I: Horizontal transmission of genes and microbiomes

II: Black Queen dynamics and how to prevent it
Who I am, and what I work on

- Bram van Dijk (he/him), assistant professor at TBB (Kruyt building, N605)
- Did my PhD with Paulien (2015-2020)
- Alkmini said I shouldn’t show a picture of myself
- The mother of Cacatoo 🦜
- I work on microbial ecology and evolution
- Mostly: spatially structured simulations that include more than 2 levels
- I am looking for students! If you like this lecture: BSc (scriptie) or MSc interns very welcome!
Horizontal vs. vertical transmission

Evolution by means of vertical transmission

Evolution by means of horizontal gene transfer
Horizontal vs. vertical gene transfer

“The walls that divide bacteria from one another are far from solid. Taken to extremes, the preponderance of HGT could even imply that microbiomes are better conceptualized as collections of locally adaptive genes, rather than communities of locally adapted species”

— James P.J. Hall, 2021
“Indeed, the estimated rates of gene family gain and loss in some groups of bacteria are such that multiple genes appear to come and go over the time required for a single nucleotide substitution to occur in an evolving gene.”

Conclusion: bacteria aren’t “waiting for beneficial mutations”, they are “waiting for beneficial genes”
Moreover: bacterial evolution is about community gene content, not species!
Nucleotides, genes, individuals, and communities

Increasing scale
HGT is often compared to sex, and seen as a side-effect

“HGT is just a side-effect of bacteria consuming DNA for resources. The adaptive benefits are secondary.”
Let’s start there. Bacteria simply take up DNA.

- Carrying a gene has a **small cost** for the bacteria.
- Toxin/resistance genes evolve their **mobility**: how likely they are to integrate into a genome after uptake (whether that happens via transposon, plasmid, *etc.*, we ignore for now).
- Genes can also flux into the system externally.
Simulating 1,000,000 time steps
Which “sets” of toxin/antitoxin survive?

Gene mobility ratio

\[ \frac{T_{MOBILITY}}{R_{MOBILITY}} \]

- Average of all genes
- Maintained toxin/resistance genes
- Extinct toxin/resistance genes
Without differential mobility, diversity crashes!
Driving up the “discovery rate” also enhanced diversity...

When we keep introducing new genes, phylogenetic diversity is lost!
Phylogenetic diversity: core- vs. accessory genomes evolved!

Toxin genes are best conceptualised as “sometimes-useful parasites”

“Accessory” genome

“Core” genome
Why don’t all genes become parasites?
Sparing close kin: without kin-recognition!

• In nature, bacteria often “spare” close kin from killing

• The model does not include kin recognition: they don’t know who they are killing

• Through the interplay of local interactions and pattern formation, however, they end up “sparing close kin” anyway

• This system is maintained due to the toxin genes transferring more frequently than their corresponding resistance genes!
Conclusions so far

On the cell-level:
- Despite HGT driving a lot of “genetic mixing”, that doesn’t mean everyone becomes the same.
- Concepts like “individuality” and “species” still persist for bacteria, but the gene- and group-level are equally (or more!) important

On the gene-level:
- Mobile genes are Darwinian entities themselves, and evolve towards “parasitism” if they get the chance!
- However: a feedback with the local environment prevents this from getting out of control!
- Toxin genes can get away with being parasites in the short term, because they are occasionally beneficial
Genes evolve to play nice... or not. What do the cells want?

“HGT is just a side-effect of bacteria consuming DNA for resources. The adaptive benefits are secondary.”

- Toxin genes give major benefits **under the right circumstances**.
- Toxin genes cost energy when not used
- On average, I measured them to be “slightly beneficial” in the model
- Do the cells then “want” to take up toxin genes?
  - **No.** No they don’t. But is that because:
    - A) HGT is not adaptive for the cells, or
    - B) Taking up toxin genes can be **extra** bad
Slightly beneficial genes

- As a proof-of-principle, we assume **HGT has a cost** (uptake of DNA, transfer machinery, etc.) rather than giving it a direct benefit for cells. (A hard-case for adaptive benefits of HGT!)
- How does this **costly** HGT impact growth rates?

\[
\frac{dC}{dt} = (1 - ch + b)C - \frac{IC}{\text{gene loss}} + \frac{hCN}{\text{HGT}} - \frac{\phi C}{\text{chemostat}}
\]

\[
\frac{dN}{dt} = (1 - ch)N + \frac{IC}{\text{gene loss}} - \frac{hCN}{\text{HGT}} - \frac{\phi N}{\text{chemostat}}
\]

\[
\phi = (1 - ch + b)C + (1 - ch)N
\]

\[C + N = 1\] (constant population size, ensured by chemostat assumption.)

