

# Equating OTUs With Species

Micah Dunthorn



OTUs ? = species

1) what is a species

2) OTU clustering as a step

3) OTU clustering approaches

What is your definition  
of a species?

eukaryote

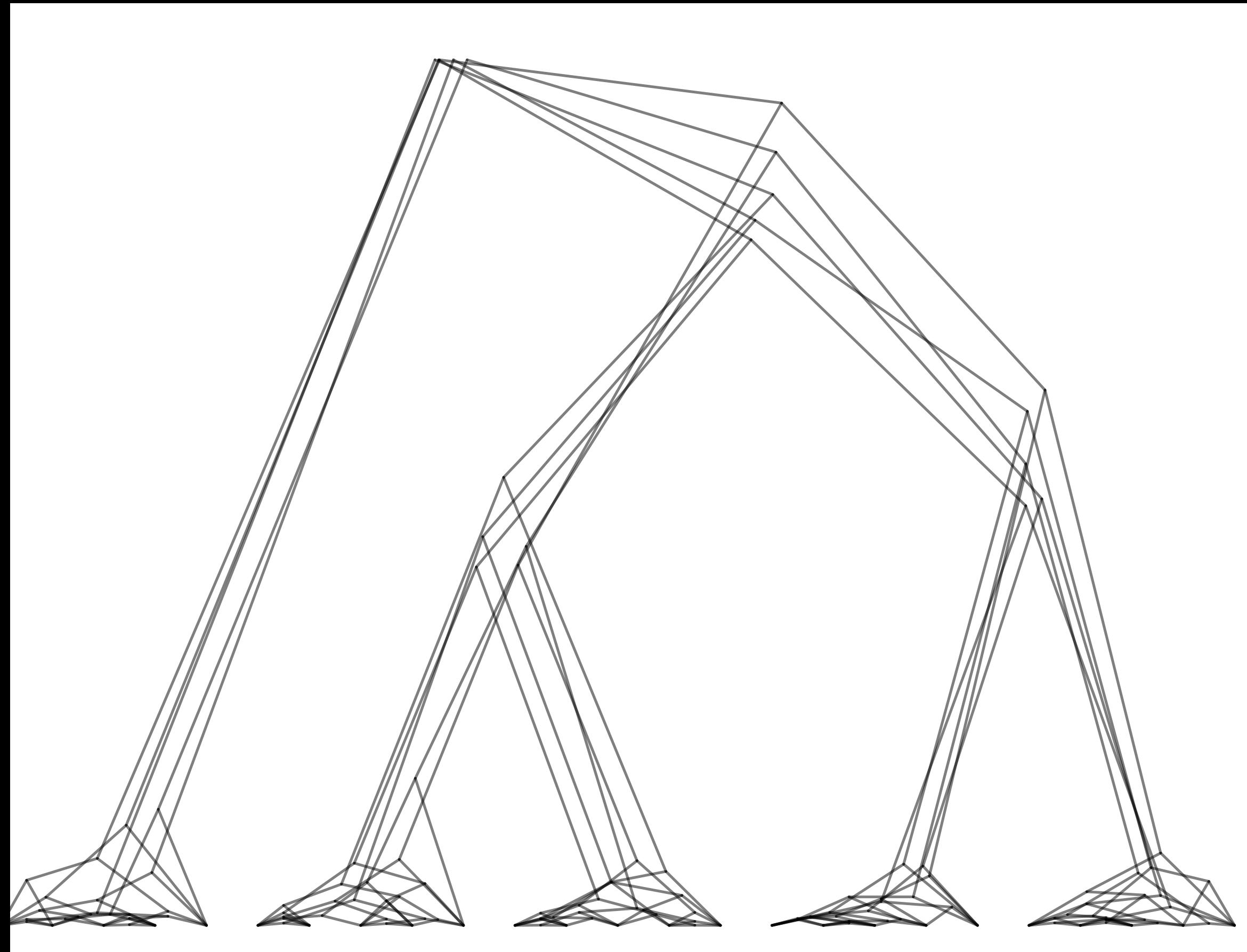
vs.

bacteria & archaea

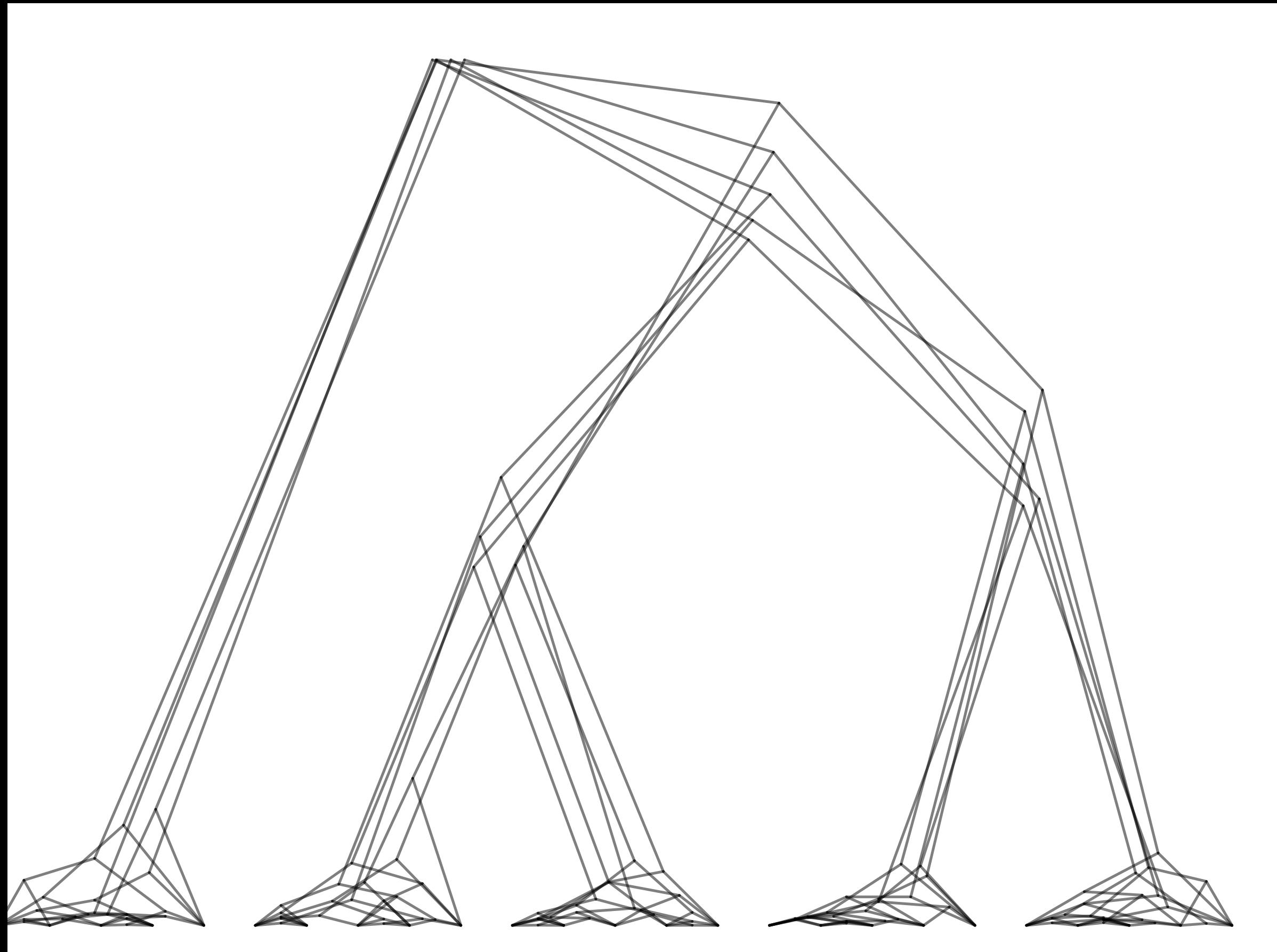
sexual

vs.

asexual

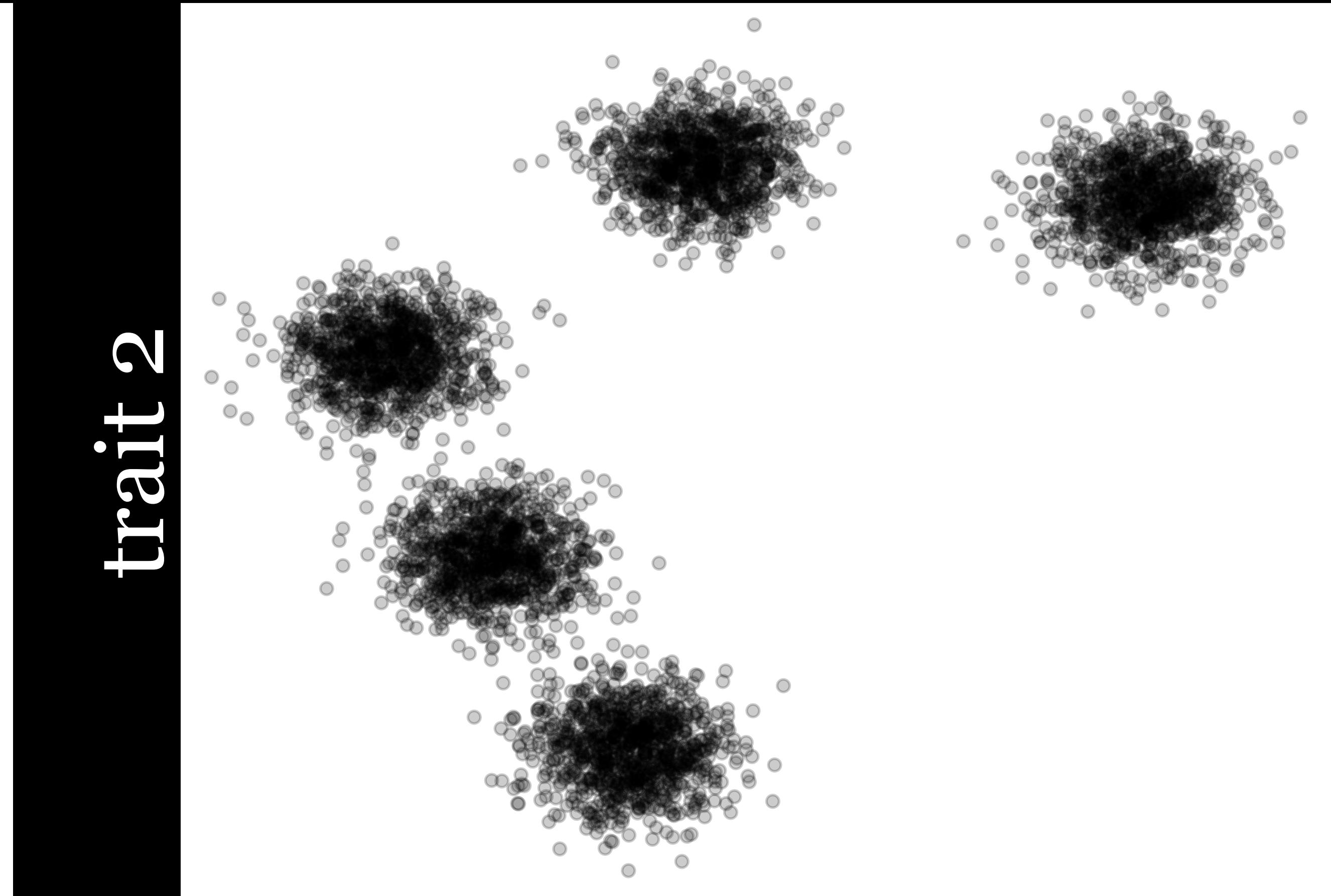
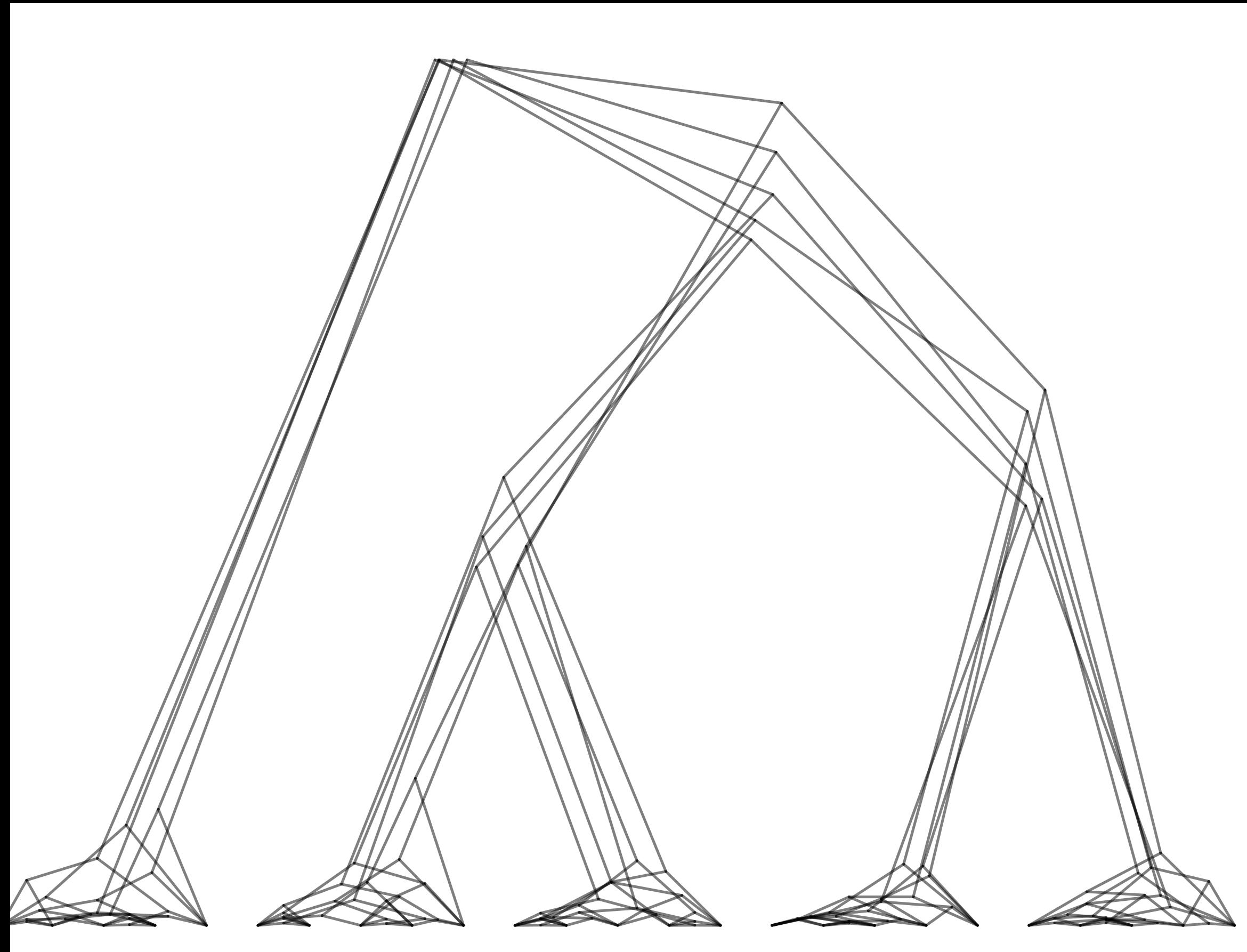


