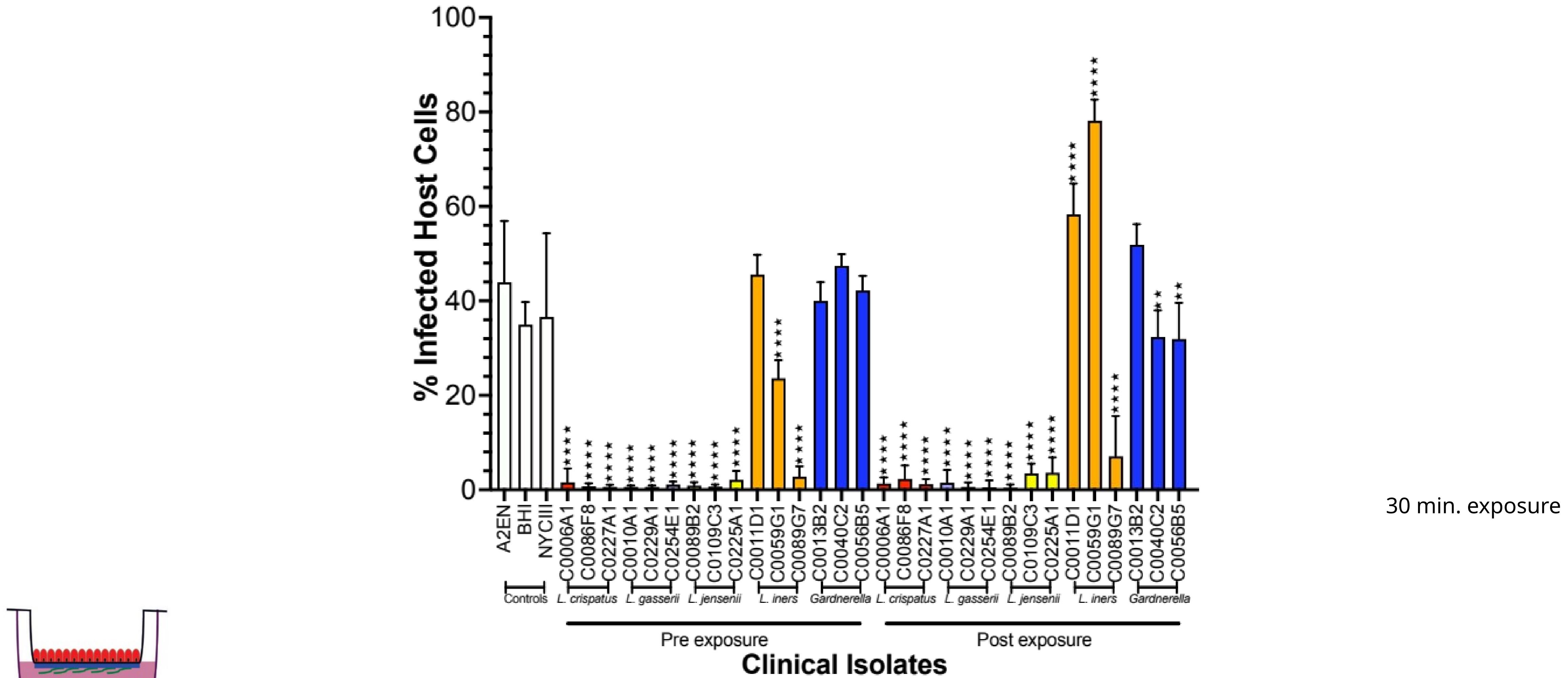
Open Access

Activation of epidermal growth factor receptor is required for *Chlamydia trachomatis* development

Achchhe L Patel<sup>1†</sup>, Xiaofei Chen<sup>1†</sup>, Scott T Wood<sup>1</sup>, Elizabeth S Stuart<sup>2</sup>, Kathleen F Arcaro<sup>2</sup>, Doris P Molina<sup>3</sup>, Snezana Petrovic<sup>3</sup>, Cristina M Furdui<sup>1\*</sup> and Allen W Tsang<sup>1\*</sup>



# *Lactobacillus* spp. differentially affect *C. trachomatis* *in vitro* infection

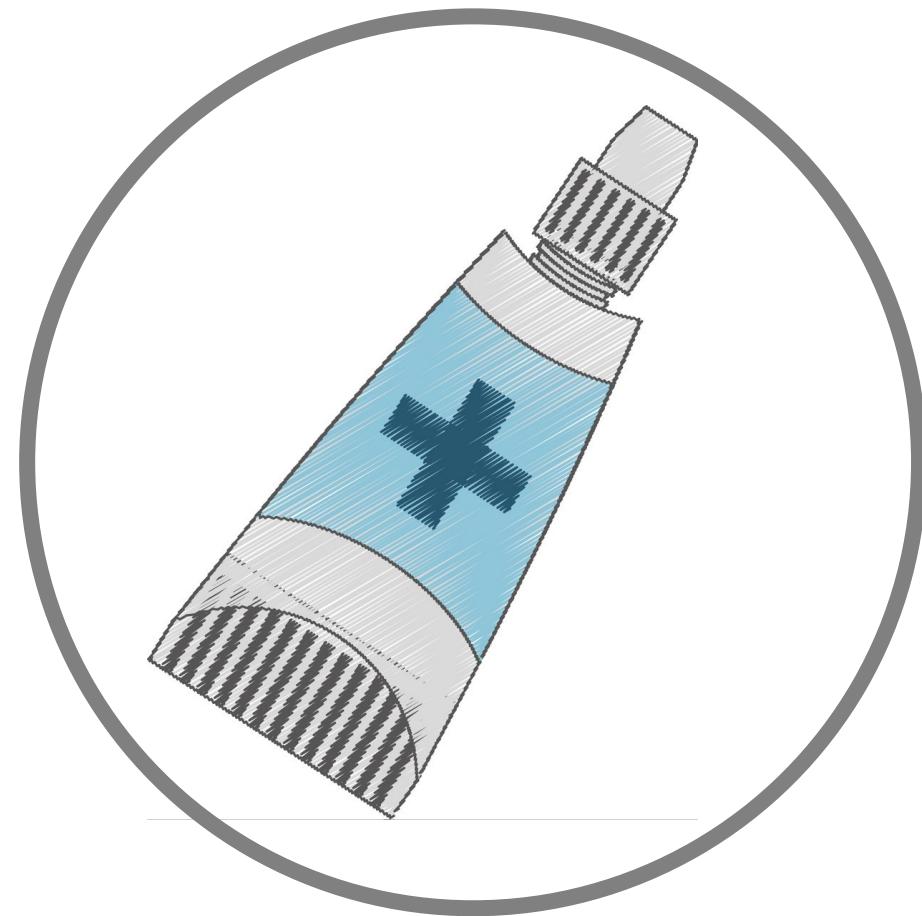


Exposure to different *Lactobacillus* species and strains can prevent and resolve chlamydial infection

# Treatment for cervicovaginal conditions, where are we now?

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Progression of treatment for bacterial vaginosis



1960's

Triple sulfa  
cream

# New therapeutics to cure and prevent infection/diseases

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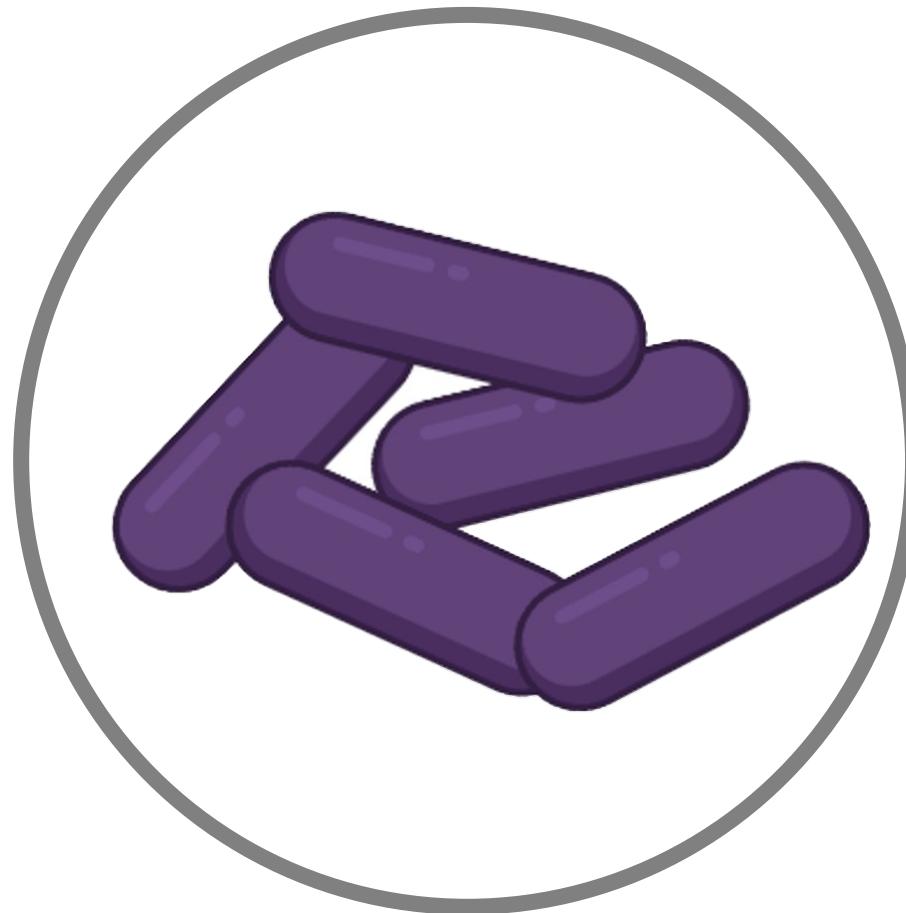
There is a need for antibiotic-sparing live biotherapeutic drugs to modulate the composition and function of the vaginal microbiota with the intent to restore an optimal microbiota to cure and prevent infection/diseases.

A vaginal microbiome-based therapeutics should at least be administered vaginally as they intend to modify the composition (colonization) and function of the vaginal microbiome to exert their effect.

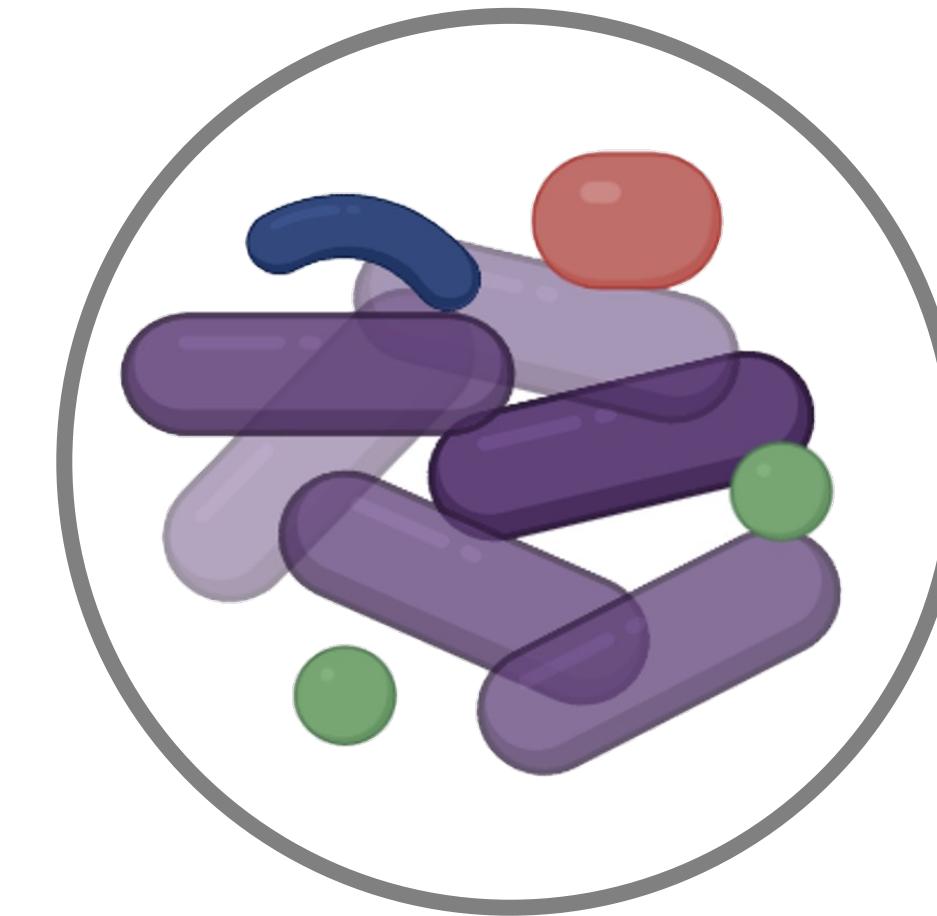
Thus, from a regulatory standpoint, microbiome-based LBPs will be regulated as drugs, and **undergo rigorous clinical trial and obtained regulatory authorization**