**Q:** How does HGT impact the population growth rate?
Maintaining beneficial genes depends on parameters

A)

But do the microbes **benefit** from maintaining the gene?
Math math math...

\[ \frac{dC}{dt} = (1 - ch + b)C - \frac{lC}{HGT} + \frac{hCN}{HGT} - \phi C \]

reproduction of C  gene loss  HGT  chemostat

\[ \frac{dN}{dt} = (1 - ch)N + \frac{lC}{HGT} - \frac{hCN}{HGT} - \phi N \]

reproduction of N  gene loss  HGT  chemostat

\[ \phi = \frac{(1 - ch + b)C}{\text{total growth of C}} + \frac{(1 - ch)N}{\text{total growth of N}} \]

\[ C + N = 1 \] (constant population size, ensured by chemostat)

\[ \phi^*(h) = \begin{cases} 
1 - ch & \text{if } h \leq (l - b) \text{ (gene cannot persist)} \\
1 - ch + b - \frac{bl}{b+h} & \text{if } h > (l - b) \text{ (gene persists).}
\end{cases} \]

The growth rate of the population in steady state
What does $\Phi^*(h)$ look like?

$$
\Phi^*(h) = \begin{cases} 
1 - ch & \text{if } h \leq (l - b) \text{ (gene cannot persist)} \\
1 - ch + b - \frac{bl}{b+h} & \text{if } h > (l - b) \text{ (gene persists).}
\end{cases}
$$
Can microbes evolve costly HGT to “rescue” a gene?

B) ODE model (carriers and non-carriers with / without HGT)

\[
\begin{align*}
C^+_{\text{carrier}} & \quad \text{loss} \quad N^+_{\text{non-carrier}} \\
\varphi &= 1 + b - ch \\
\text{HGT} & \quad \varphi = 1 - ch
\end{align*}
\]

Proportional to total carriers \((C^+ + C^-)\)

\[
\begin{align*}
C^-_{\text{carrier}} & \quad \text{loss} \quad N^-_{\text{non-carrier}} \\
\varphi &= 1 + b \\
\varphi &= 1
\end{align*}
\]

Dilution by total growth \(\phi = (\varphi_{C^+} + \varphi_{N^+} + \varphi_{C^-} + \varphi_{N^-})\)
Evolve costly HGT to “rescue” a gene?

Maybe I shouldn’t have

Put them in space!
Almost identical model. In space.

C) Individual-based, eco-evolutionary model

Phew minor differences:

- Individual-based model: individual lineages can evolve their rate of eDNA uptake!
- Both “good” and “bad” genes are possible in 1 system (\(b\) and \(\beta\)-parameter)!  
- Mobile vs. Selfish genetic elements

Q: What is the impact of spatial structure?

\[ \phi = 1 - ch_i \]
\[ h_i = 0.023 \]

\[ \phi = 1 + b - \beta - ch_i \]
\[ h_i = 0.043 \]

\[ \phi = 1 + b - ch_i \]
\[ h_i = 0.012 \]
What about space though?
Cells maintain costly HGT, in the presence of costly SGEs

- Proof-of-principle: HGT can be adaptive for microbes, even under genuinely terrible circumstances
Conclusions Part Ia

- While the toxin genes were “occasionally” beneficial, similar observations for constantly (“slightly”) beneficial genes
- Spatial structure can overcome apparent “paradoxes” caused by positive frequency dependence (“Allee effects”)
- Microbes engage in HGT under terrible circumstances. It can still benefit them!
- To understand this, spatial heterogeneity is relevant:
What about the vehicles of HGT?

Evolution by means of horizontal gene transfer

- **Transduction**
  - Infection by bacteriophage
- **Transformation**
  - Cell lysis and uptake of DNA
- **Conjugation**
  - Direct contact via conjugation pilus

These are the “text-book” examples of HGT, but there are more mechanisms and vehicles.

- Membrane vesicles, gene transfer agents, transposons, integrative and conjugative elements (ICEs), integrons, BORGs, starships and voyagers, even mobile chromosomes...

The list is endless...