macro-evolutionary  
processes of phylogeny



macro-evolutionary  
processes of phylogeny

micro-evolutionary  
processes of population  
genetics



1997 in “Species: The units of biodiversity”

19

# A hierarchy of species concepts: the denouement in the saga of the species problem

---

*R. L. Mayden*

Contacting address: Department of Biological Sciences, P.O. Box 0344, University of Alabama, Tuscaloosa, AL 35487, USA

# Biological Species

= groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups

- Mayr 1942

1937 23:378-384

PNAS

*SEX, SEX INHERITANCE AND SEX DETERMINATION IN  
PARAMECIUM AURELIA*

BY T. M. SONNEBORN

DEPARTMENT OF ZOOLOGY, JOHNS HOPKINS UNIVERSITY

Communicated June 9, 1937

Opinion

2010 18:183-188

Cell  
PRESS

# Secretive ciliates and putative asexuality in microbial eukaryotes

Micah Dunthorn<sup>1,\*</sup> and Laura A. Katz<sup>1,2</sup>

**HYPOTHESIS**

Insights & Perspectives

2019 41:e1800246

 BioEssays

[www.bioessays-journal.com](http://www.bioessays-journal.com)

## All Eukaryotes Are Sexual, unless Proven Otherwise

Many So-Called Asexuals Present Meiotic Machinery and Might Be Able to Have Sex

*Paulo G. Hofstatter and Daniel J. G. Lahr\**

2008 8:98

# BMC Evolutionary Biology



Research article

Open Access

## Gene flow persists millions of years after speciation in *Heliconius* butterflies

Marcus R Kronforst

# Morphological Species

= the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means

- Cronquist 1978

Trends in Microbiology

2019 27:197-205

Opinion

# Are We Overestimating Protistan Diversity in Nature?

David A. Caron<sup>1,\*</sup> and Sarah K. Hu<sup>1</sup>

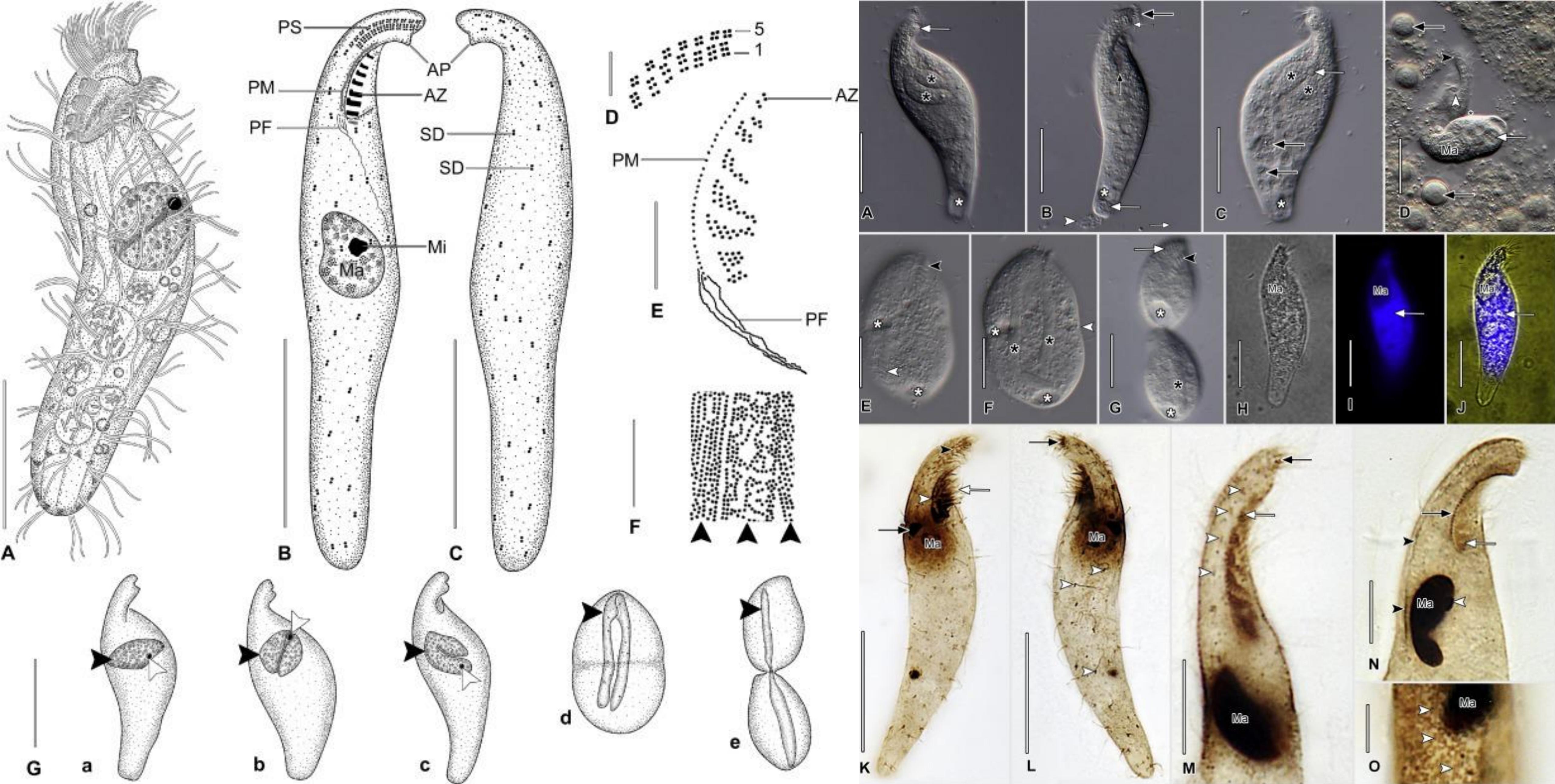
**CellPress**  
REVIEWS

Protist, Vol. 169, 362–405, July 2018  
<http://www.elsevier.de/protis>  
Published online date 21 April 2018

Protist

# Tropidoatractidae fam. nov., a Deep Branching Lineage of Metopida (Armophorea, Ciliophora) Found in Diverse Habitats and Possessing Prokaryotic Symbionts

Johana Rotterová<sup>a,1</sup>, William Bourland<sup>b</sup>, and Ivan Čepička<sup>a</sup>



*Tropidoatractus ariella*

## TAXONOMIC DESCRIPTION

Boscaro *et al.*, *Int J Syst Evol Microbiol* 2017;67:3570–3575  
DOI 10.1099/ijsem.0.002169

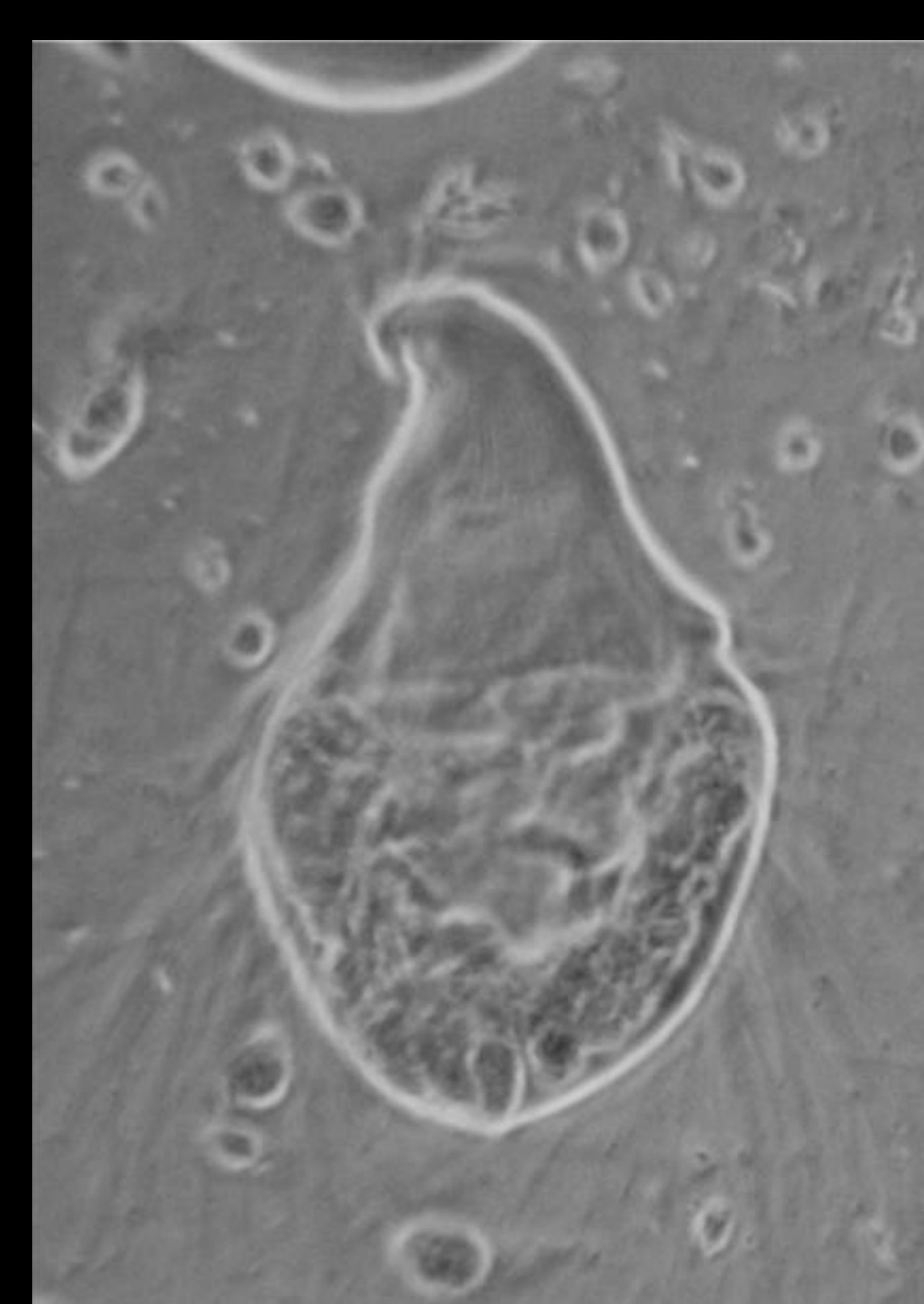


# Molecular characterization and phylogeny of four new species of the genus *Trichonympha* (Parabasalia, Trichonymphea) from lower termite hindguts