# Live Biotherapeutics Products (LBP) development

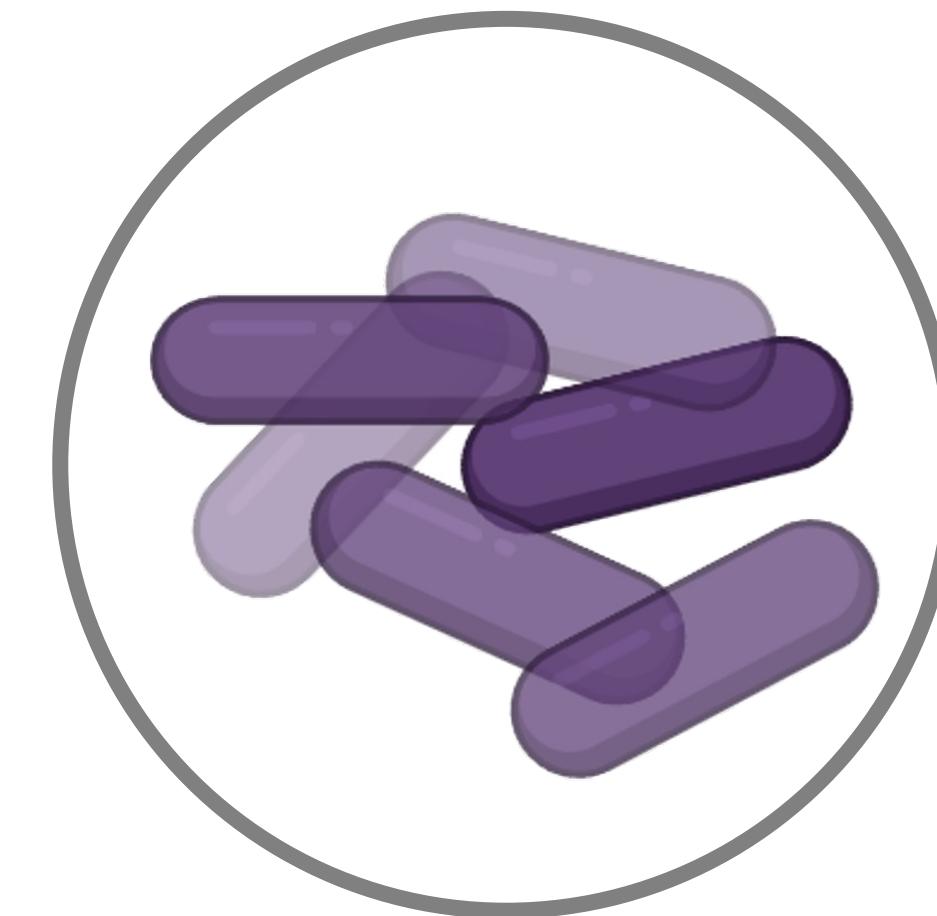
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**Lactin-V:**  
Single species  
Single strain



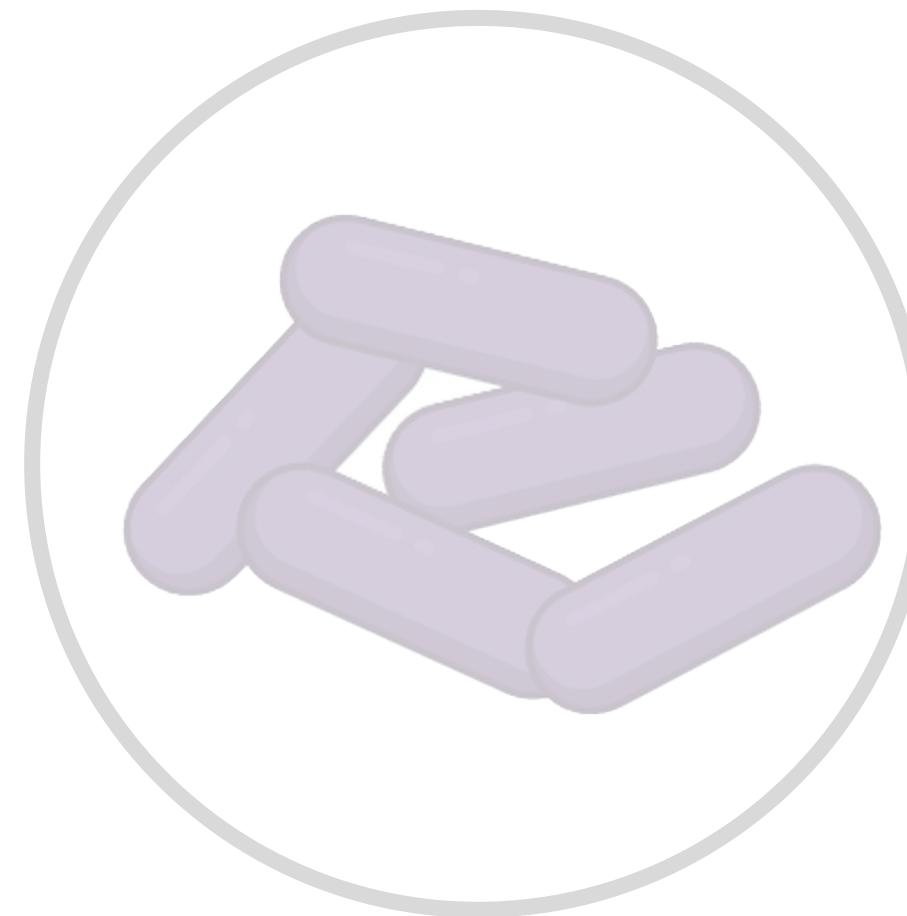
**Vaginal Microbiota  
Transplant:**  
Dominant species  
Whole community



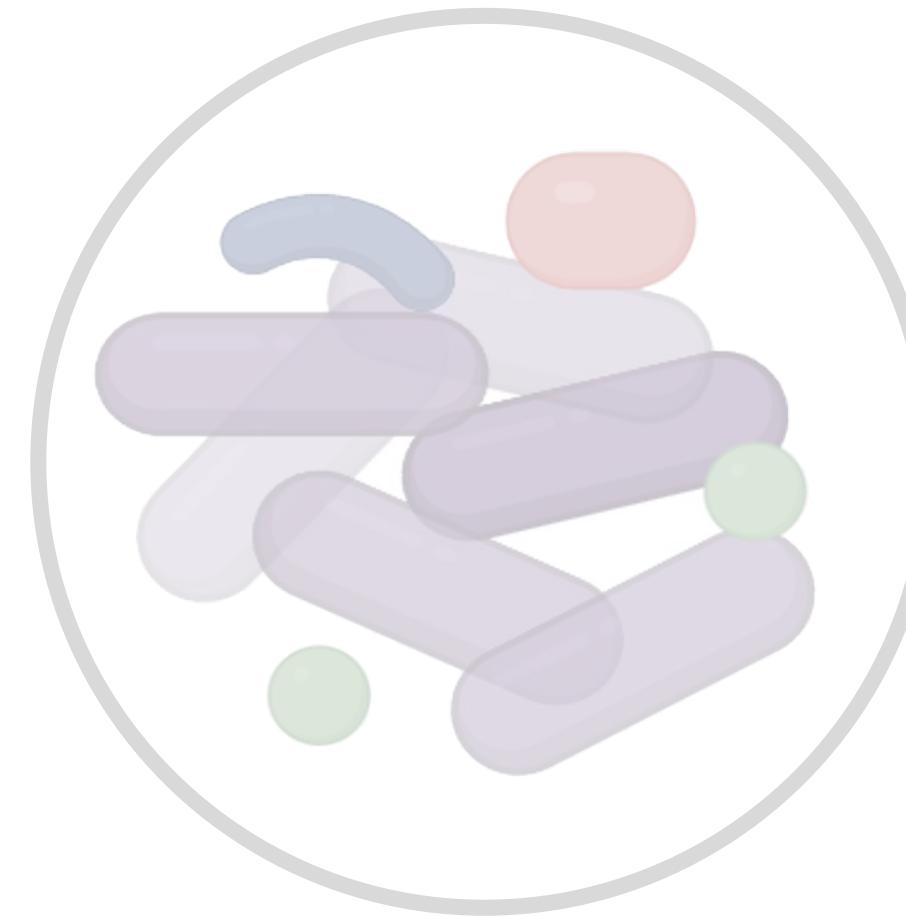
**Multi-strain  
LBP:**  
Single species  
Multiple strains

# Live Biotherapeutics Products (LBP) development

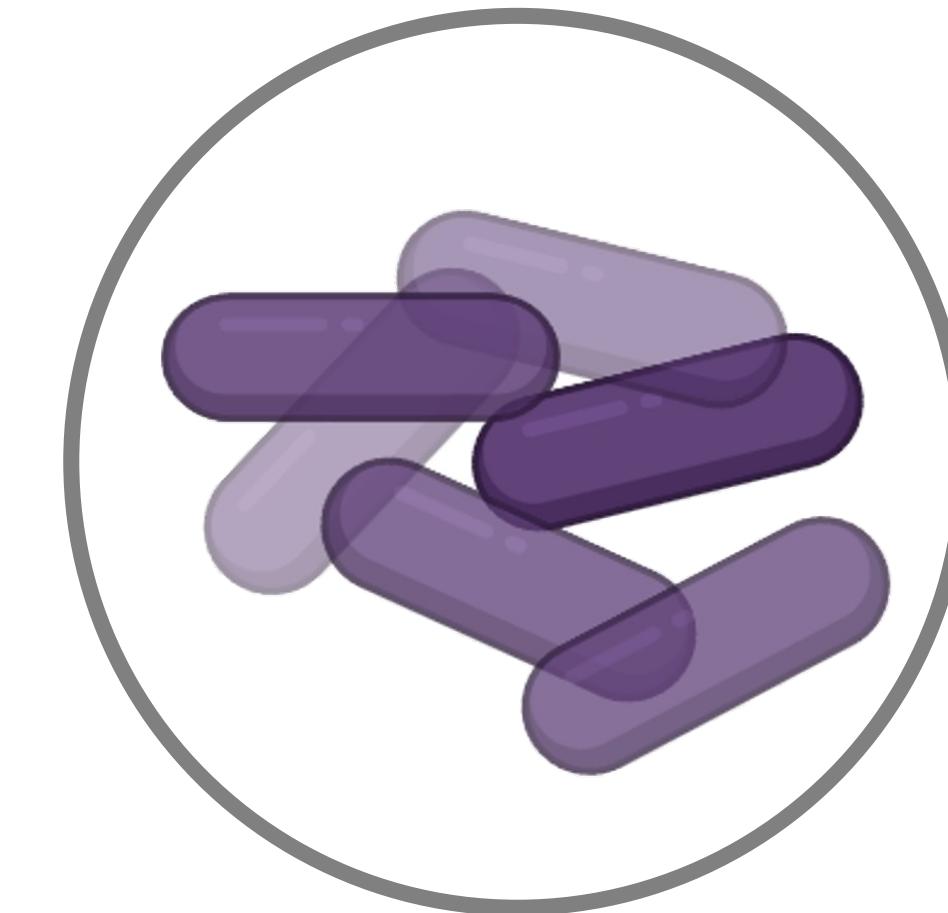
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**Lactin-V:**  
Single species  
Single strain



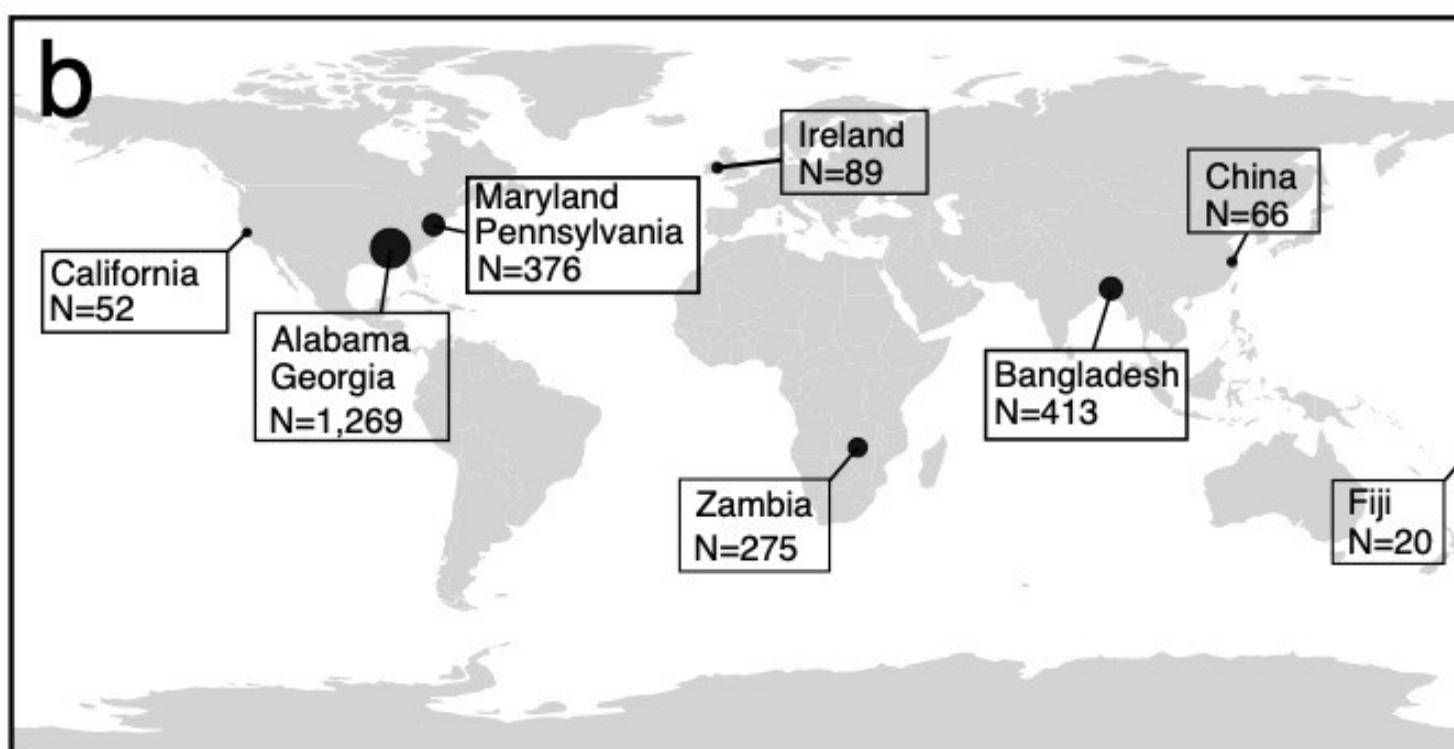
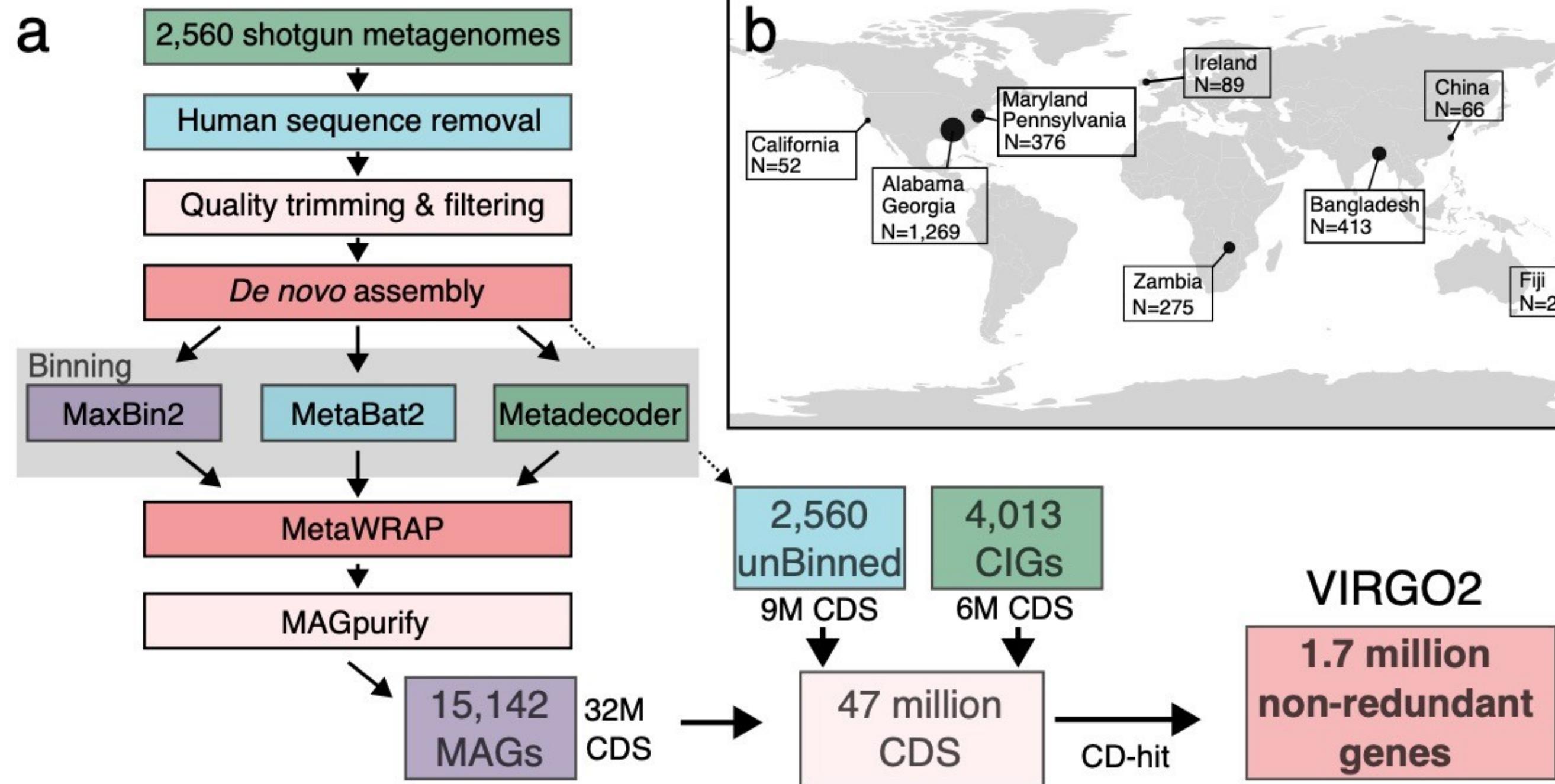
**Vaginal Microbiota Transplant:**  
Dominant species  
Supporting community