“Mobile elements are entities that evolved to persist and replicate through adaptations that move DNA.”

— James P.J. Hall, 2021; “The secret lives of Mobile Genetic Elements”
Friends or foes?

Mobile elements are diverse and evolve on a parasitism-mutualism continuum.
Friends or foes?

- Transposons are the simplest “nested replicator”, they replicate inside chromosomes.

- They can also jump from cell to cell, after uptake from the environment.

- Occasionally, they carry useful genes in nature, such as antimicrobial resistance genes.

- So what drives parasitism vs mutualism for these very simple entities?
Genome evolution 101

• More models of **genome evolution** will follow later in the course, but here's a quick intro

In earlier models, bacteria are “bags of genes”…

But real genes are on a chromosome!

• Suddenly, the word “integration” means something else...
• Genes have to **insert somewhere** (more on this later!)
The pearls-on-strings (PoS) model of genome evolution
A versatile model

Gene clustering (Crombach et al., 2007)

Gene regulatory network evolution (Vroomans et al., 2017)

Combine with artificial chemistry (van Dijk et al. 2019)

Add self-replicating pearls —> mobile genetic elements! (van Dijk et al., 2021, 2024 wip)
Part 1 - Horizontal- vs vertical transmission of genes and microbiomes

PoS gives “linkage” of genes

Can a selfish genetic element get “linked” to a good gene? (transposons carry AMR genes, phages carry virulence genes, ...)

Toy model of co-evolving genomes and transposons

Warning: the figures on the next slides never made it to publication, and are not super pretty :)

TEs are not predefined, they have to emerge!
TEs emerge after some time (no AMR selection)
System persists because of spatial structure

Host replicator (microbial cell)

a. negative selection > replication rate: transposable element lost

b. negative selection < replication rate: exponential growth of transposable elements
Adding selection for AMR genes (constant)

- AMR did emerge (blue line), but it is not linked to a transposon at all... (not shown)
Adding selection for AMR genes (pulsing)

• Pulsing for antibiotic resistance: still TEs and AMR do not get linked...
Okay, so getting SGEs “linked” to beneficial genes is really hard...

Then why do so many SGEs (transposons, phages) carry ecologically relevant genes?
Part 1 - Horizontal- vs vertical transmission of genes and microbiomes

Replication-rate of transposons determined by $\phi$ of flanking DNA:

- $\phi$ (evolves per non-coding gene)
- Transposase flanked by nearby repeats?
  - No
  - Yes
  - Every time step (rate $\beta$), replicate within host with chance $p = 4e^{-6}$
  - After uptake (rate $\nu$), integrate with chance $p = 4e^{-6}$

You?
What if jumping causes damage?

Transposon-induced mutations (TIMs)
What if jumping causes damage?

Genomes become more “streamlined” (smaller, less non-coding DNA)
This became a whole new story!
How long does it take to go extinct?

- a. negative selection > replication rate: transposable element lost
- b. negative selection < replication rate: exponential growth of transposable elements

**time-to-extinction**
Evolving a shorter extinction time...?

But wait, isn’t it better to take very long to go extinct?!
Immediately dying pays off: altruistic suicide!

(c) Streamlined genomes outcompete non-streamlined genomes by preventing TE proliferation (cartoon)
Not the same with sex!

- With sex, a TE doesn’t need to "jump into a gene" to transfer to another lineage!
- So streamlining doesn’t benefit the cells either!
- HGT and sex are very distinct processes!
if (this.lecture.time <= 11:20){
    skip_slides(0);
}
else{
    skip_slides(6);
}
What about more “clever" TEs?

a. Simulation of TE/host co-evolution (van Dijk et al., 2021)

Every “pearl” carries a insertion-site parameter between 0 and 1
Every transposon additionally has a target-site between 0 and 1
Highly specific TEs care about this (has to match). Non-specific TEs don’t.