Vittorio Boscaro,<sup>1,\*</sup> Erick R. James,<sup>1</sup> Rebecca Fiorito,<sup>1</sup> Elisabeth Hehenberger,<sup>1</sup> Anna Karnkowska,<sup>1,2</sup> Javier del Campo,<sup>1</sup> Martin Kolisko,<sup>1,3</sup> Nicholas A. T. Irwin,<sup>1</sup> Varsha Mathur,<sup>1</sup> Rudolf H. Scheffrahn<sup>4</sup> and Patrick J. Keeling<sup>1</sup>



*Trichonympha hueyi*



*Trichonympha deweyi*



*Trichonympha louiei*

1937 23:378-384

PNAS

*SEX, SEX INHERITANCE AND SEX DETERMINATION IN  
PARAMECIUM AURELIA*

BY T. M. SONNEBORN

DEPARTMENT OF ZOOLOGY, JOHNS HOPKINS UNIVERSITY

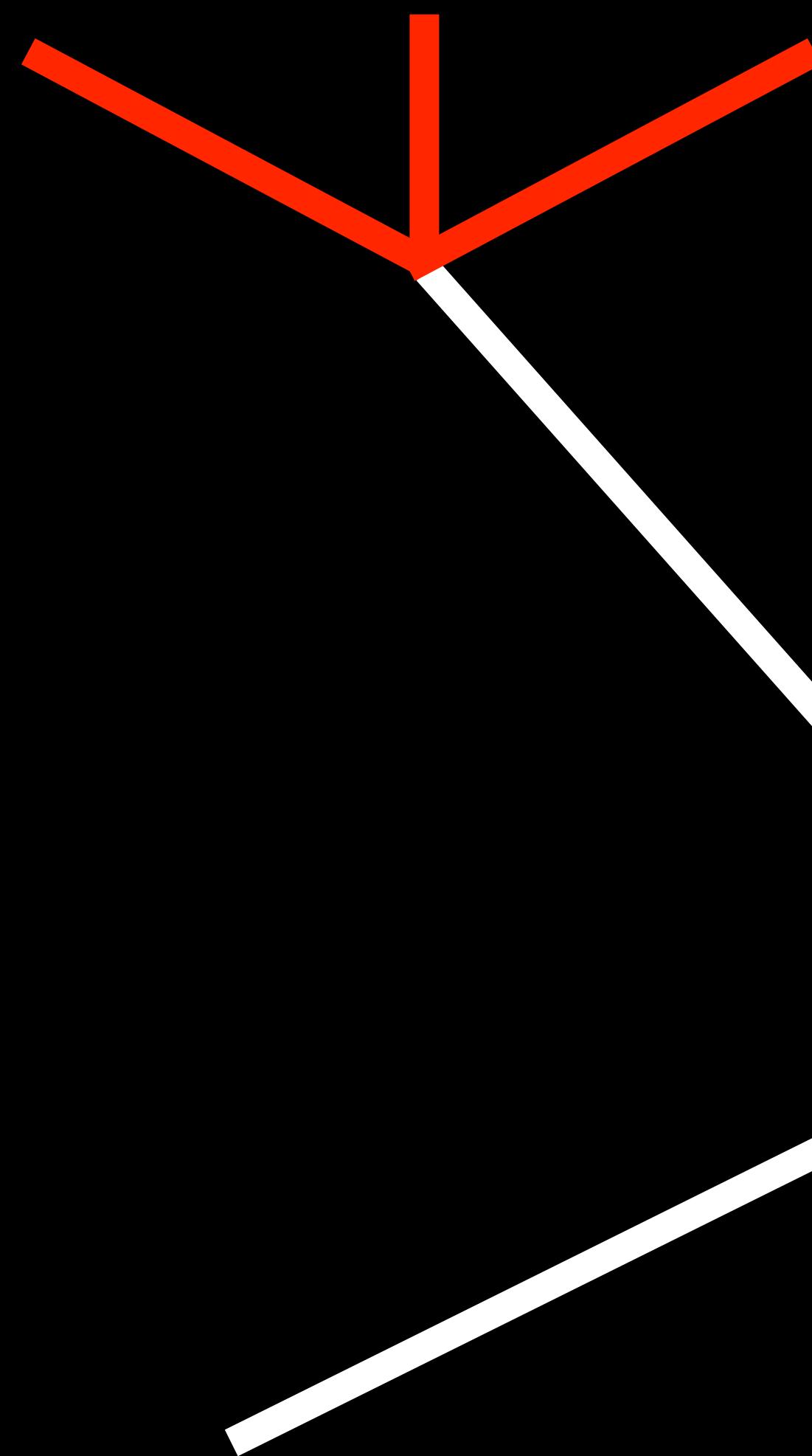
Communicated June 9, 1937

# Phylogenetic Species

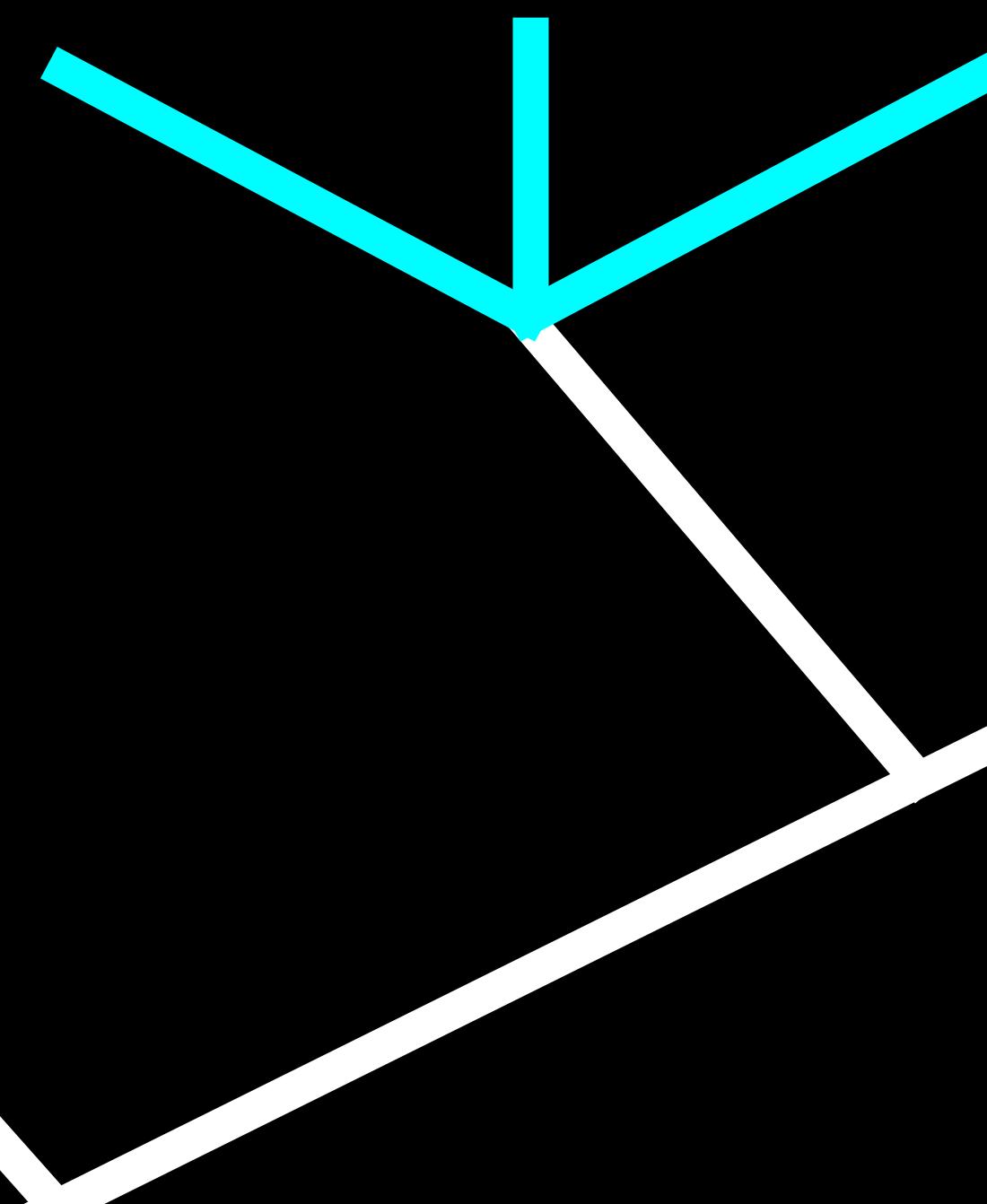
= the smallest biological entities that are diagnosable and/or monophyletic

- Mayden 1997

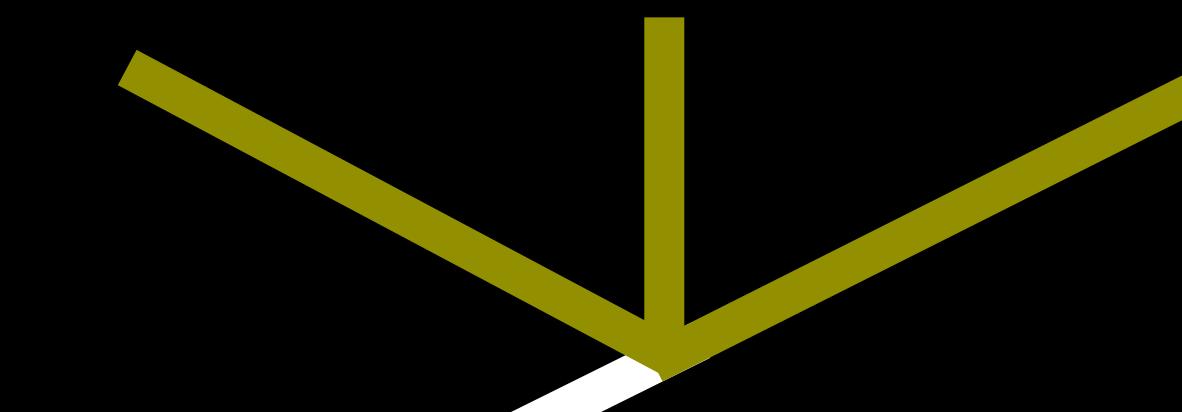
species 1

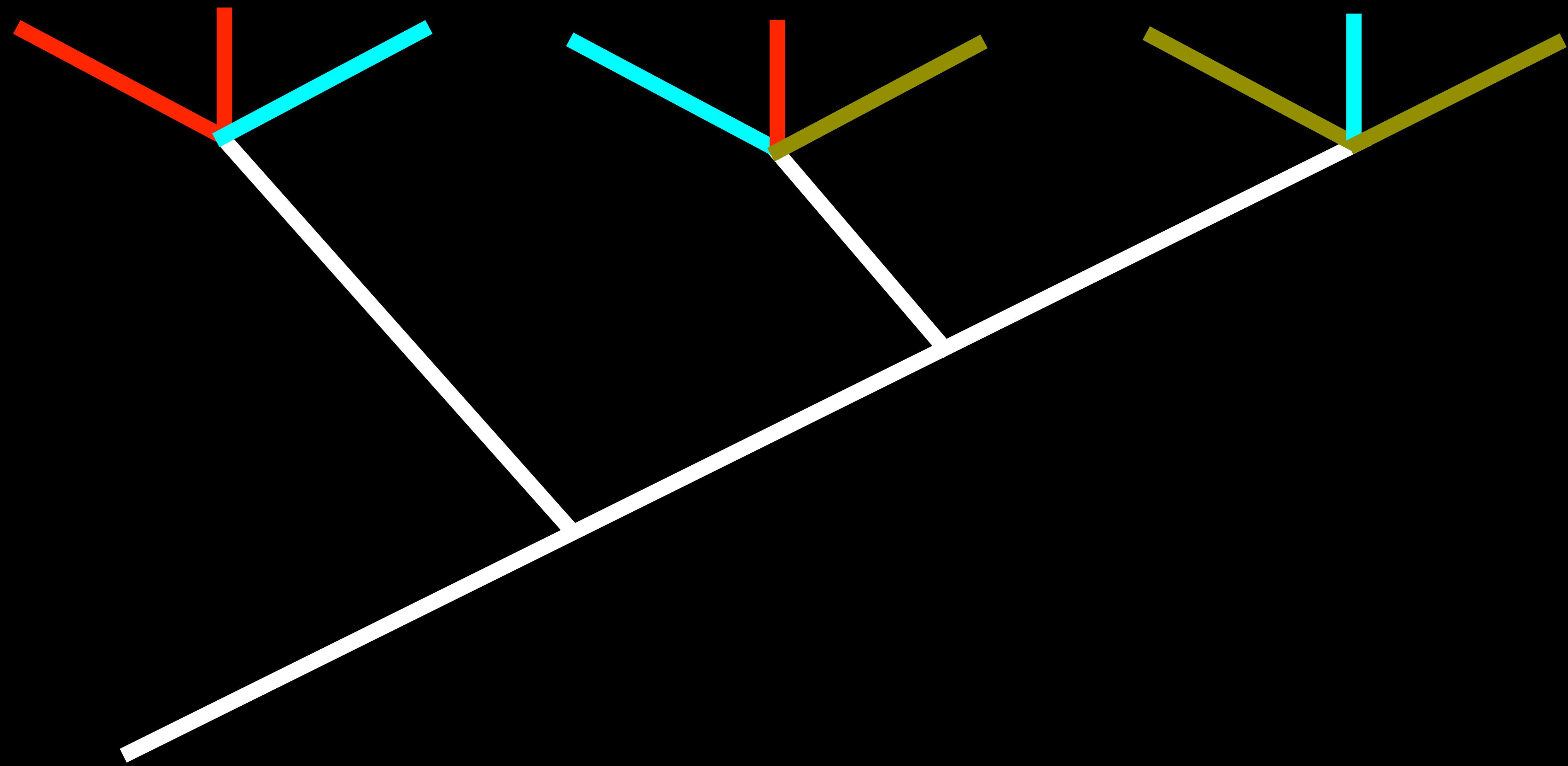


species 2



species 3





*Syst. Biol.* 56(6):879–886, 2007

Copyright © Society of Systematic Biologists

ISSN: 1063-5157 print / 1076-836X online

DOI: 10.1080/10635150701701083

# Species Concepts and Species Delimitation

KEVIN DE QUEIROZ

*Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington,  
DC 20560-0162, USA; E-mail: dequeirozk@si.edu*