**Multi-strain LBP:**  
Single species  
Multiple strains

# Metagenomics exploration of the vaginal microbiome functions

a



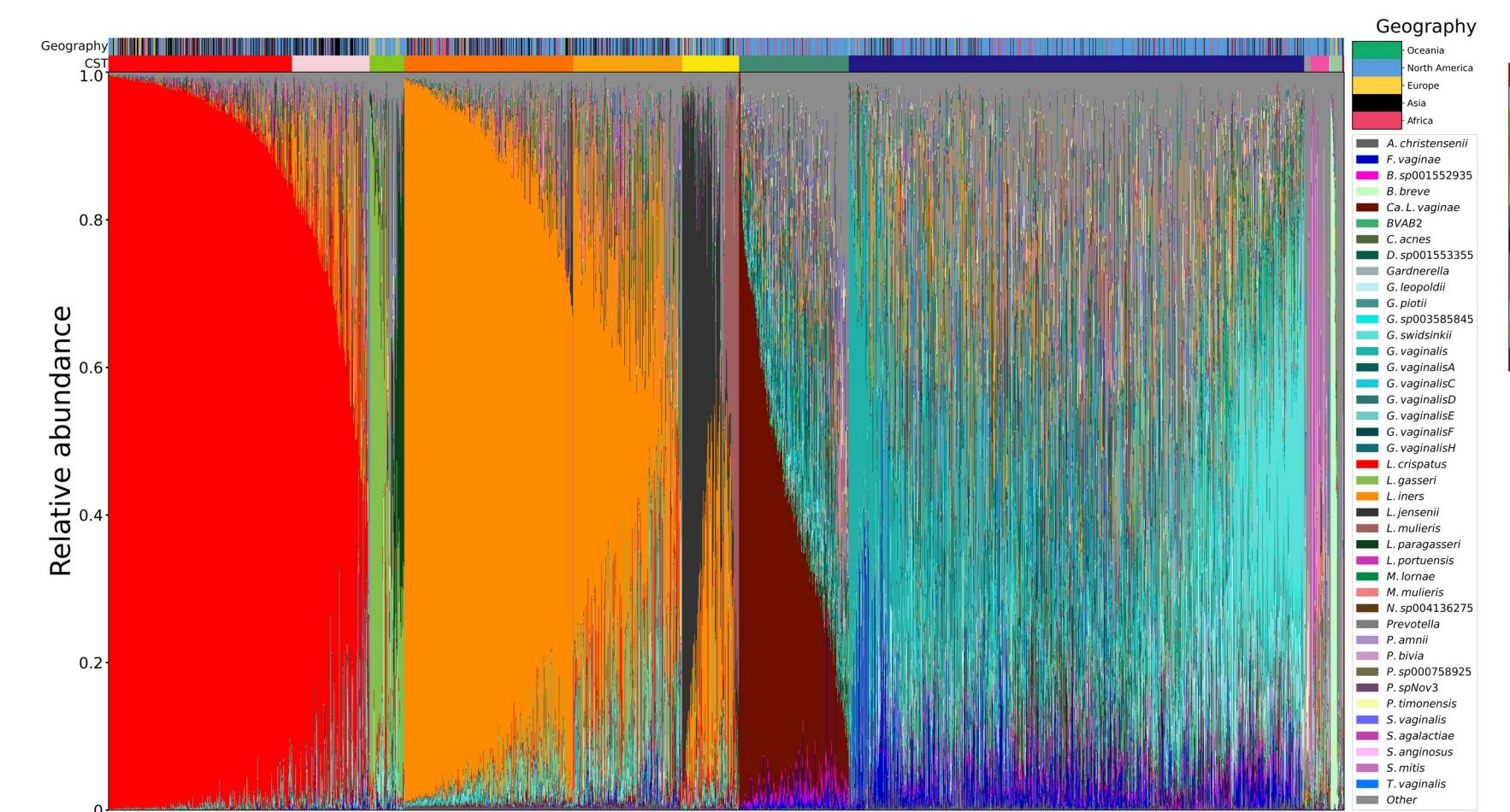
VIRGO2  
1.7 million  
non-redundant  
genes

**Virgo**

Human Vaginal  
Non-redundant  
Gene Catalog

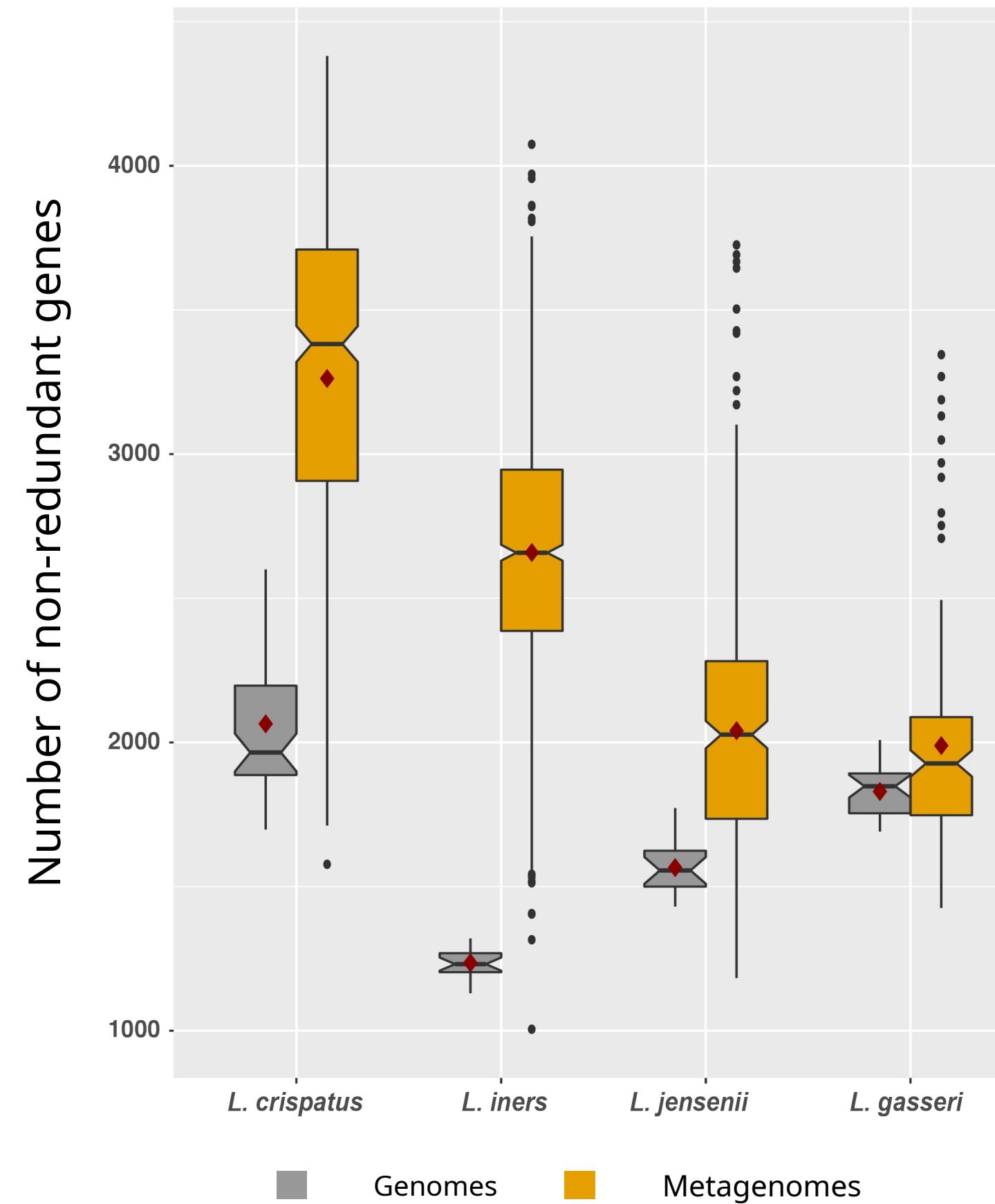
[virgo.igs.umaryland.edu](http://virgo.igs.umaryland.edu)

- 2,560 metagenomes
- 15,221 genomes



# Within women intraspecies diversity

Under dominance of a single *Lactobacillus* species, multiple strains co-exist and there is a high within-community intraspecies diversity.