Transposons can evolve to be nasty (non-specific) or show some restraint (high specificity)
Dynamics with/without a beneficial gene

- Every TE copy reduces fitness with 0.02 (starting from 1)
- Optionally, having 1 or more copies give a 0.12 fitness benefit (cargo gene)

This is with high uptake of eDNA
With lower HGT rates...

d. At low eDNA uptake (0.01), highly specific TEs evolve

Can anyone guess why this didn’t happen at high HGT?
Without HGT altogether

e. Without eDNA uptake, the fate of TEs is no different than any other gene

![Diagram showing the fate of TEs with and without a beneficial cargo gene.](image-url)
Part 1 - Horizontal- vs vertical transmission of genes and microbiomes

Side-by-side comparison

c. With high eDNA uptake (0.05), non-specific TEs evolve regardless of the beneficial cargo gene

d. At low eDNA uptake (0.01), highly specific TEs evolve

e. Without eDNA uptake, the fate of TEs is no different than any other gene

Both types coexist...? :o
Sneak peak:

Cacatoo – IS-elements and their host

Histogram of TE-counts at T=1743

Histogram of TE-properties at T=1743

Help pls!
Conclusions Part Ib

• Unclear why TEs are often associated with useful (or at least ecologically relevant) genes. What are my models missing?
• The “resource” on which TEs grow is non-coding DNA
• Reducing non-coding DNA can prevent TEs from taking over
• This requires group-level effects (it only works in space)
Intermezzo: setting yourself up for surprise

• As a modeller, it can be hard to decide what **NOT** to put into your model (especially if you enjoy programming, talking to you ALKMINI!!1!!)

• But: by adding complexity and degrees of freedom, you allow a model to surprise you!

• This can reduce your bias (we can’t nullify it of course, but it helps)
Horizontal vs. vertical transmission of microbiomes, similar rules?

• Microbiomes can be inherited from your parents (vertical inheritance) or from the environment (horizontal inheritance)
• Do the rules we found for HGT apply here too?
Getting microbes from your mom, or from the environment

IBM of symbiont-host coevolution
(three nested biological entities: genes, bacteria, and hosts)

Level I: genes
- Individual bacteria with a genome (many genes model)
- Gene transfer (per gene)
- Gene loss (per gene)
- Gene gain (per generation)

Level II: bacteria
- Bacterial population dynamics (freeliving in within host)
- Local populations with birth/death
- Gene loss/gain/transfer
- Microbial migration (freeliving)

Level III: hosts
- Host population dynamics
- Individual birth/death events
- Microbiome spilling upon death
- Host migration
- Microbiome inheritance regimes
- Distinct inheritance regimes (or mixed)

Virulence only emerges with horizontal (H) transmission, and only when microbial migration is high
Environmental and horizontal transmission are different things!

Animals need an adaptive immune system?
Once again: **horizontal** vs. **vertical** transmission modes have a massive impact on the **phylogeny**

This impact on phylogeny is visible PRIOR to the disease —> it is an effect of horizontal transfer, not of the disease!
Signatures of horizontal vs. vertical transmission, early indicators of disease?
Conclusions Part Ic

- While “horizontal transmission” and “via the environment” are often used interchangeably in the literature, **environmental transmission can be vertical in space**!
- Horizontal transmission gives lower-level entities (genes in microbes, or microbes in hosts) **an opportunity to be nasty**
- This interplay gets even more interesting when considering **more than just 2 levels** (e.g. genes, microbes, groups of microbes)
- Phylogenies could be **predictors of conditions that promote nastiness** (not the nastiness itself!!)
- *(could be: this is work in process!)*
“Cheaters and cooperators”

• Altruism is common in nature

• Many papers on this, but most focus on a “cheater-cooperator” framework...

• Cooperators provide a public good. Cheaters don’t.

• Often **unstable** in well-mixed environments, but the system survives in space

Local extinction but global persistence
Black Queen dynamics

- Benefits are not always exclusive
- Microbial communities contain many “public goods” (cellulase, peroxidase, elastase, amylase, beta-lactamase, heavy metal detoxification, wss operon (cellulose production!))
- Why provide this costly public service, if someone else can do it for you?
- Refers to “Game of Hearts”, where players do not want to hold hearts (-1), and especially not the queen of spades! (-13)
Strong BQ looks like "cooperation", but the route there could hardly be more different!

If you took these to the lab, what would you call them?

"Cheater cooperator"

This doesn’t need to follow the "cheater-cooperator" narrative.
Part II: Black Queen ecosystems and a race to the bottom

Matt Fullmer’s model of BQ dynamics

A) Spatially structured model of evolution with multiple public goods

B) Classifying emergent ecologies
Part II: Black Queen ecosystems and a race to the bottom

Both “classic” and “strong” Black Queen observed

A) Strong Black Queen

- non-producers
- 1-producers
- all others

B) Classic Black Queen

- non-producers
- 6-producer
- all others

1-1-1-1-1-1 ecosystem

2-0 ecosystem

genome_state:
- 0,0,0,0,0,0
- 0,0,0,0,1
- 0,0,0,1,0
- 0,0,1,0,0
- 0,1,0,0,0
- 1,0,0,0,0
- undefined
Stable yet redundant ecosystems...?