concept of a species

= separately evolving metapopulation lineages

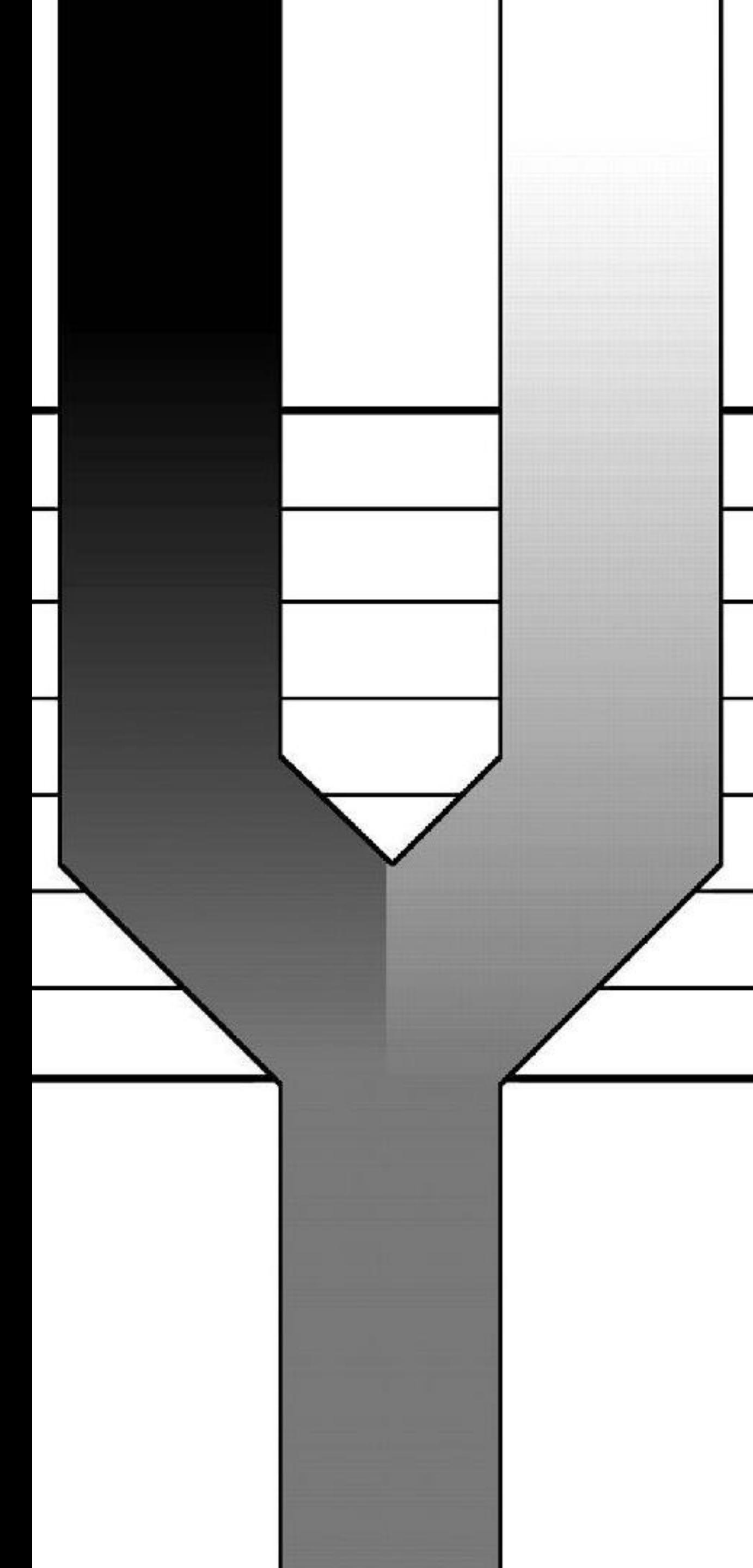
concept of a species

= separately evolving metapopulation lineages

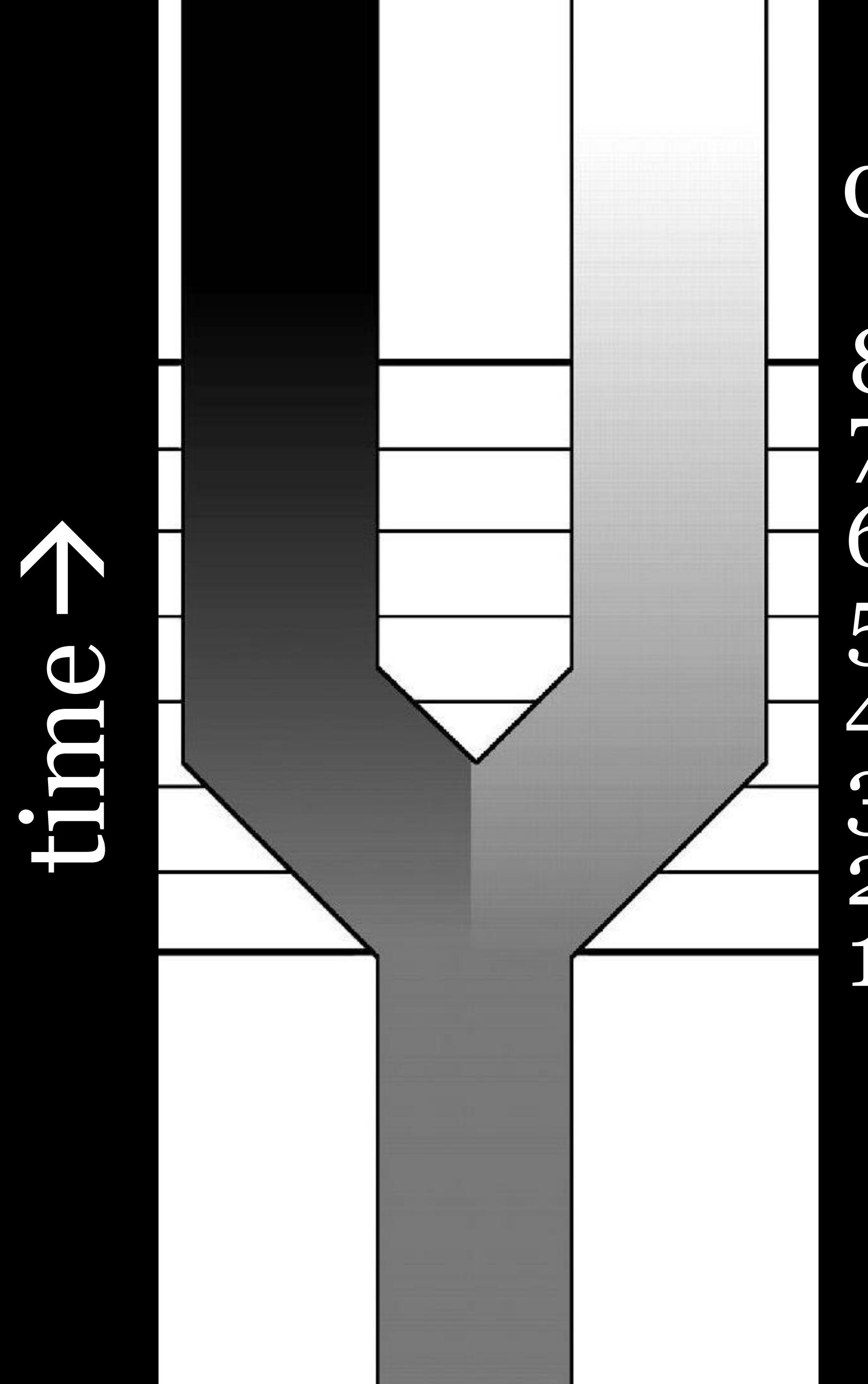
operational criteria for species delimitation

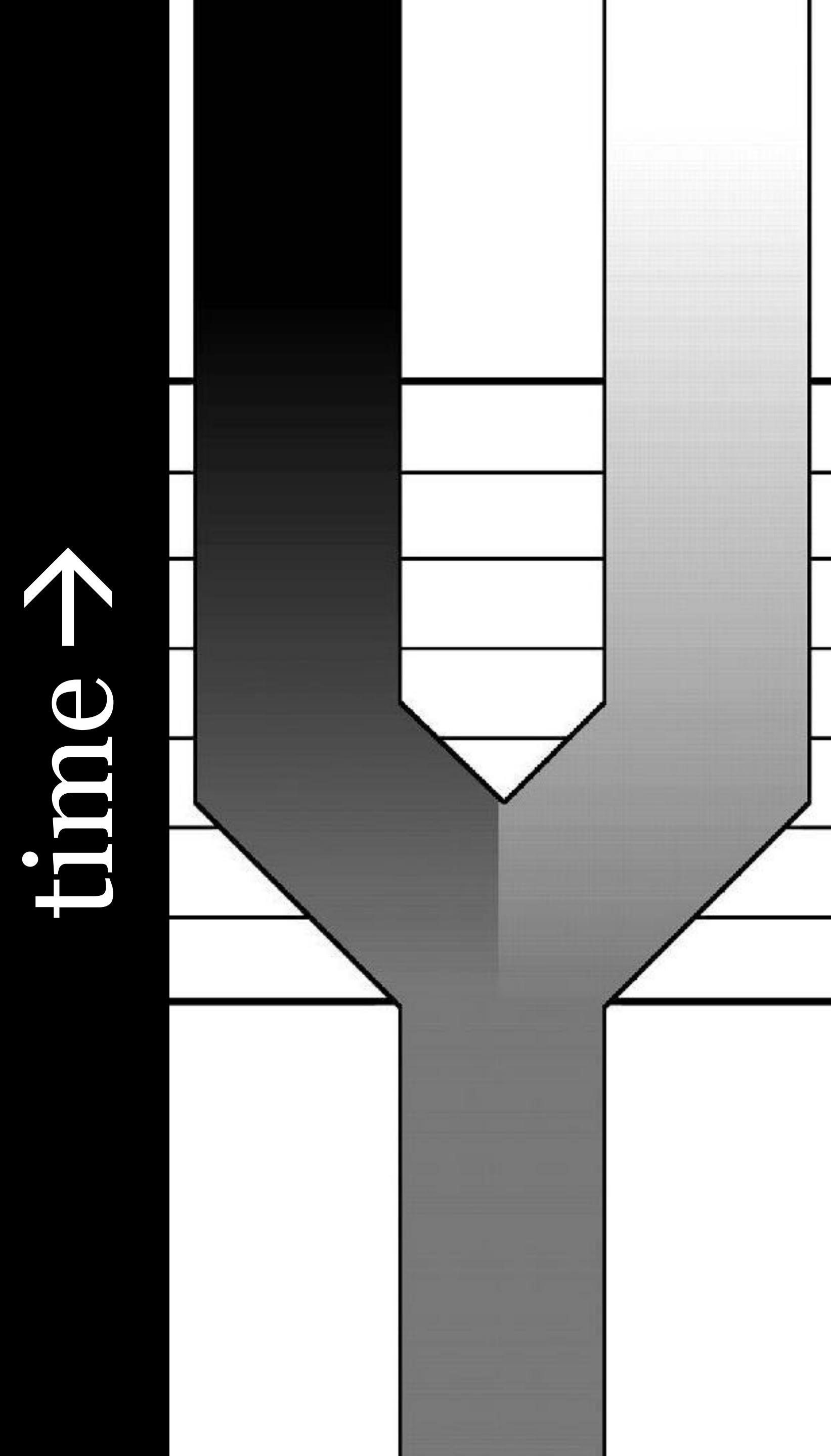
= Biological Species, Morphological Species,  
Phylogenetic Species, and many more...