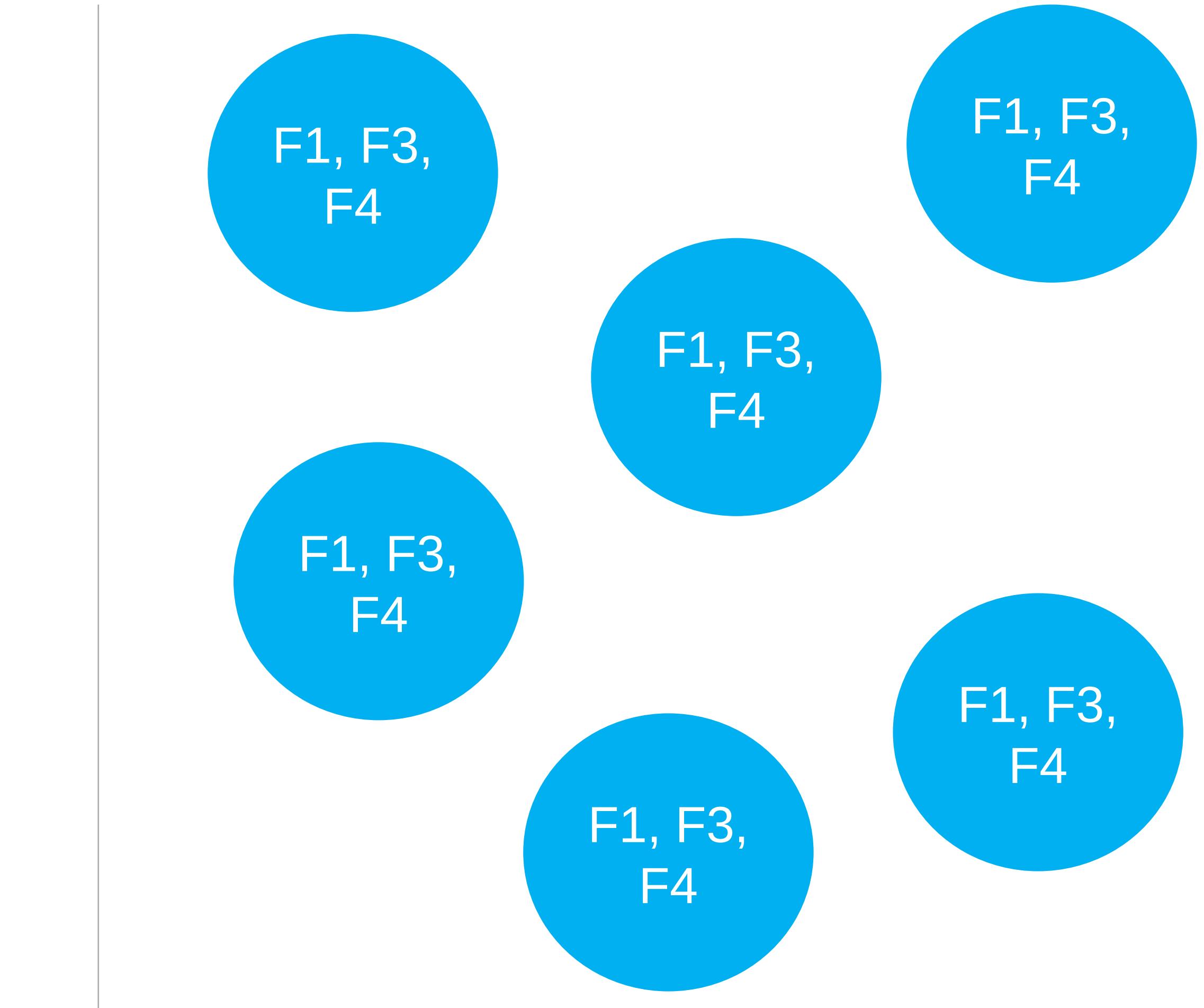
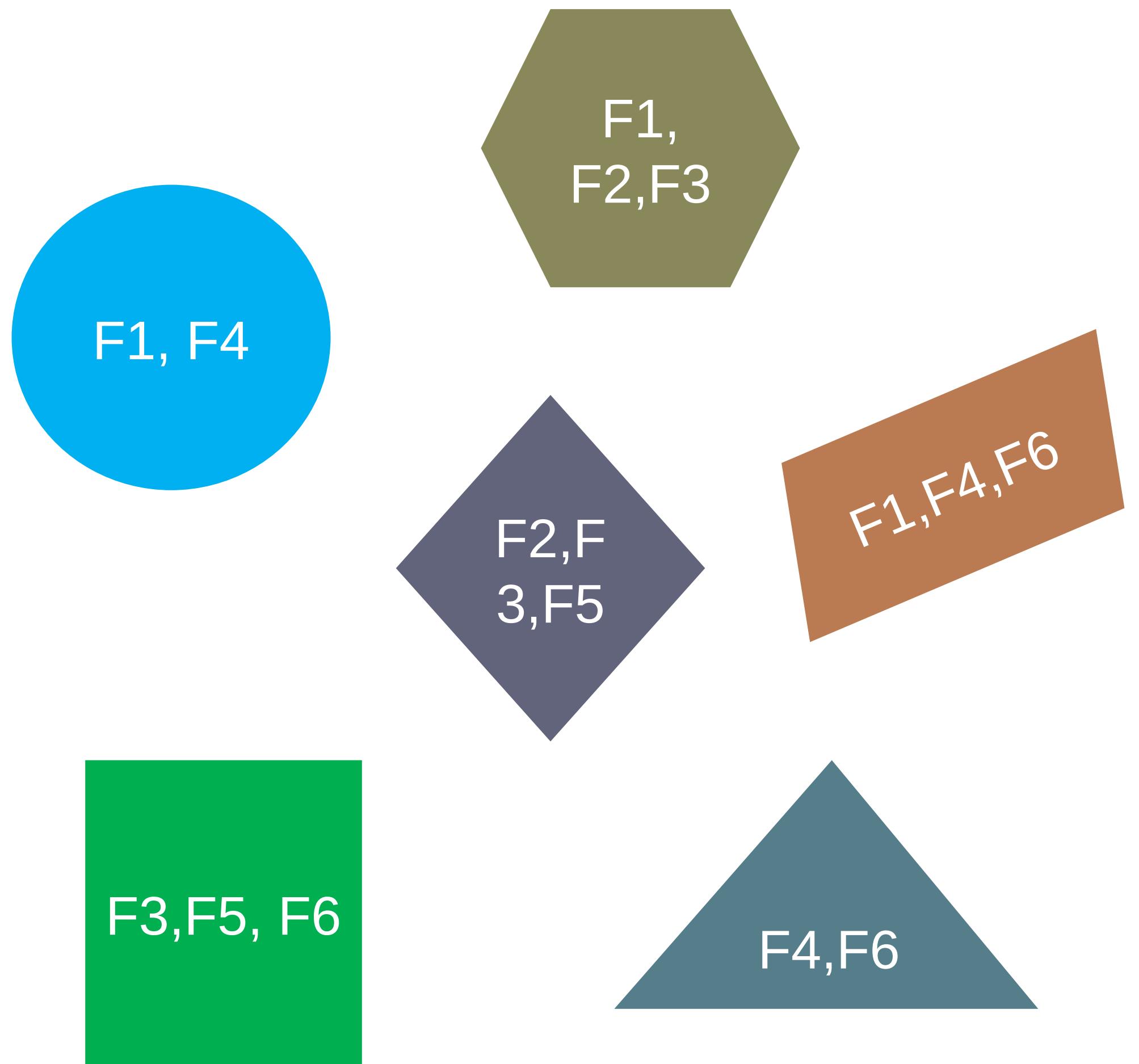


Assemblages of con-specific genotypes  
(strains of the same species) in vaginal  
microbiomes

Intraspecies diversity affords  
functional redundancy

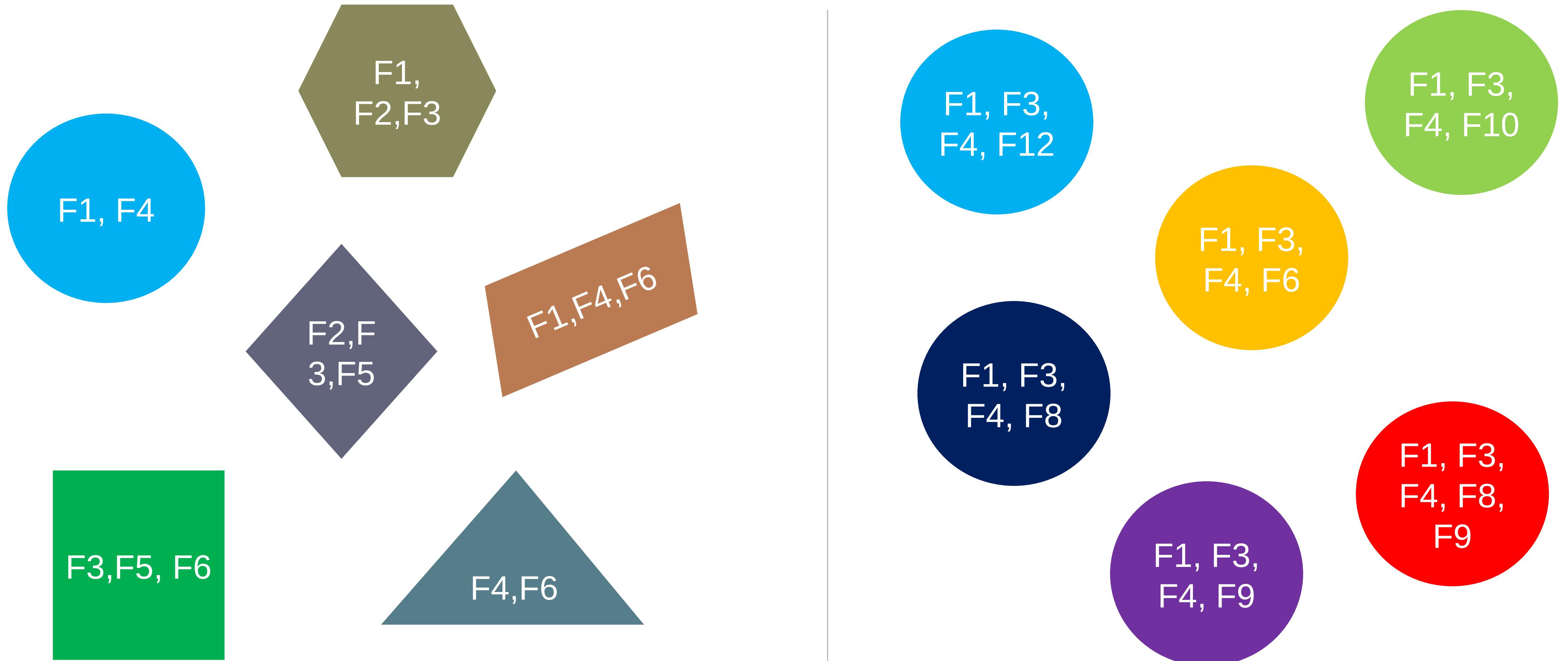
# Functional redundancy

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# Functional redundancy

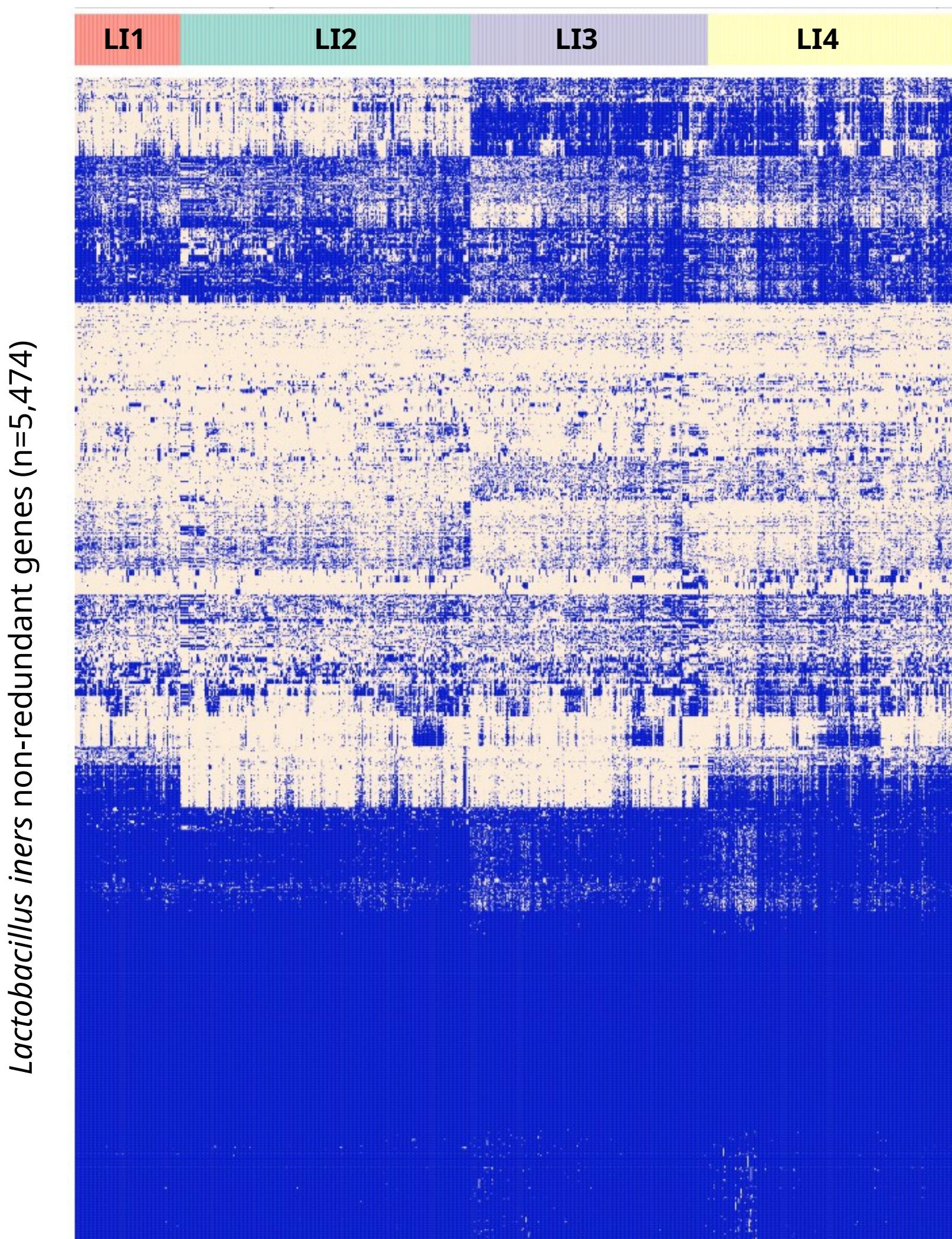
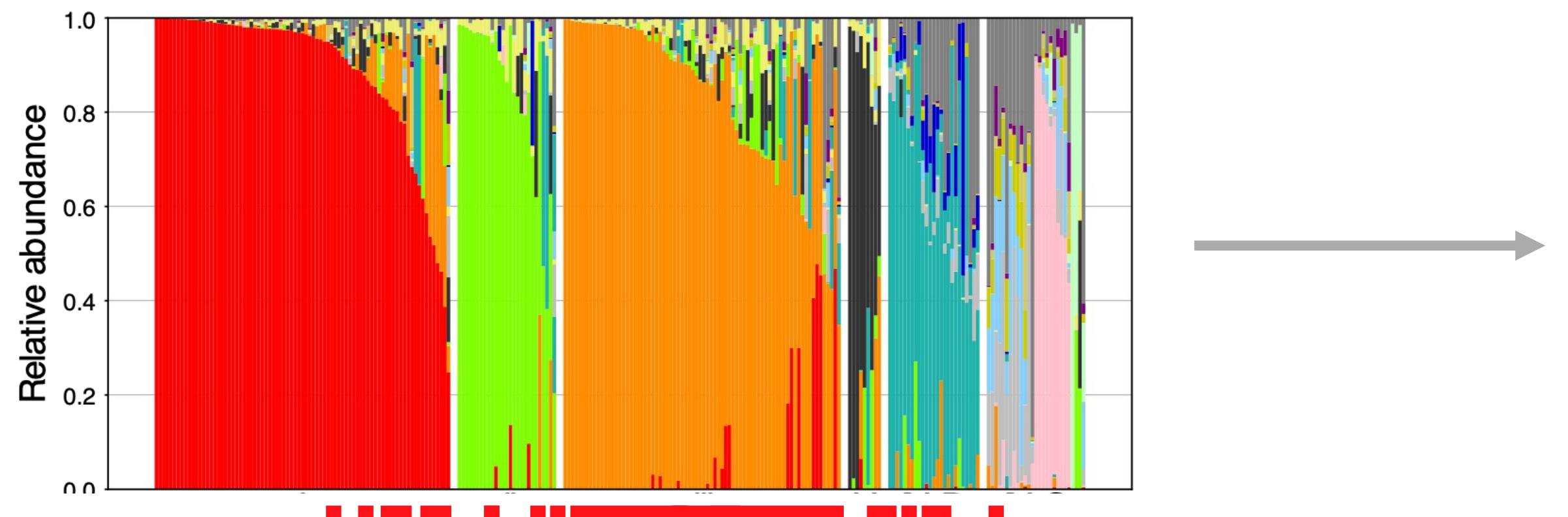
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# Metagenomic subspecies - Define by gene content