A) Ecosystem of redundant types (9)-(8)-(7)

B) Ecosystem of redundant types 6-(6)-(4)-(2)

What the heck?!
Late shifts in ecosystem structure

A 3-3-0 ecosystem transitions to a 3-(2)-(1) after millions of time steps

(2)-(1) was frequently present, but failed to invade

After they DID invade, the non-producers are nearly pushed out
Why can ecosystems “be stuck”, not dividing labour, for so long?

Two hypotheses:

i) Non-producers (or lesser producers) keep preventing it because of their fitness benefits

ii) Non-producers (or lesser producers) keep preventing it because they simply take up space!
Introducing “infertile blocks”

Exactly the type of “experiment” that would be impossible in the lab

With 10% blocks, 3-3 successfully transforms into 3-2-1
Introducing “infertile blocks”

Exactly the type of “experiment” that would be impossible in the lab

With 33% blocks, 3-3 persisted!
Neighbourhood certainty shapes BQ dynamics!

B) Without non-producers, producers can nearly always reproduce, promoting Black Queen dynamics

C) With non-producers or "blocks", producers are often reproductively isolated, stalling Black Queen dynamics
Are costs even all that relevant?
No! BQ can also work without any costs!

• “Selection is blind” for as long as someone provides the CG

• So without a cost, drift can promote dependencies!

• So once again: fitness effect for public goods are not the only important thing!

• The Black Queen hypothesis was defined as this social dilemma.

• The neutral variant has been phrased as “Grey Queen” by Ford Doolittle, but I like purple more.

• Let’s explore this a little more (WIP)
Grey Queen dynamics in patches

Within populations (patches of 200 cells)
- **Strong** black queen dynamics ("evolutionary race to the bottom")
- Five essential public goods (A,B,C,D,E)
- Death of random cells
- Birth of (viable) cells
- **Gene loss** upon reproduction
- **No costs** for public good production ("Grey Queen")

Between populations (patches)
- Differential **persistence** (resilience to noise)
- Differential **spread** (colonisation capacity)
- "Survival of the systems"?
Grey Queen dynamics in patches
Community-level properties

• Patches (microbial communities!) have the following properties:
  “Aging” → Within-population conflict ages patches
  “Death” → “Aged” communities die
  “Birth” → Founding a new colony,
  “Rejuvenation” → Decrease in mutual dependencies upon community-reproduction

I.e. patch-level properties, but without “patch-level” parameters!

• These dynamics can avoid the evolutionary race to the bottom, sustaining public good production
Community-level selection?
Strong community-level selection!

Earlier models have only shown selection for lower growth rate when considering limited resources! (If we’re going to use words like “cheaters”, THIS is cheating! :P)

“Recent theoretical progress highlights that natural selection can occur based solely on differential persistence of biological entities, without the need for conventional replication. This calls for a reconsideration of how ecosystems and social systems can evolve, based on identifying system-level properties that affect their persistence.”
- Tim Lenton on “Survival of the Systems”

This still requires some unpacking.
Conclusions Part II

- **Black Queen dynamics** can promote an evolutionary race to the bottom
- With limited interaction range, strange (“redundant”) ecosystems can form and be maintained for long evolutionary timescales
- Non-producers can “stall” the race to the bottom by taking up space -> neighbourhood uncertainty!

- BQ still happens **even when there are no costs** to producing the common goods (Grey Queen)
- If the race to the bottom ends in catastrophe, we can observe **selection for lower growth rates**, without any limiting resource!
General conclusions

• Evolutionary dynamics with horizontal transmission create **conflicts between lower- and higher-level entities**

• The conflict between levels of selection (gene and microbe, microbe and host) becomes extra interesting when including **more than two levels**

• Sometimes it can help to **not** start with the simplest model (this is how we accidentally discovered the streamlining in response to transposons!)

• While many models do not include the fact that organisms “take up space”, this turns out to be very important for ecosystem function (BQ dynamics)

• Besides spatial structure there is also **genome structure** to consider when thinking about evolution. Paulien will discuss more on this later in the course.