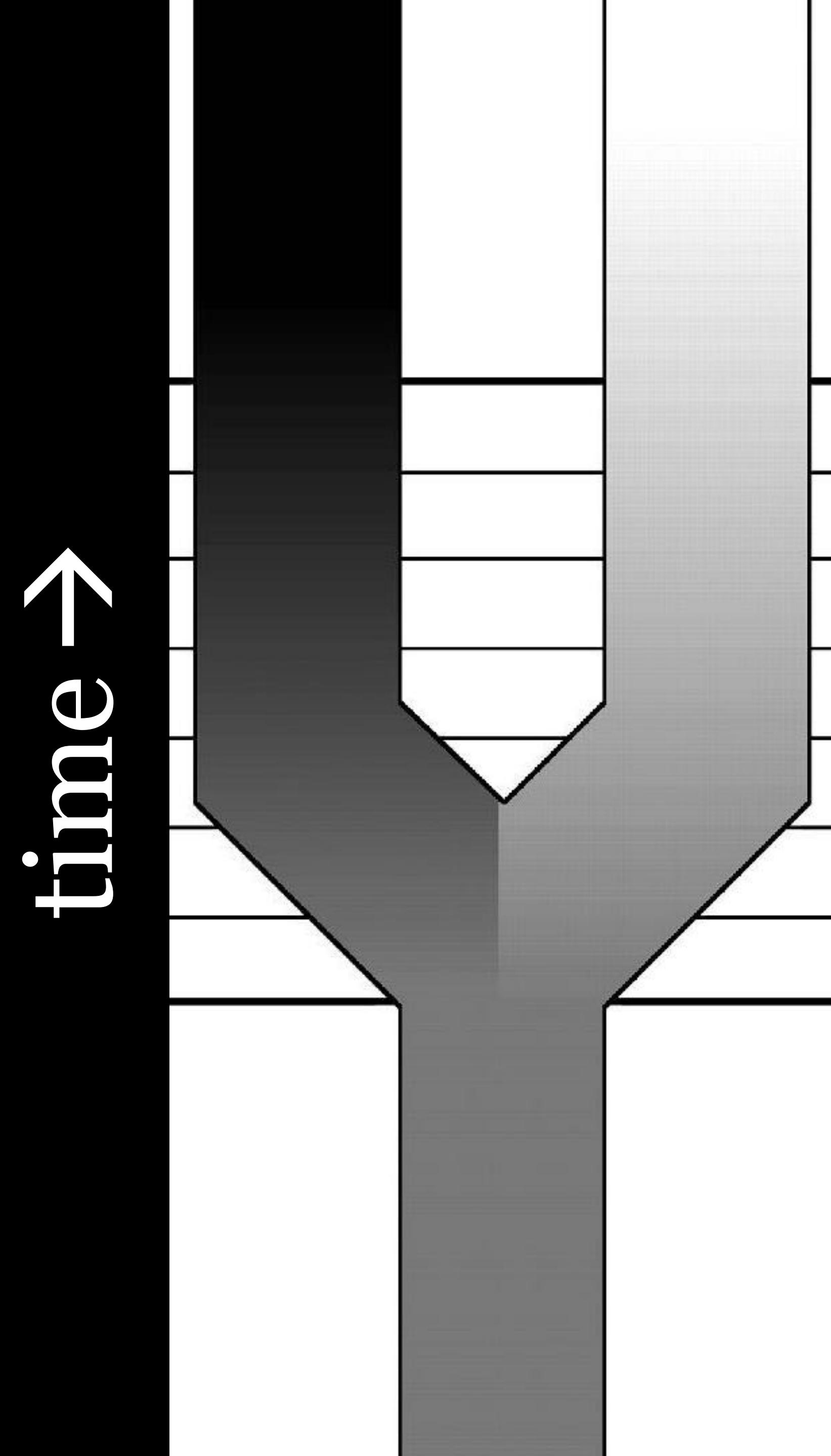
time →



# operational criteria







operational criteria

Phylogenetic

Morphological

Biological

- 1) what is a species
- 2) OTU clustering as a step
- 3) OTU clustering approaches

# clustering

= grouping of similar amplicons

# clustering

sampling,  
storage

clustering

## Opinion

# Replicate or lie

**James I. Prosser\***

*Institute of Biological and Environmental Sciences,  
University of Aberdeen, Cruickshank Building, St.  
Machar Drive, Aberdeen, AB24 3UU, UK.*

sampling,  
storage



nucleotide  
extraction

clustering

sampling,  
storage



nucleotide  
extraction



amplification,  
sequencing

clustering

sampling,  
storage



nucleotide  
extraction



amplification,  
sequencing

cleaning,  
dereplication



clustering

sampling,  
storage



nucleotide  
extraction



amplification,  
sequencing

cleaning,  
dereplication



clustering



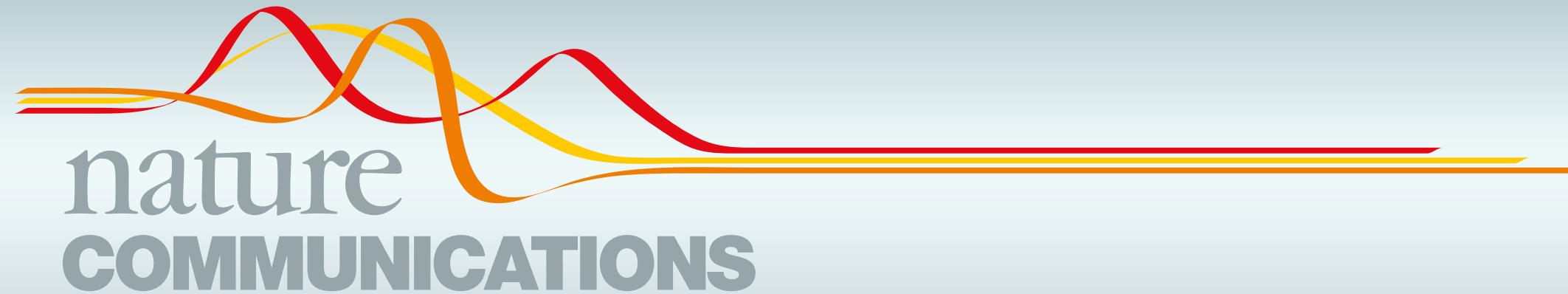
more  
cleaning

## Opinion

# Replicate or lie

**James I. Prosser\***

*Institute of Biological and Environmental Sciences,  
University of Aberdeen, Cruickshank Building, St.  
Machar Drive, Aberdeen, AB24 3UU, UK.*



2017 8:1188

ARTICLE

DOI: 10.1038/s41467-017-01312-x

OPEN

program: LULU

# Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates

Tobias Guldberg Frøslev<sup>1,2</sup>, Rasmus Kjøller<sup>1</sup>, Hans Henrik Bruun<sup>1</sup>, Rasmus Ejrnæs<sup>3</sup>, Ane Kirstine Brunbjerg<sup>3</sup>, Carlotta Pietroni<sup>2</sup> & Anders Johannes Hansen<sup>2</sup>

sampling,  
storage



nucleotide  
extraction



amplification,  
sequencing

cleaning,  
dereplication



clustering



more  
cleaning

taxonomic  
assignments

sampling,  
storage



nucleotide  
extraction



amplification,  
sequencing

cleaning,  
dereplication



clustering



more  
cleaning

taxonomic  
assignments



phylogenetic  
placements

sampling,  
storage



nucleotide  
extraction



amplification,  
sequencing

cleaning,  
dereplication



clustering



more  
cleaning

taxonomic  
assignments



phylogenetic  
placements



$\alpha, \beta$  diversities,  
co-occurrences

- 1) what is a species
- 2) OTU clustering as a step
- 3) OTU clustering approaches

just clustering

vs.

clustering and cleaning

*de novo* clustering  
vs.  
closed-reference clustering

metabarcoding data  
vs.  
meta-genomic/-transcriptomic

2002 75:509-516

Biol. J. Linn. Soc.

# **On the use of genetic divergence for identifying species**

J. WILLEM H. FERGUSON\*

*Department Zoology and Entomology, University of Pretoria, 0002 Pretoria, South Africa*

global clustering  
thresholds

local clustering  
thresholds

pairwise  
comparisons

phylogenetic  
comparisons

global clustering  
thresholds

local clustering  
thresholds

pairwise  
comparisons

phylogenetic  
comparisons

global clustering  
thresholds

local clustering  
thresholds

VSEARCH/  
Mothur  
DADA

Swarm

multi-rate  
PTP

pairwise  
comparisons

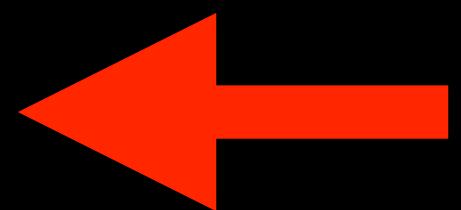
phylogenetic  
comparisons

global clustering  
thresholds

local clustering  
thresholds

VSEARCH/  
Mothur  
DADA

Swarm



multi-rate  
PTP

# VSEARCH: a versatile open source tool for metagenomics

Torbjørn Rognes<sup>1,2</sup>, Tomáš Flouri<sup>3,4</sup>, Ben Nichols<sup>5</sup>, Christopher Quince<sup>5,6</sup> and Frédéric Mahé<sup>7,8</sup>

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2009, p. 7537–7541

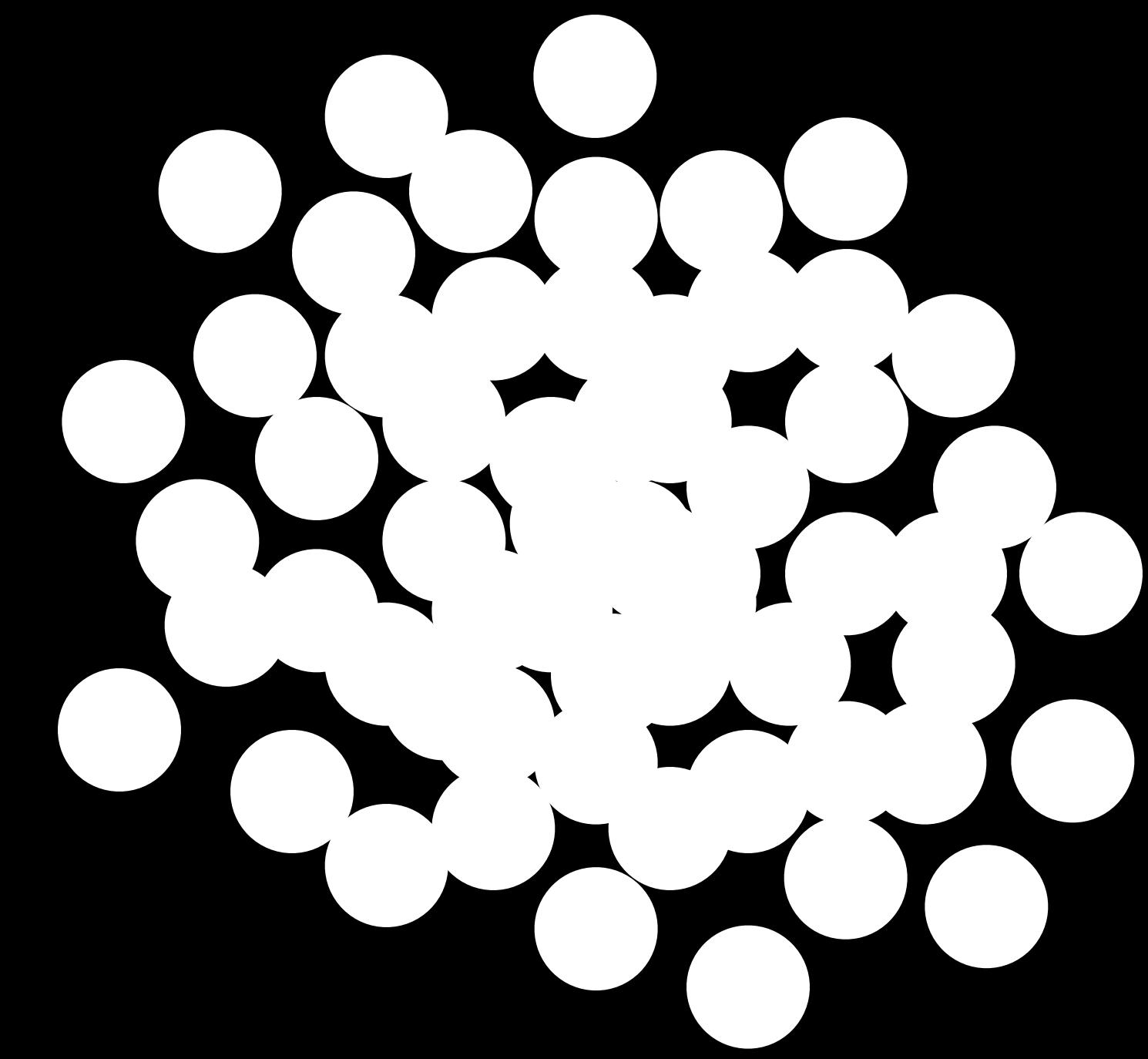
0099-2240/09/\$12.00 doi:10.1128/AEM.01541-09