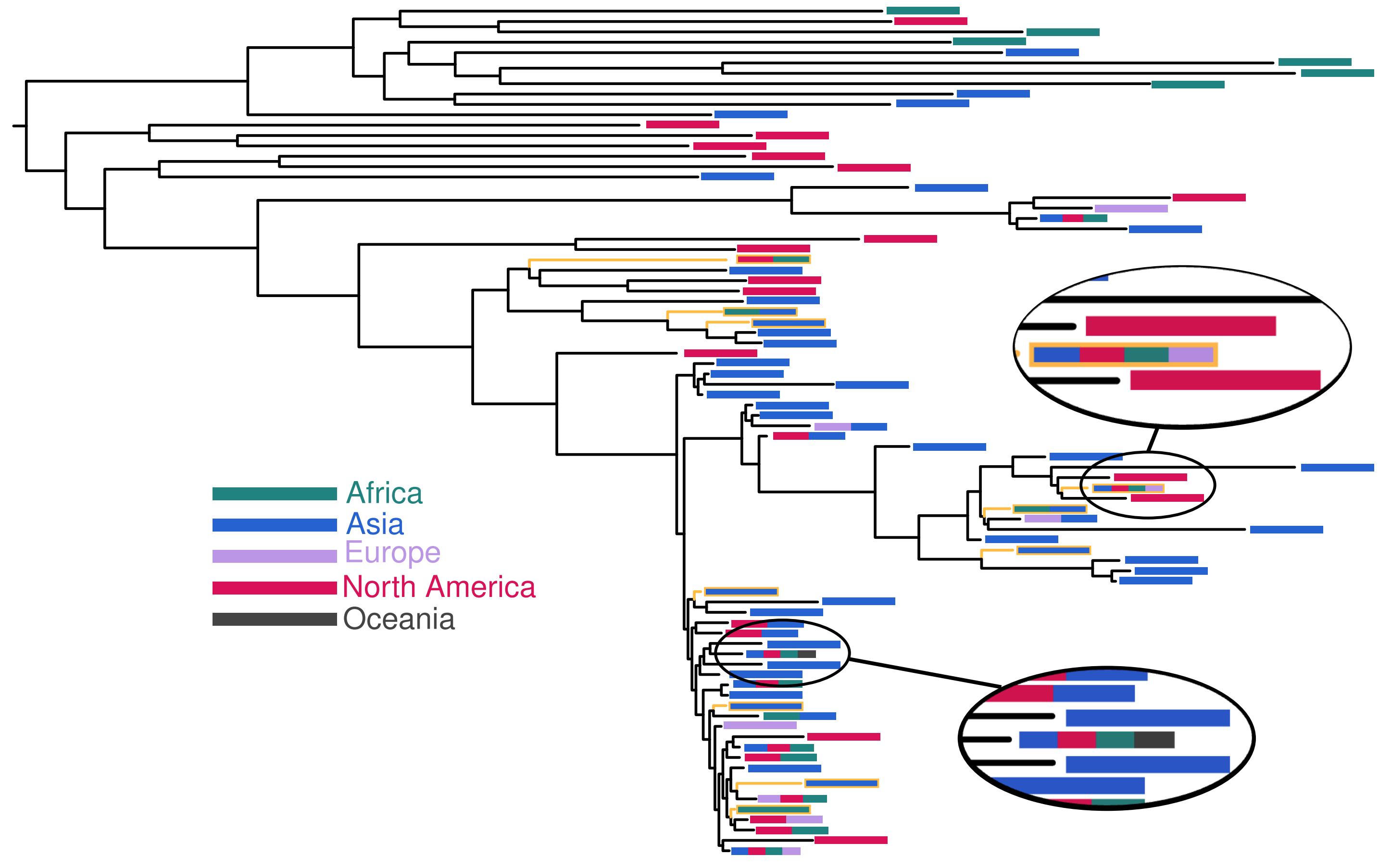
Con-specific genotypes assemblages (metagenomic subspecies) differ in the genetic content of the encompassing strains.

The overall functional content of the assemblages are different.



# Biogeography of the vaginal *L. crispatus*

*L. crispatus* lineages display biogeographic patterns, with some appearing endemic to certain geographic locales and others displaying more cosmopolitan ranges



Genome SNP tree of 81 worldwide *L. crispatus* lineages  
represented in VIRGO2

# Leveraging knowledge to develop microbiome-based vaginal LBPs

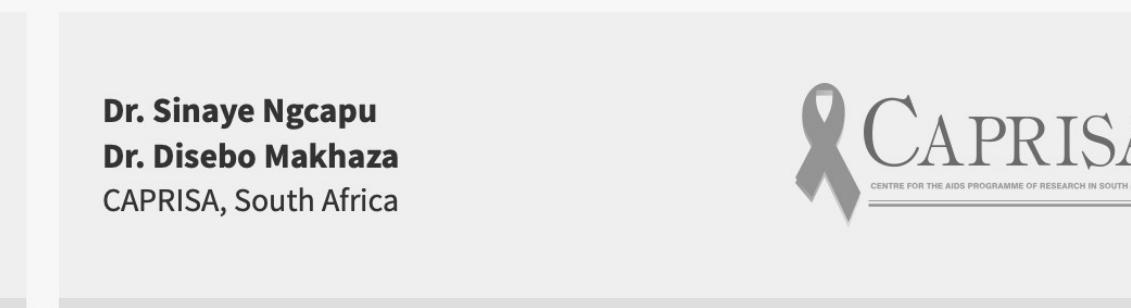
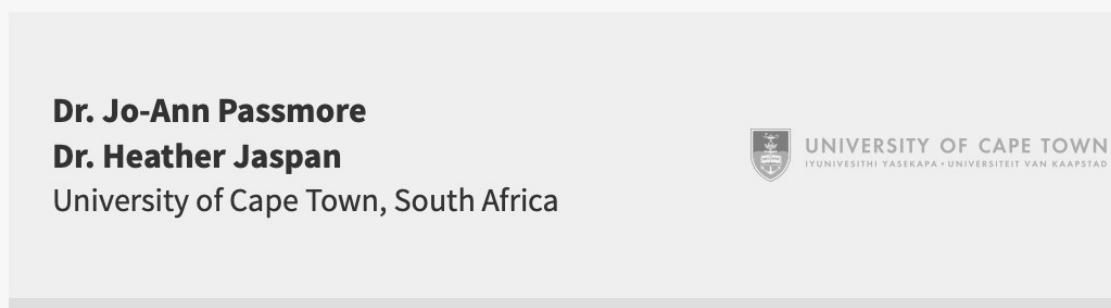
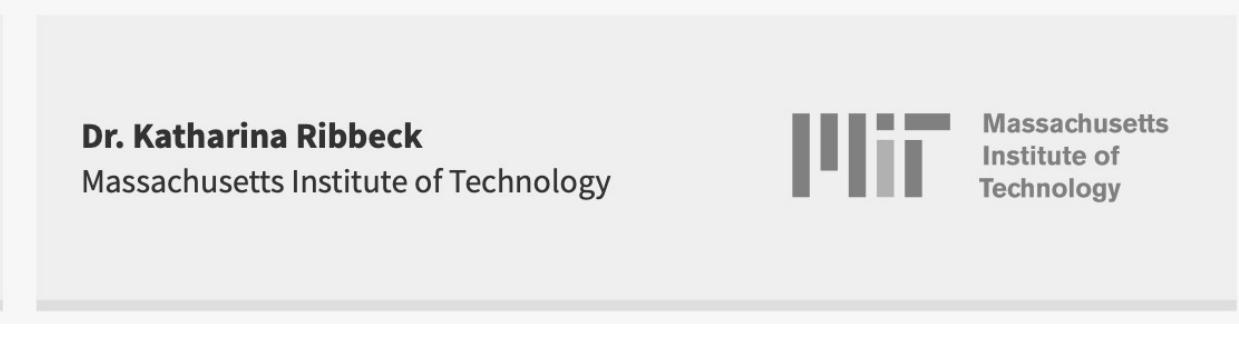
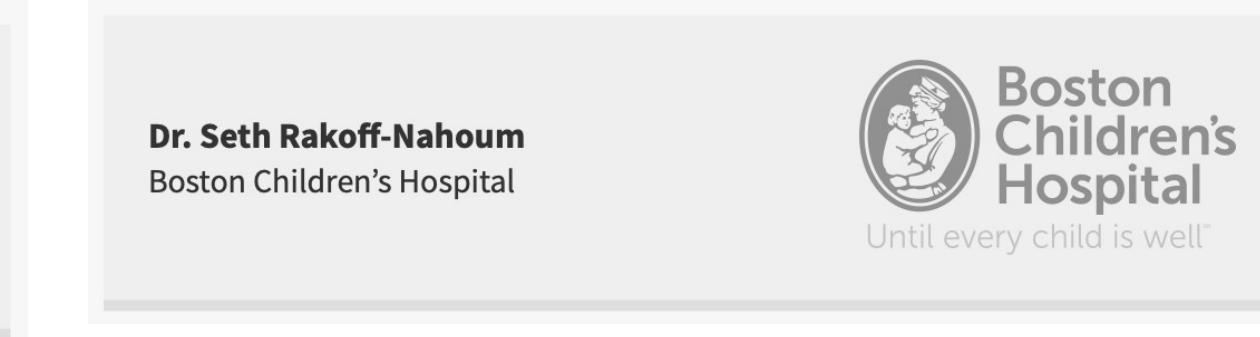
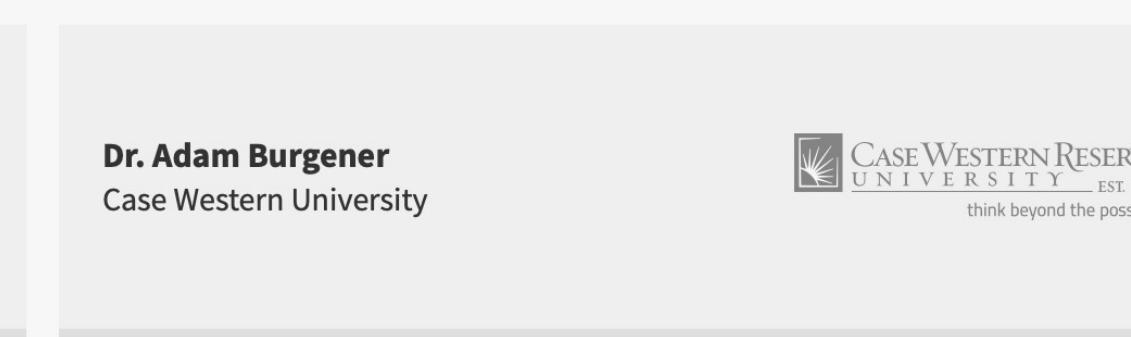
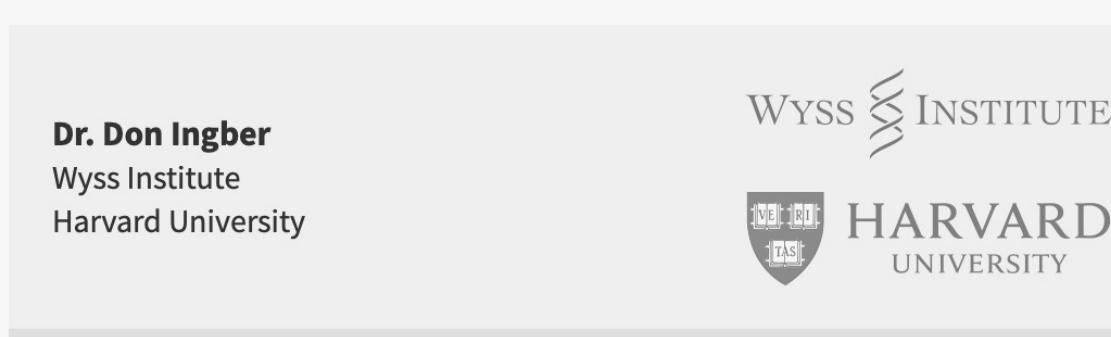
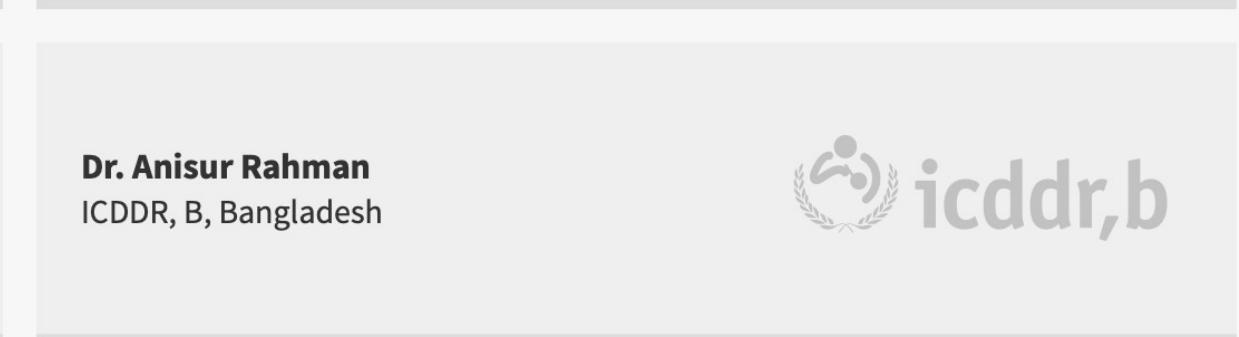
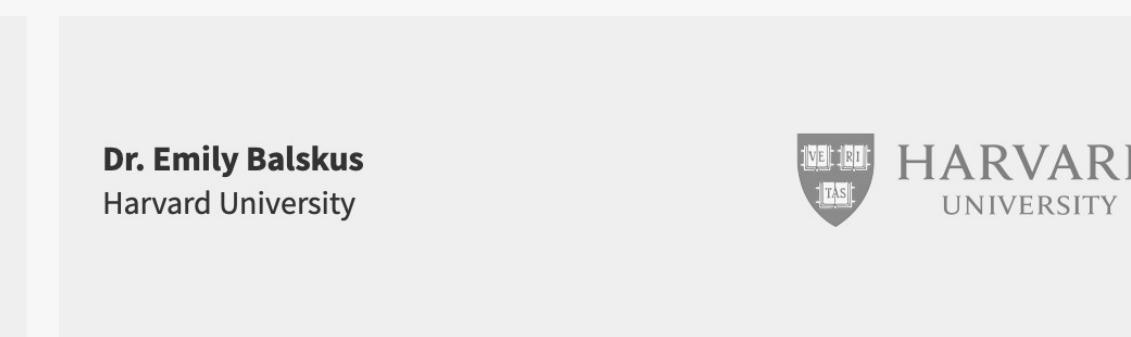
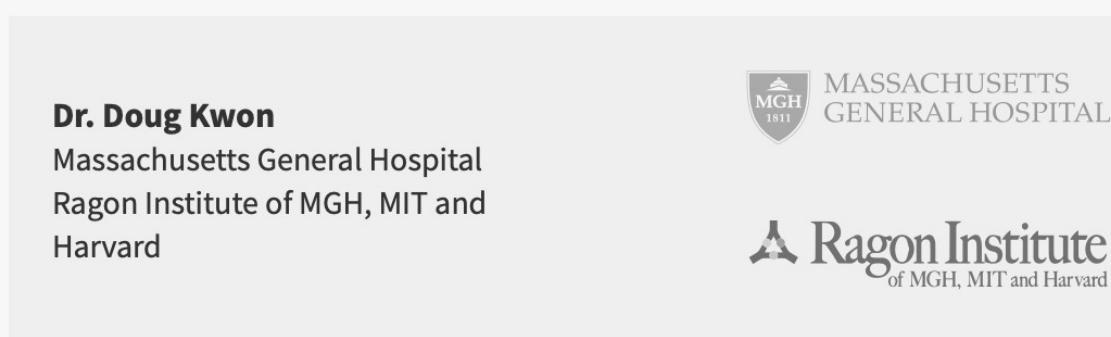
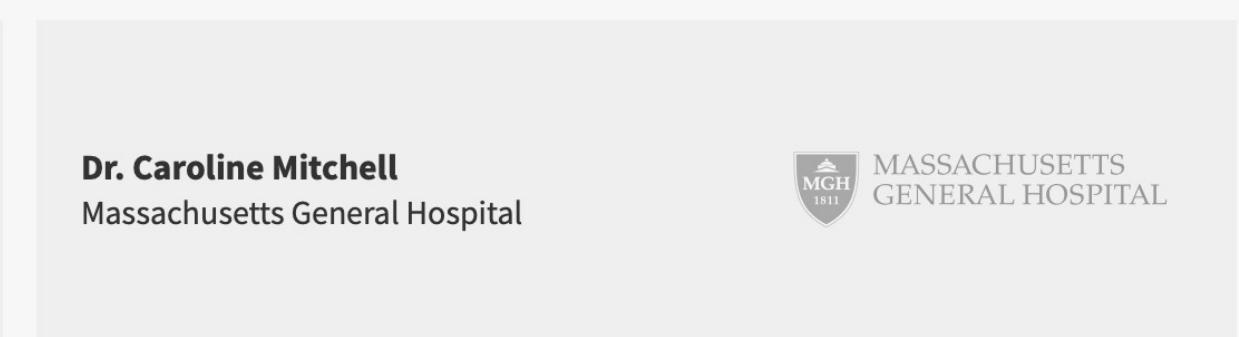
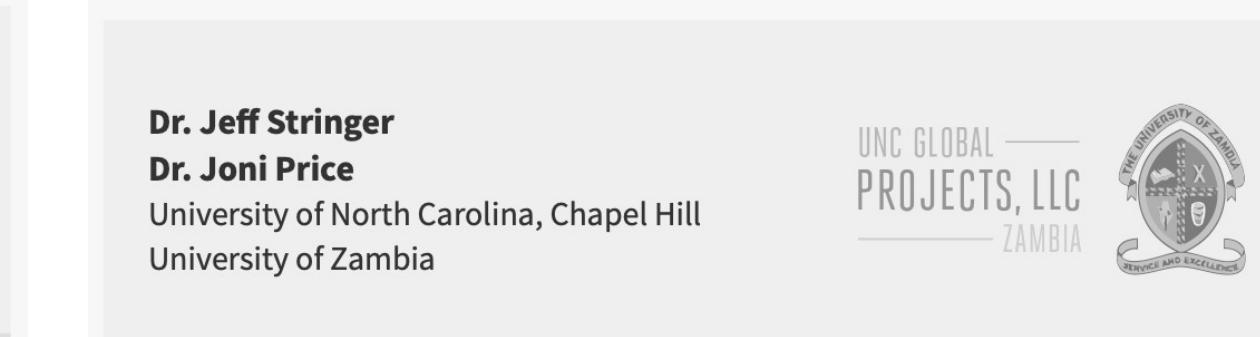
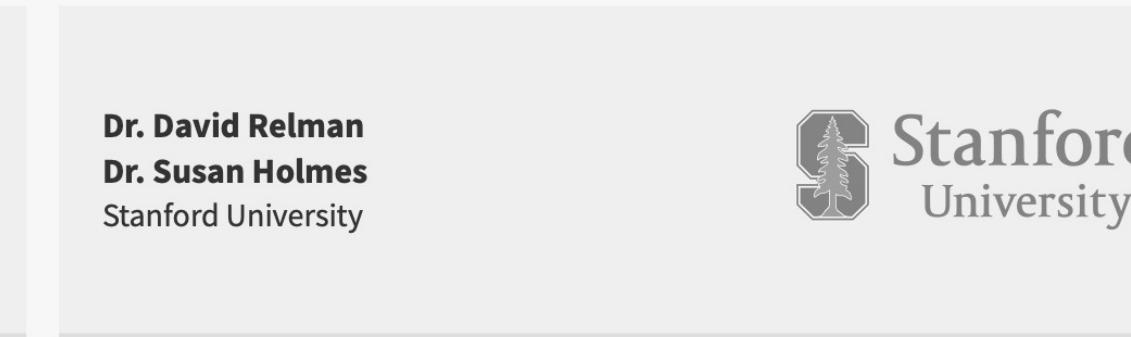
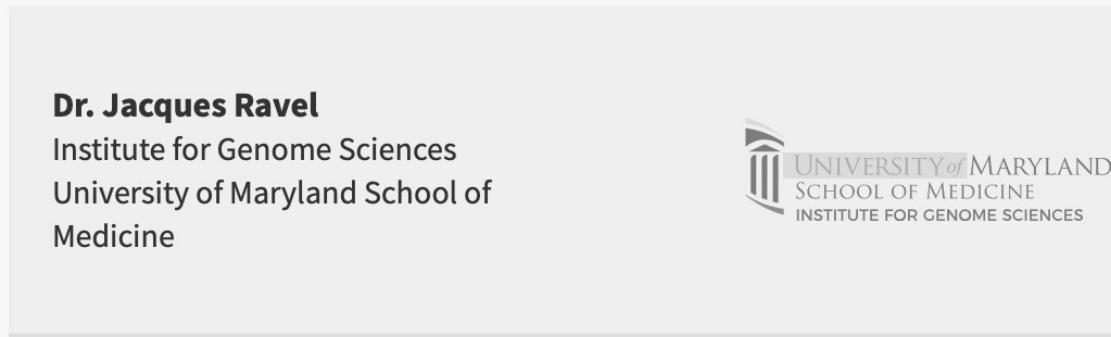
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Microbiome-based LBPs should include multiple strains (of *L. crispatus*) or even multiple species and might need to be developed considering local biogeography for optimal efficacy.

Selected strains should be associated with stable vaginal microbiota, have beneficial properties, have beneficial genotypic characteristics, and potentially geo-adapted to the target population

Consortia should encompass the genetic diversity comprised in stable and optimal vaginal microbiota to maximize functional properties

# The Bill and Melinda Gates Foundation Vaginal Microbiome Research Consortium

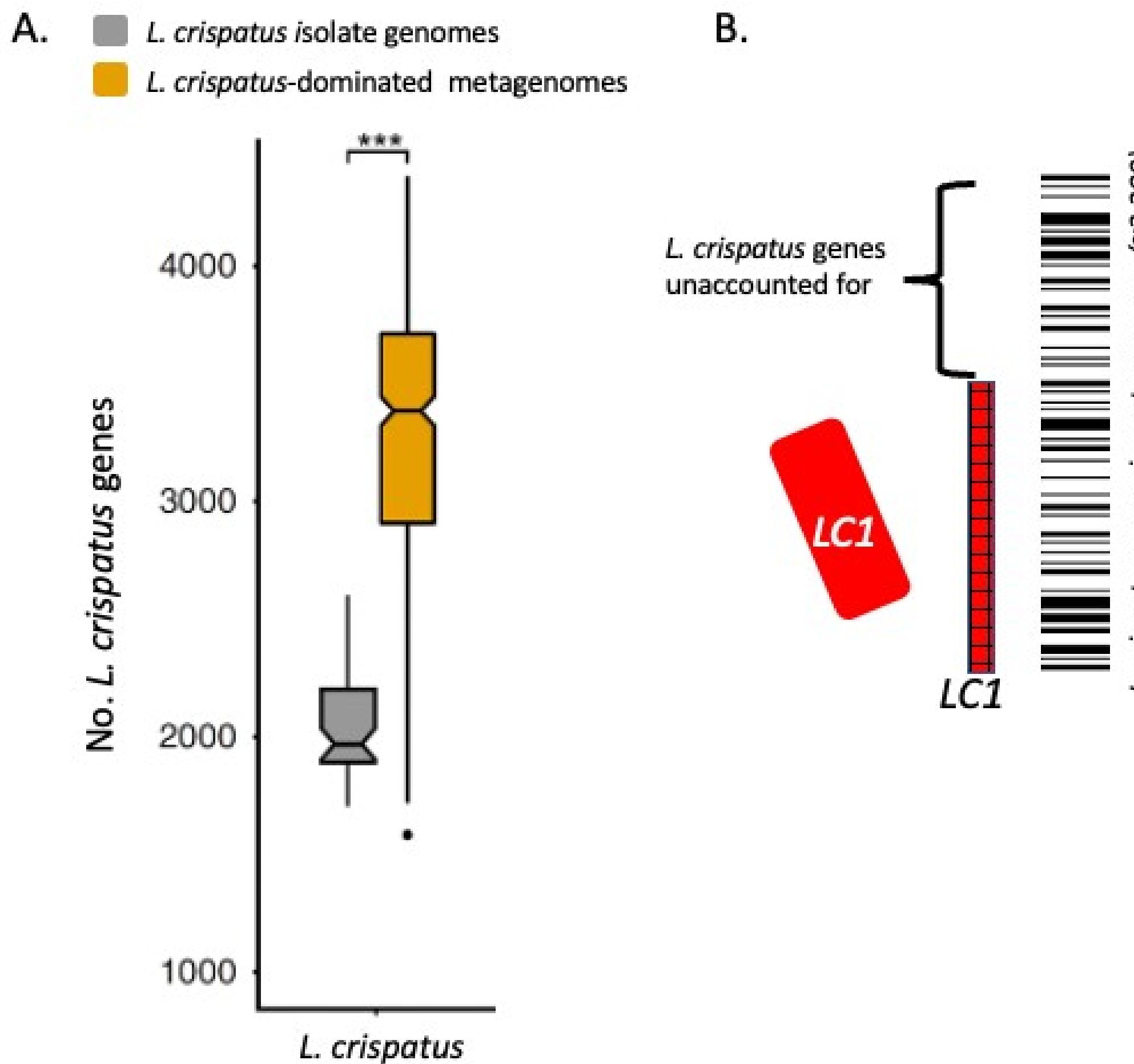


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**Goal:** Develop an innovative Live Biotherapeutic Products for bacterial vaginosis/CST IV to ultimately reduce the risk of reproductive and gynecological adverse outcomes in Africa and South Asia.