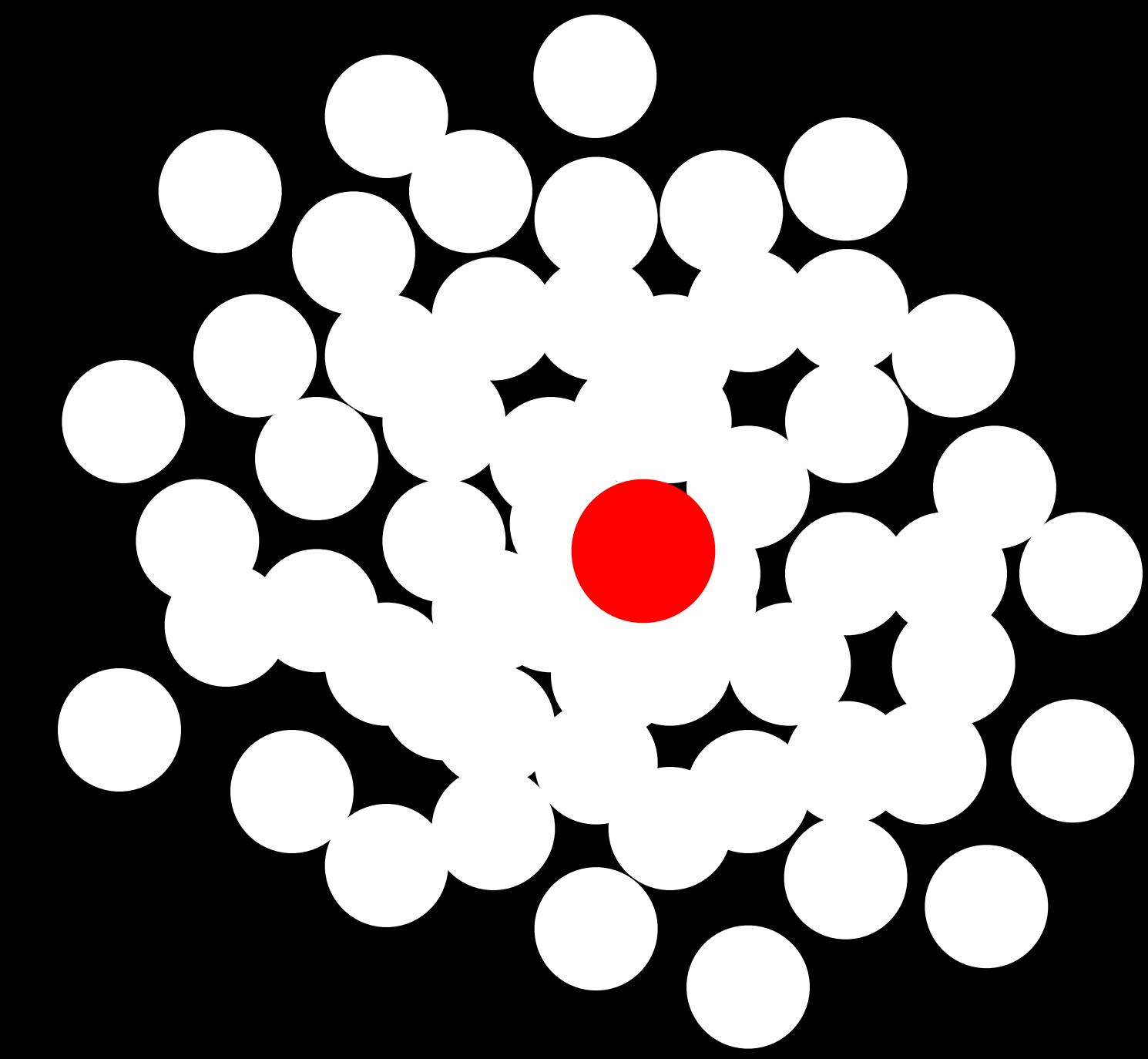
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

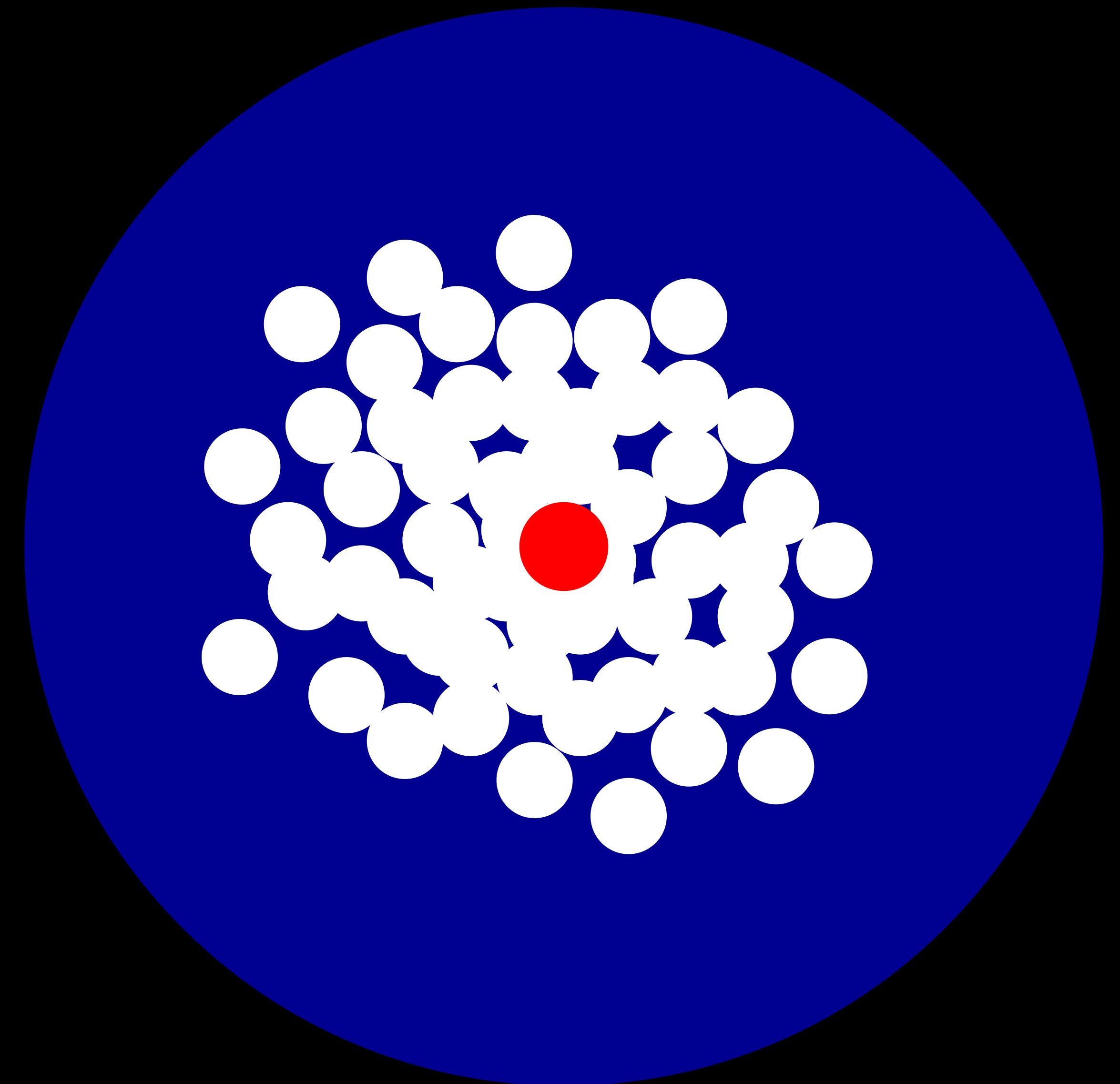
Vol. 75, No. 23

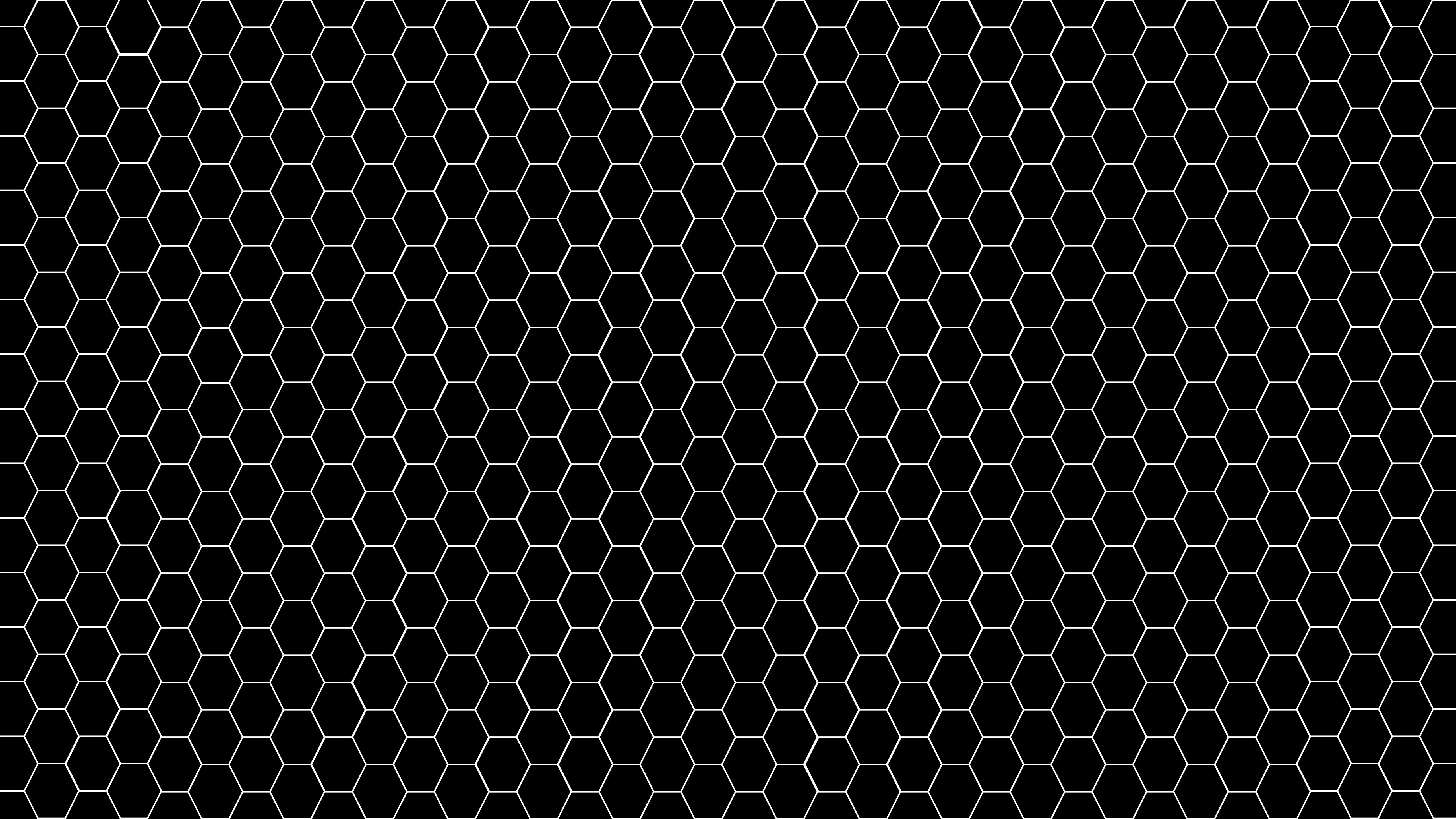
## Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities<sup>▽</sup>