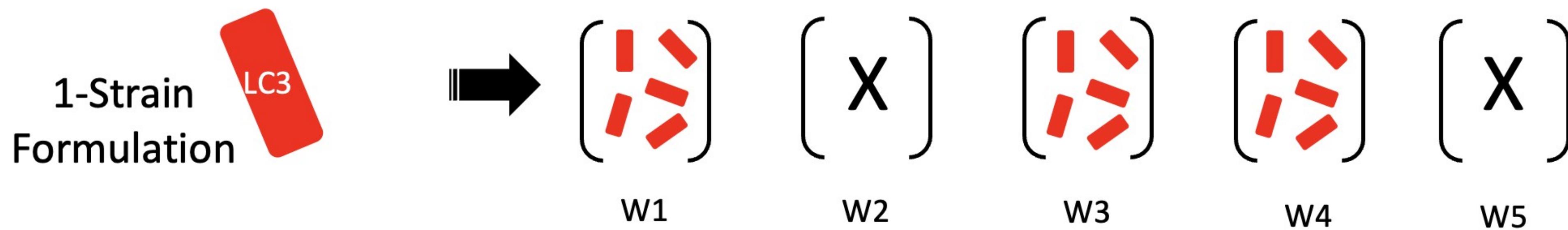
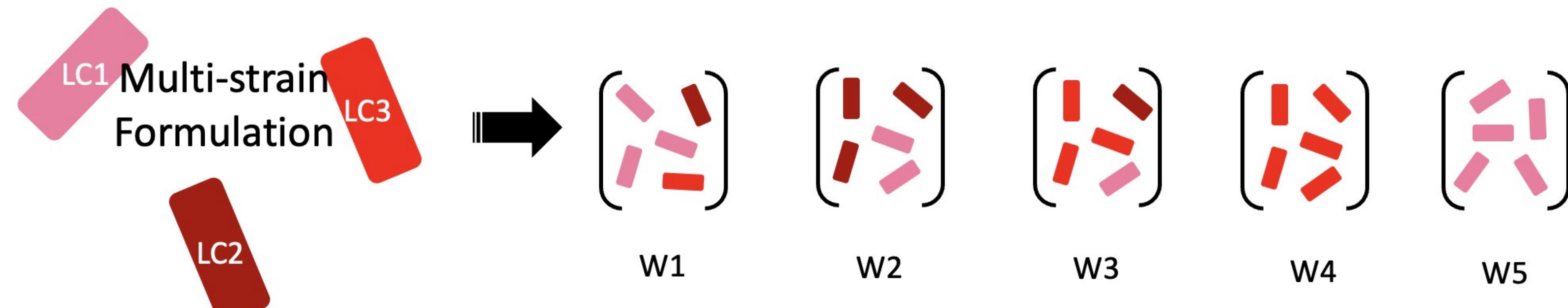
# Design approach: multiple strains consortium



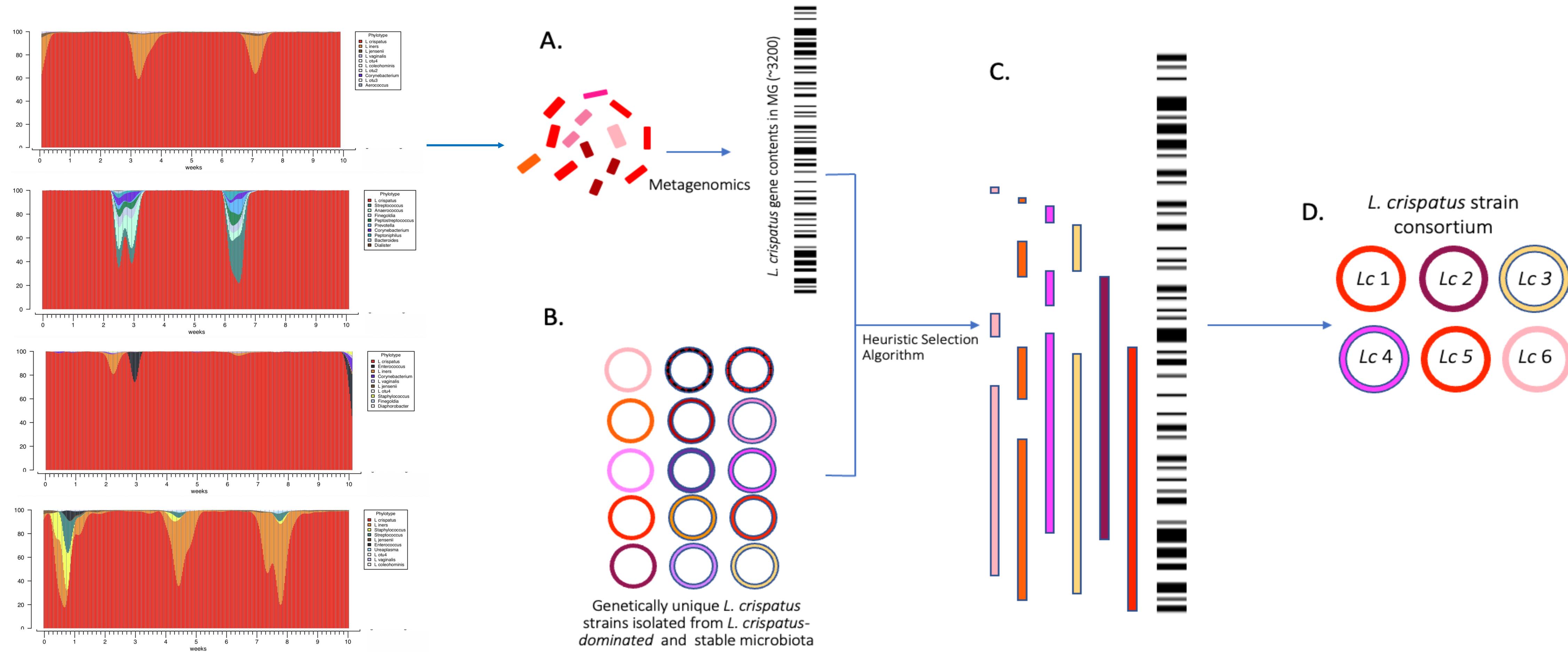
Use the information to design a vaginal live biotherapeutic product that comprise of multiple conspecific genotypes of *Lactobacillus crispatus*.

# Multi-strain consortia may adapt to differences in host response

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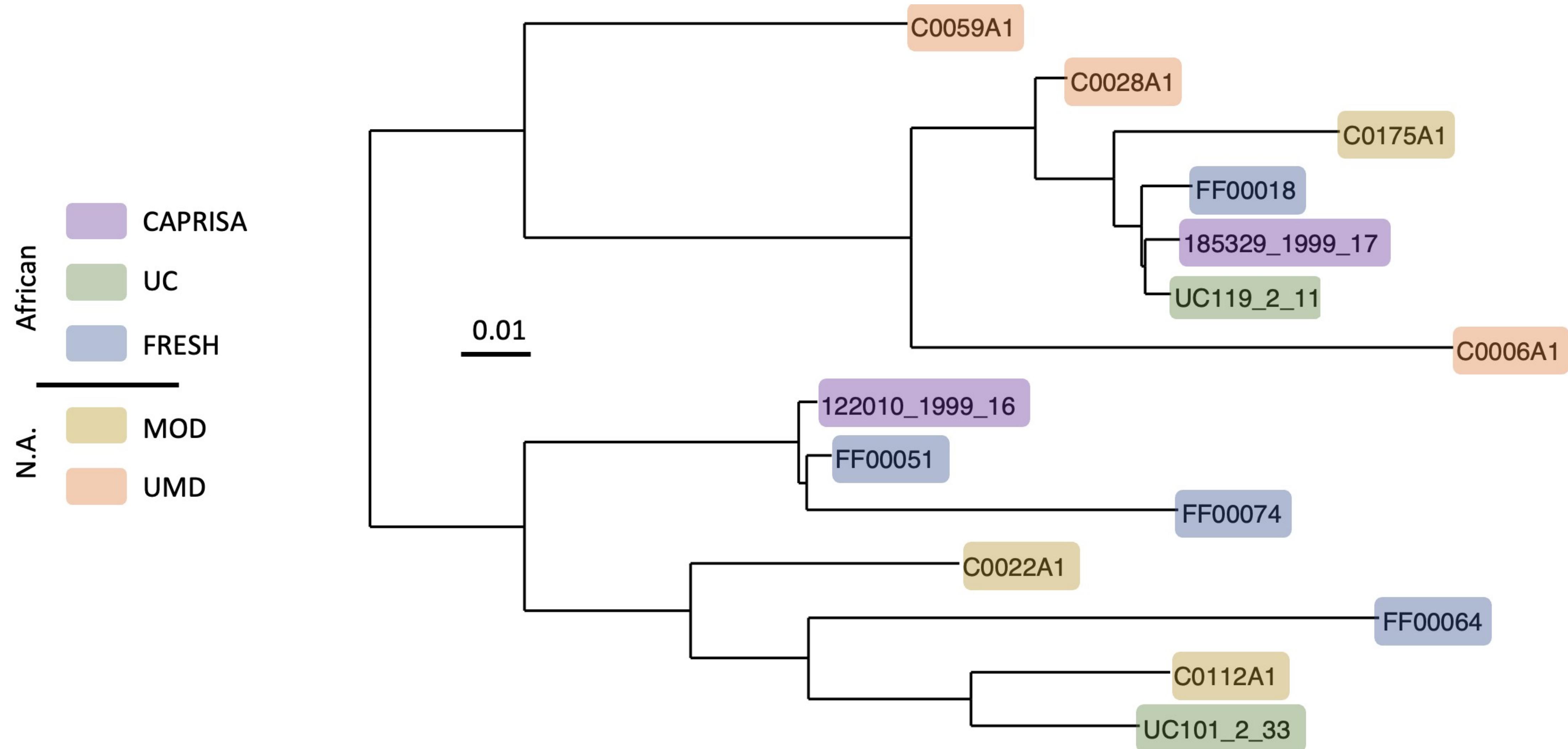


# Building microbiota-informed live biotherapeutics



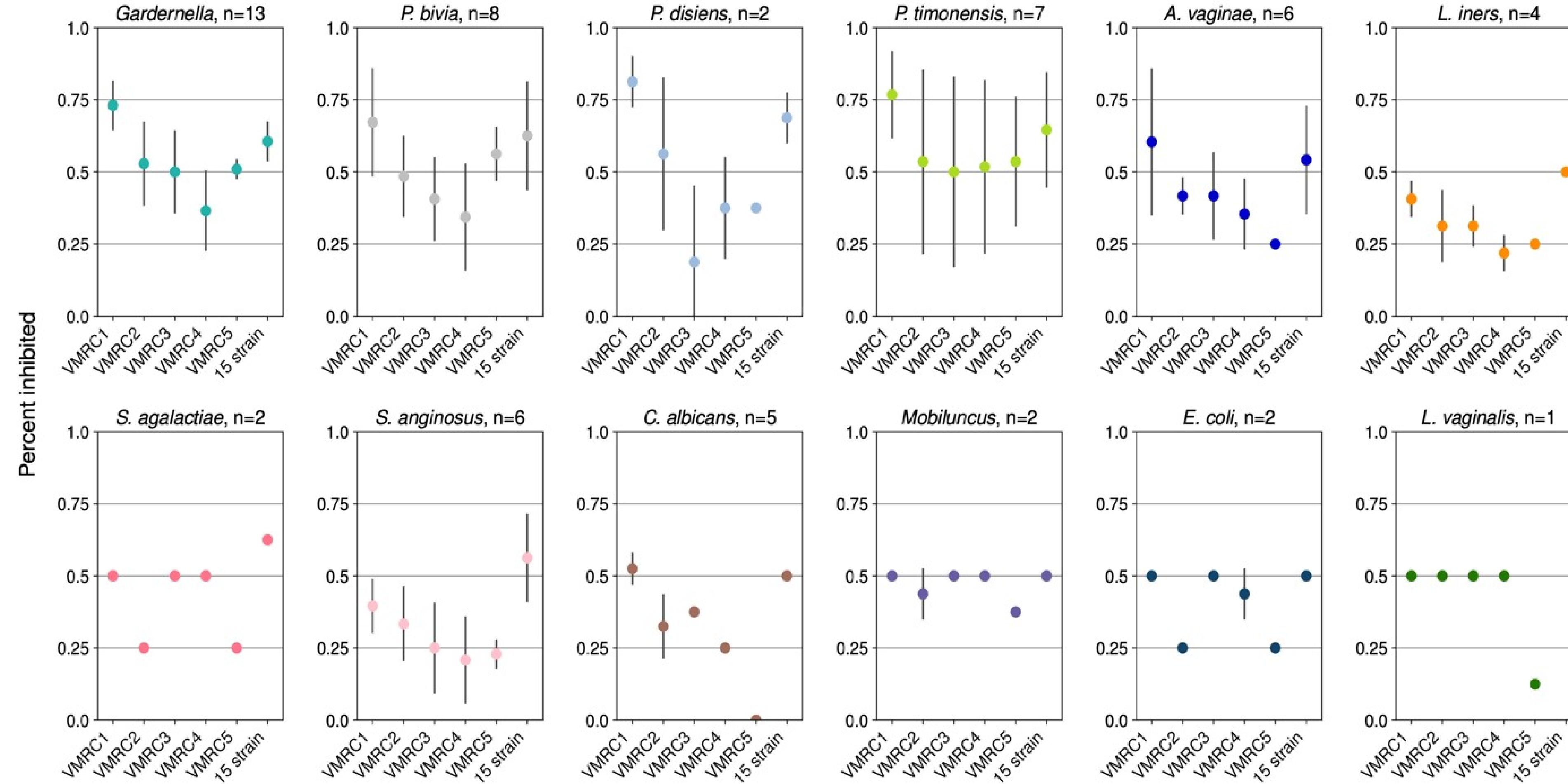
The algorithm generated several candidate consortia of 6 *L. crispatus* strains

# Considering biogeography



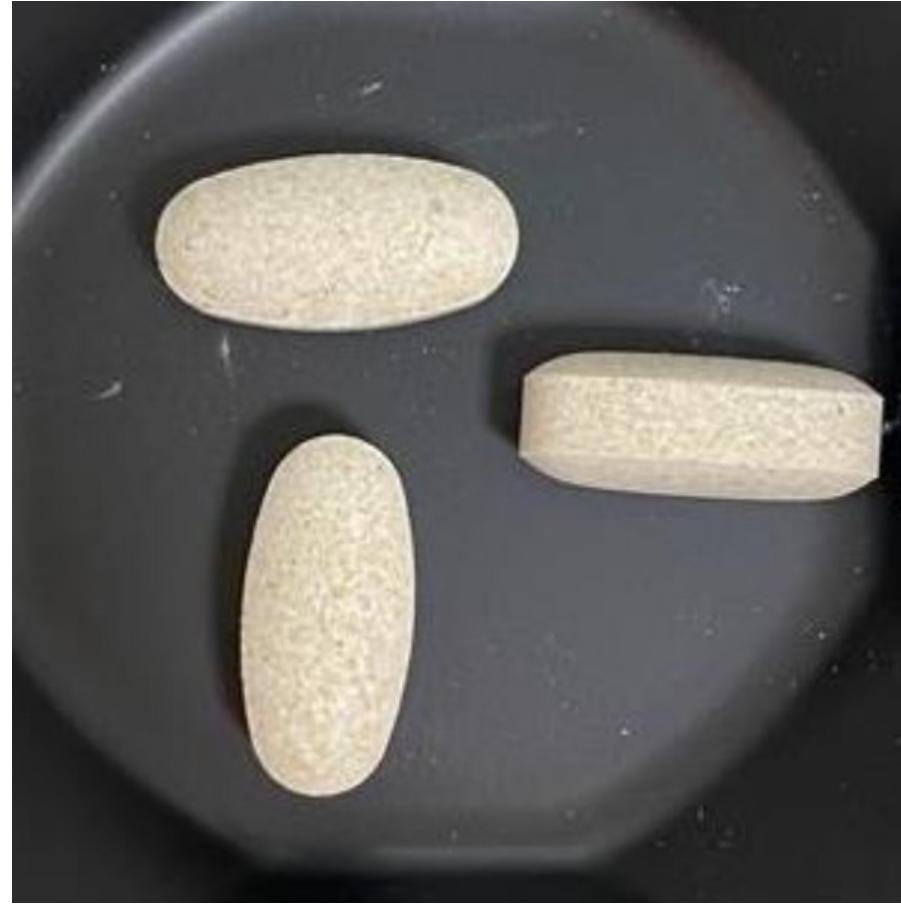
# Building microbiota-informed live biotherapeutics

## Antimicrobial Assays – Consortium Selection



# Manufacturing a microbiota-informed live biotherapeutics

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Two cGMP formulations have been manufactured:

A 6-strain (LC106) and a 15-strain (LC115) formulations:

1 g mucoadhesive pills

Disintegration time is 5-6 hours

Packaged into blister packs

Delivered with an applicators

# Clinical Trial for the Prevention of Recurrent Bacterial Vaginosis

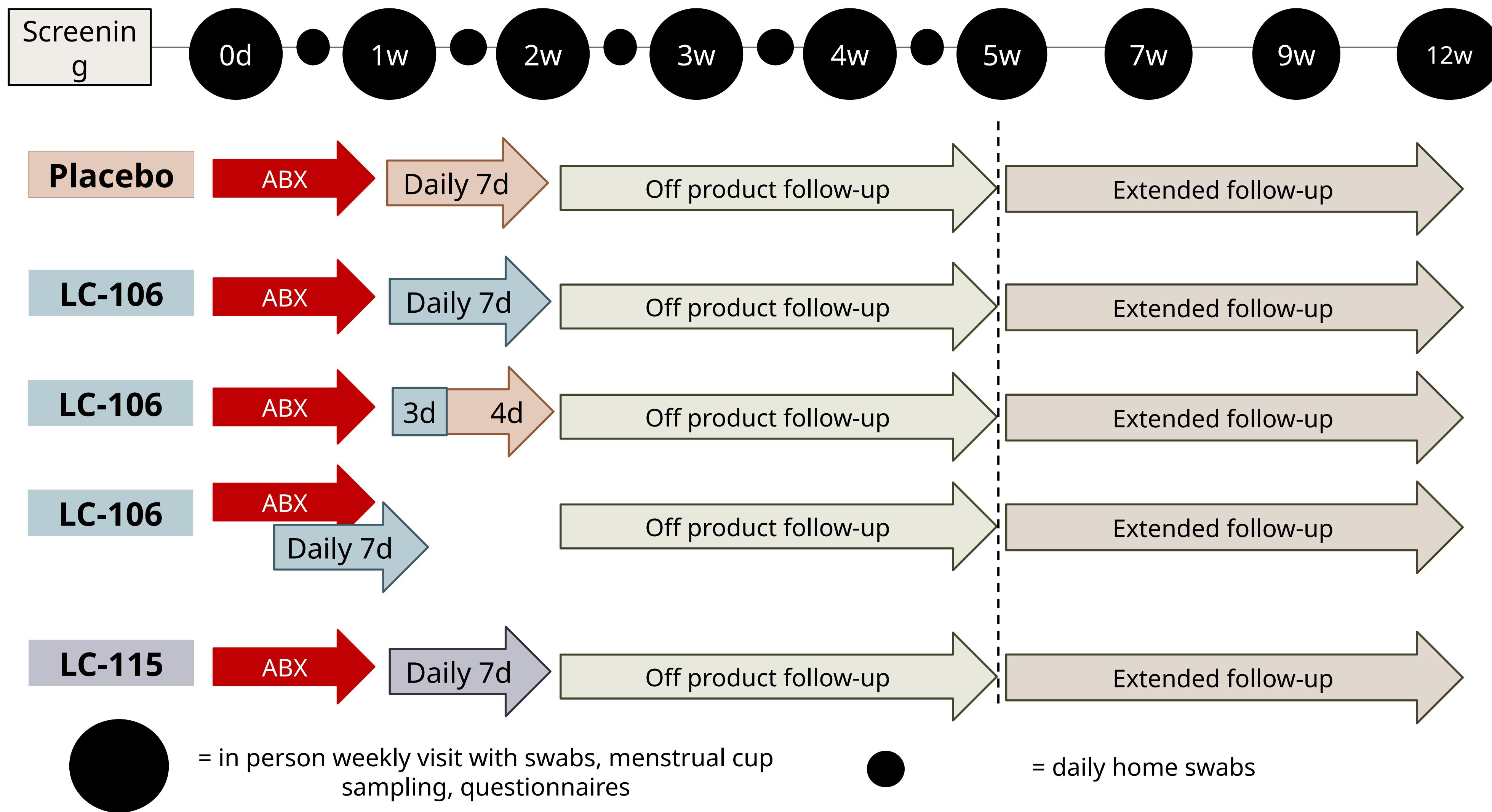
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VIBRANT

Vaginal lIve Biotherapeutic RANdomized Trial



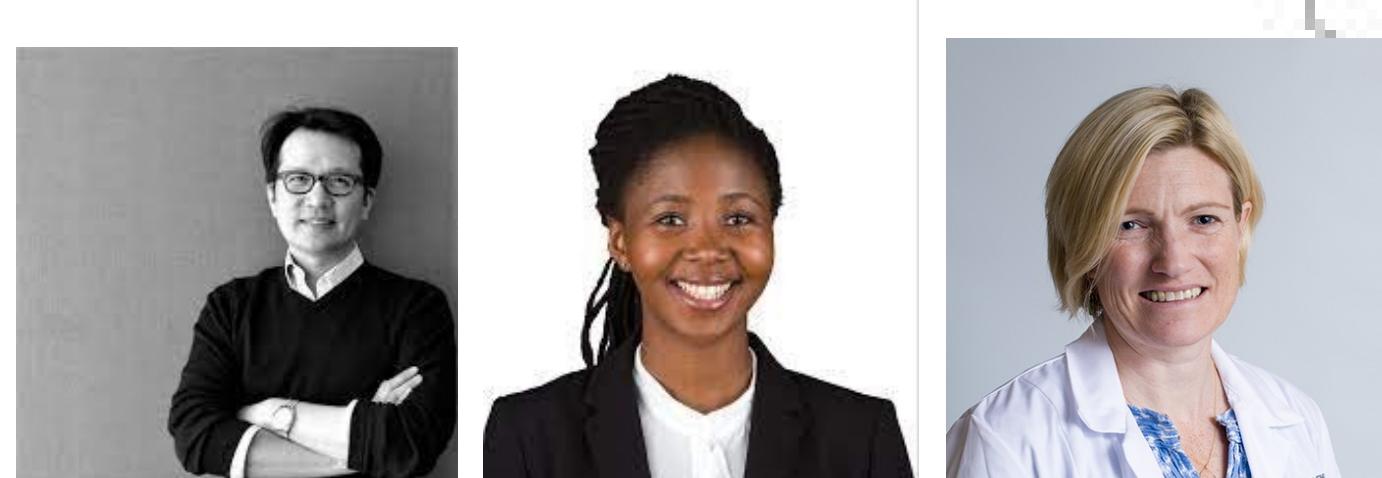
# VIBRANT Clinical Trial Design



# VIBRANT Clinical Trial Sites



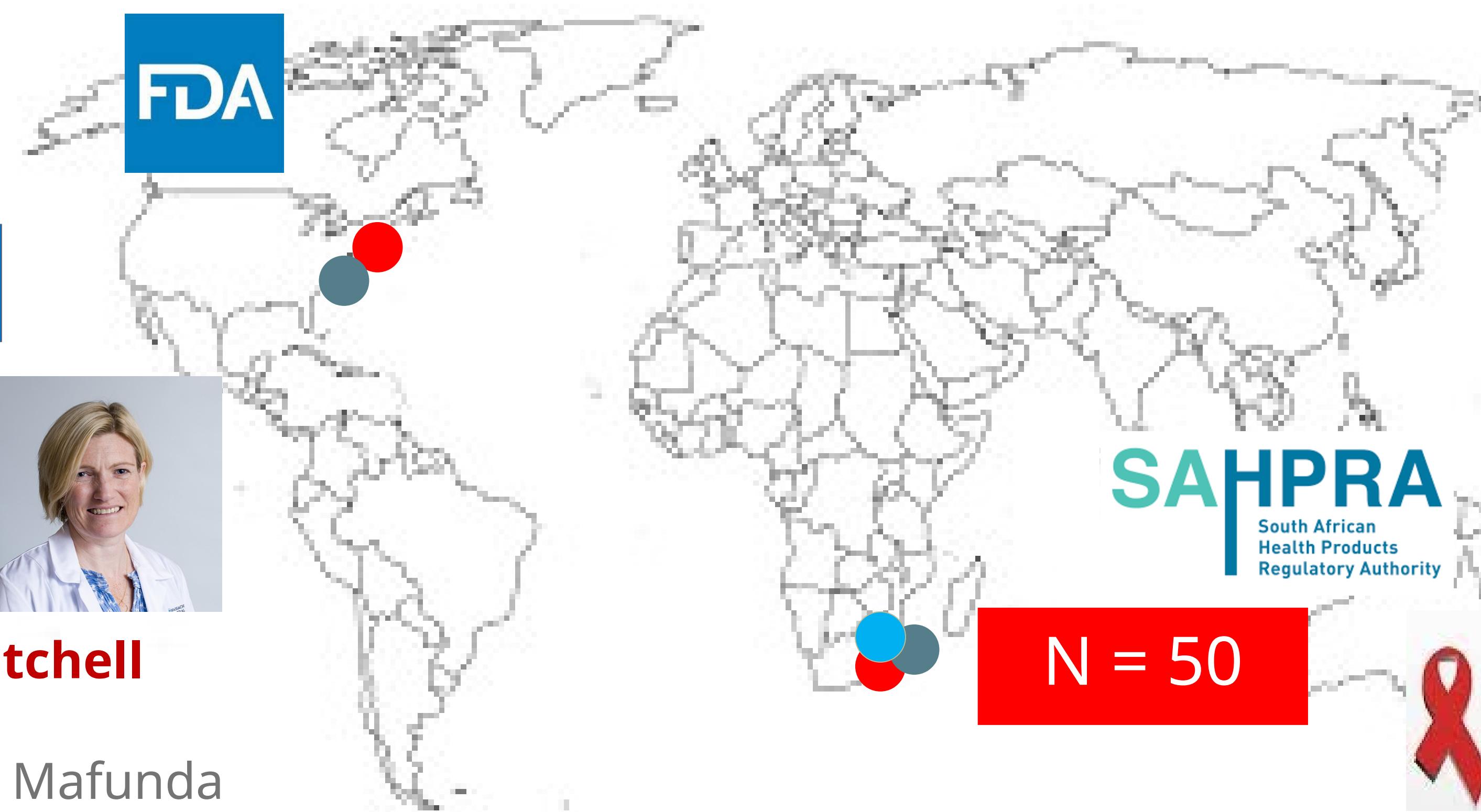
N = 50



- **Caroline Mitchell**
- Doug Kwon
- Nomfuneko Mafunda

**Laboratory Sites:** University of Maryland School of Medicine, MGH

BILL & MELINDA GATES foundation



- **Disebo Potloane**
- Sinaye Ngcapu
- JoAnn Passmore Lenine
- Leibenberg

# Conclusions

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We need innovation in the development of live biotherapeutic products with established clinical efficacy to restore *Lactobacillus crispatus* dominance and address major reproductive, obstetric and gynecological outcomes associated with CST IV types of microbiota.

Several initiatives (academic or private) are ongoing. There are reasons to be optimistic!

These products will need to be tested through rigorous and well-powered clinical trials (Phase 1 through 3) and obtain regulatory (FDA/EFSA...) approval.

We need to continue innovating until optimal solutions are identified that finally provide women ways to optimize their vaginal microbiota and protect themselves.

# Acknowledgments



[virgo.igs.umaryland.edu](http://virgo.igs.umaryland.edu)



[https://github.com/ravel-lab/speciate\\_IT](https://github.com/ravel-lab/speciate_IT)



Bing  
Ma

Micha  
el  
France

Pawel  
Gajer



- Vonetta Edwards
- Lindsay Rutt
- Bern Monari
- Breanna Shirtliff
- Christina Barrett
- Sarah Brown
- Melanie Quain
- Maddy Alizadeh
- Issac Chaudry
- Amaury Maros
- Kayla Carter



- **Disebo Potloane**
- Sinaye Ngcapu
- JoAnn Passmore
- Lenine Leibenberg
- **Caroline Mitchell**
- **Doug Kwon**
- Nomfuneko Mafunda

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Johanna Holm



# Exploring and translating the female genital tract microbiota

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Questions?