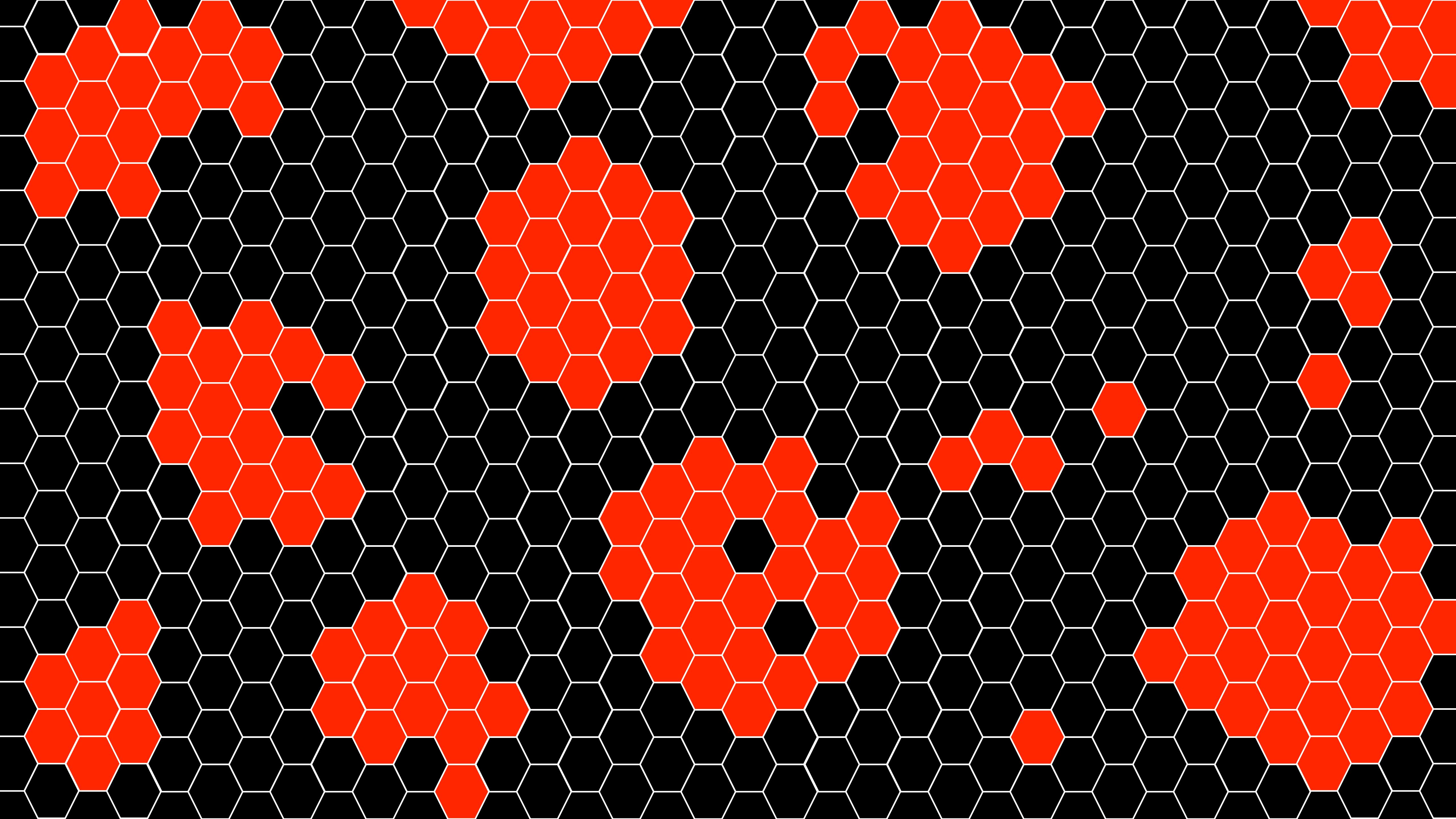
Patrick D. Schloss,<sup>1,2\*</sup> Sarah L. Westcott,<sup>1,2</sup> Thomas Ryabin,<sup>1</sup> Justine R. Hall,<sup>3</sup> Martin Hartmann,<sup>4</sup> Emily B. Hollister,<sup>5</sup> Ryan A. Lesniewski,<sup>6</sup> Brian B. Oakley,<sup>7</sup> Donovan H. Parks,<sup>8</sup> Courtney J. Robinson,<sup>2</sup> Jason W. Sahl,<sup>9</sup> Blaz Stres,<sup>10</sup> Gerhard G. Thallinger,<sup>11</sup> David J. Van Horn,<sup>2</sup> and Carolyn F. Weber<sup>12</sup>

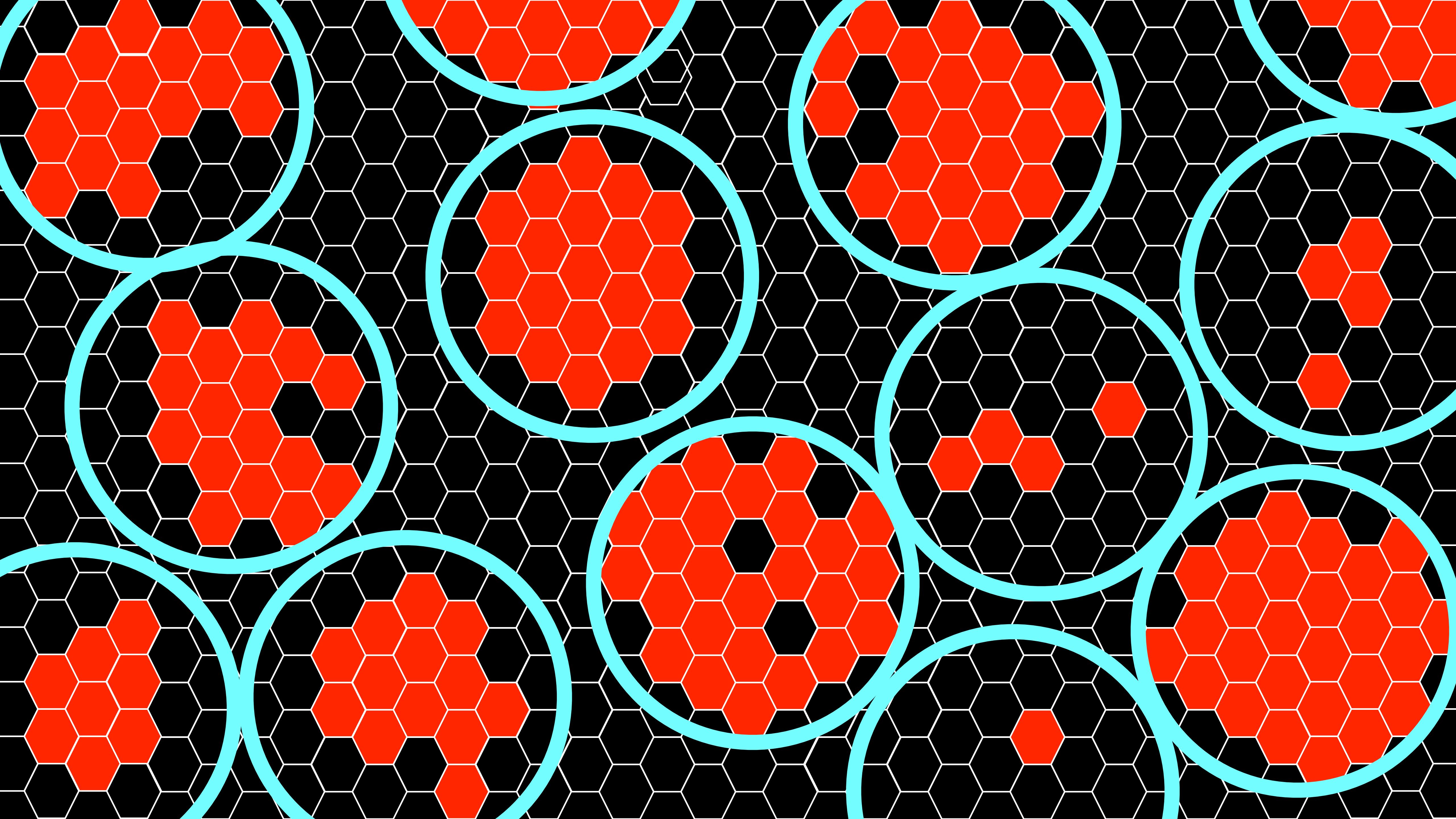


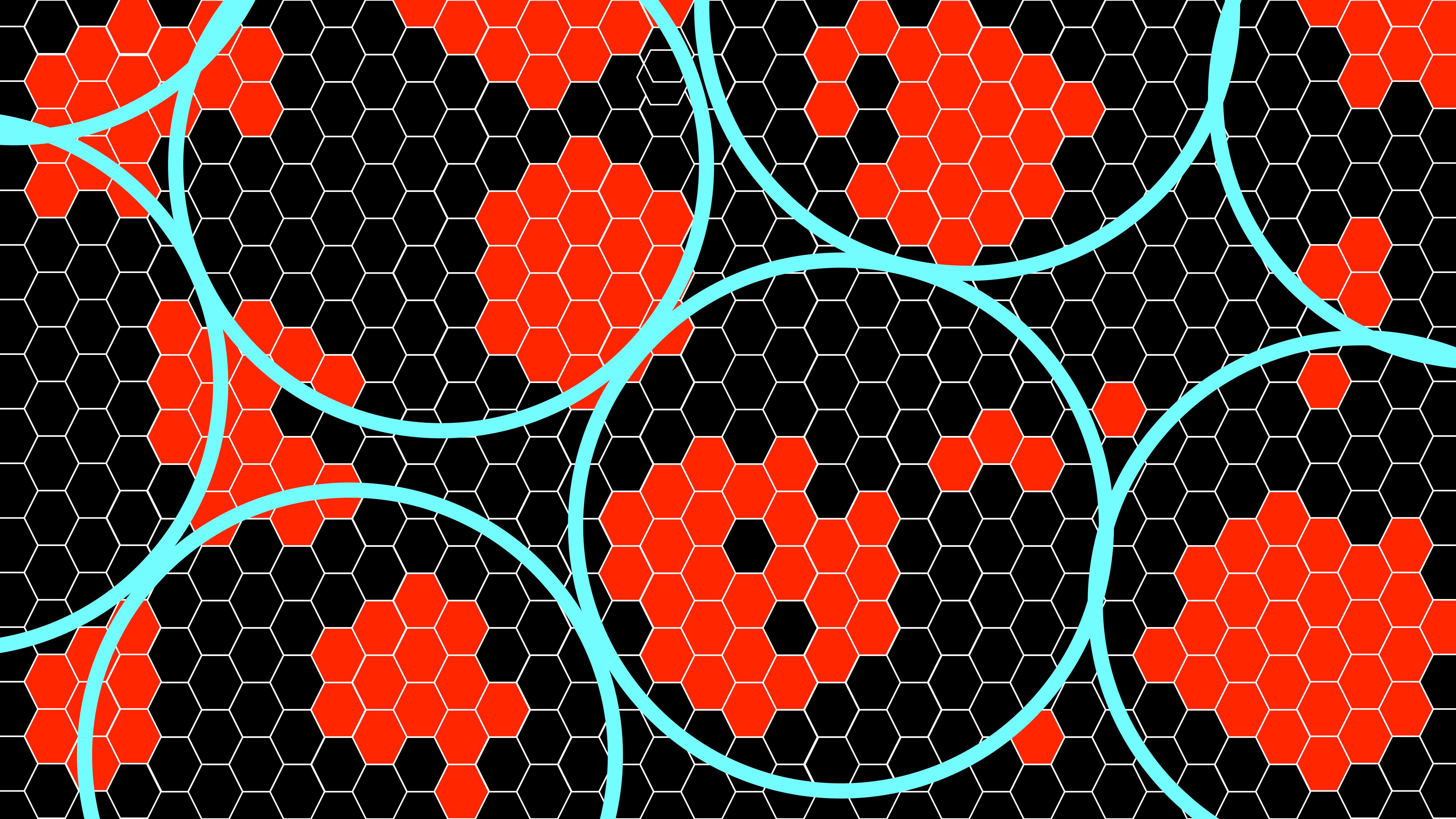


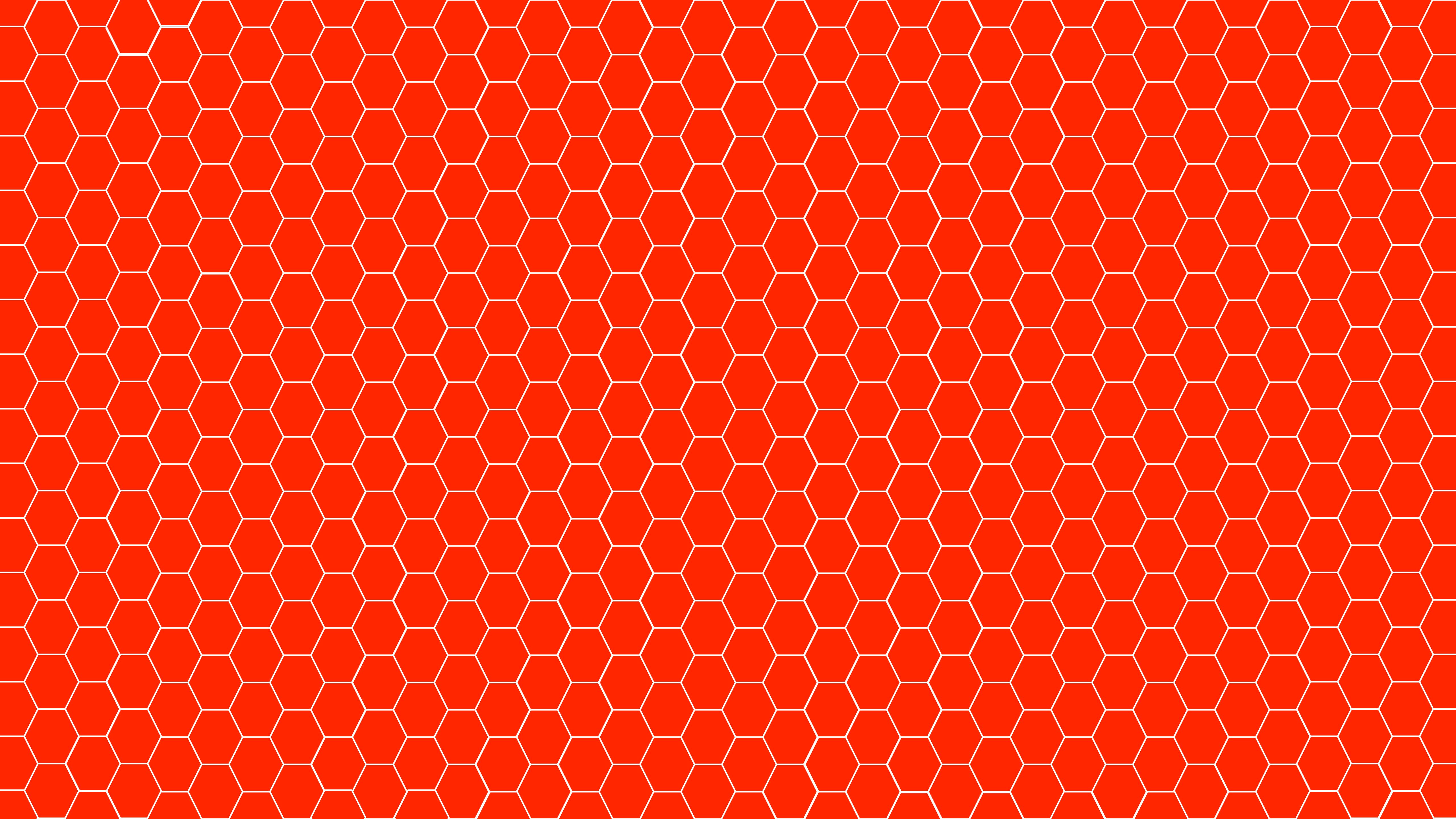


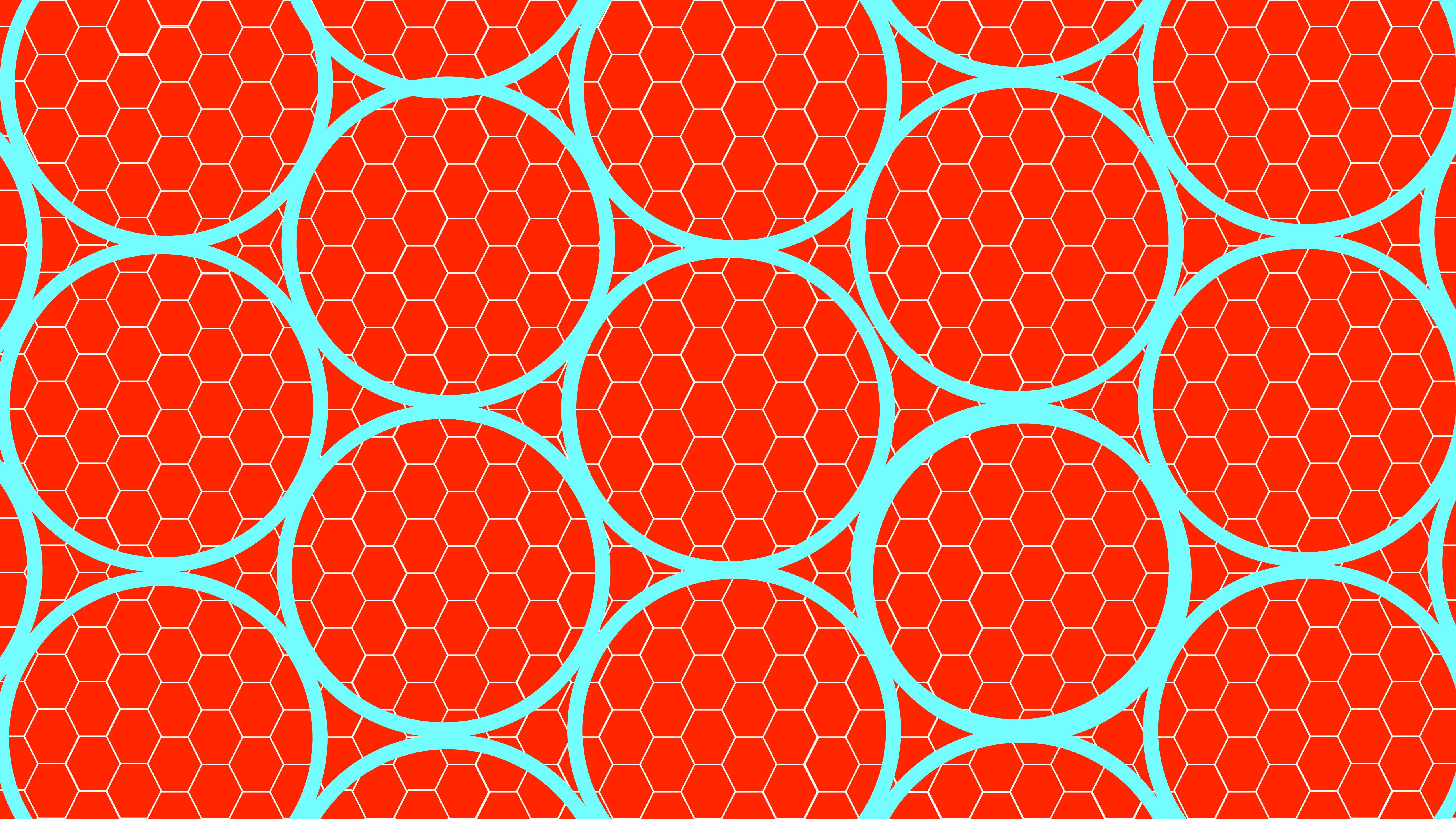


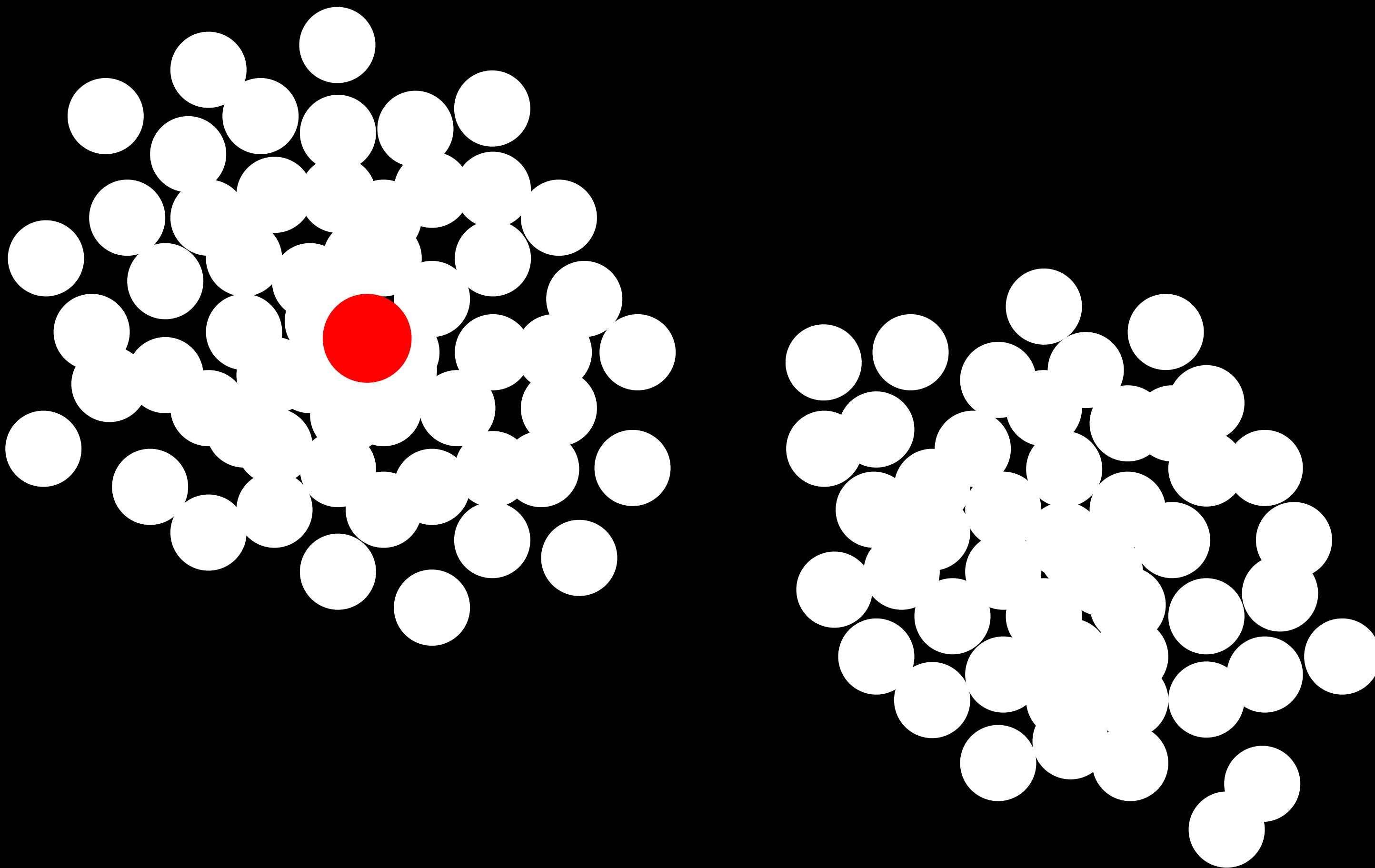


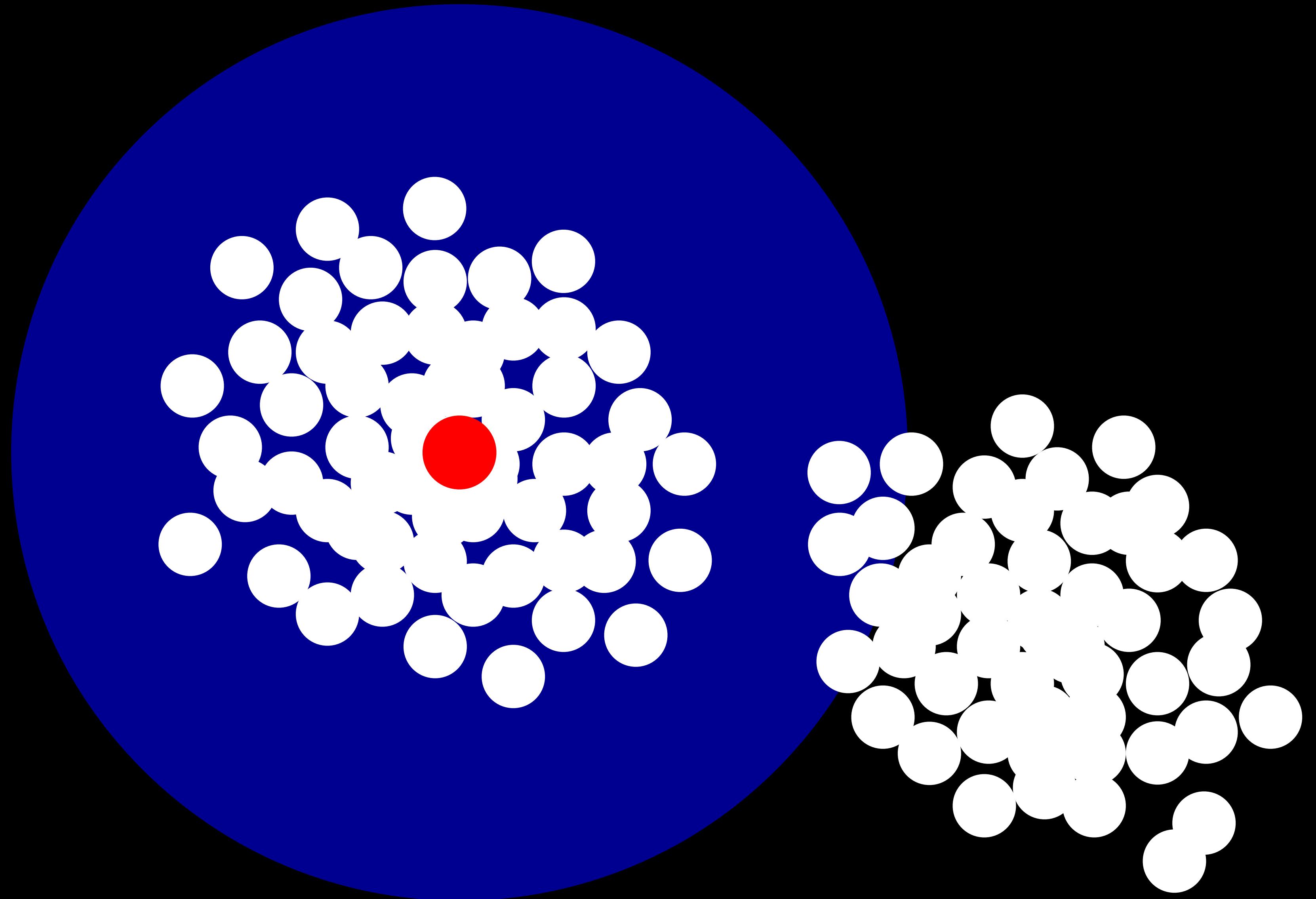


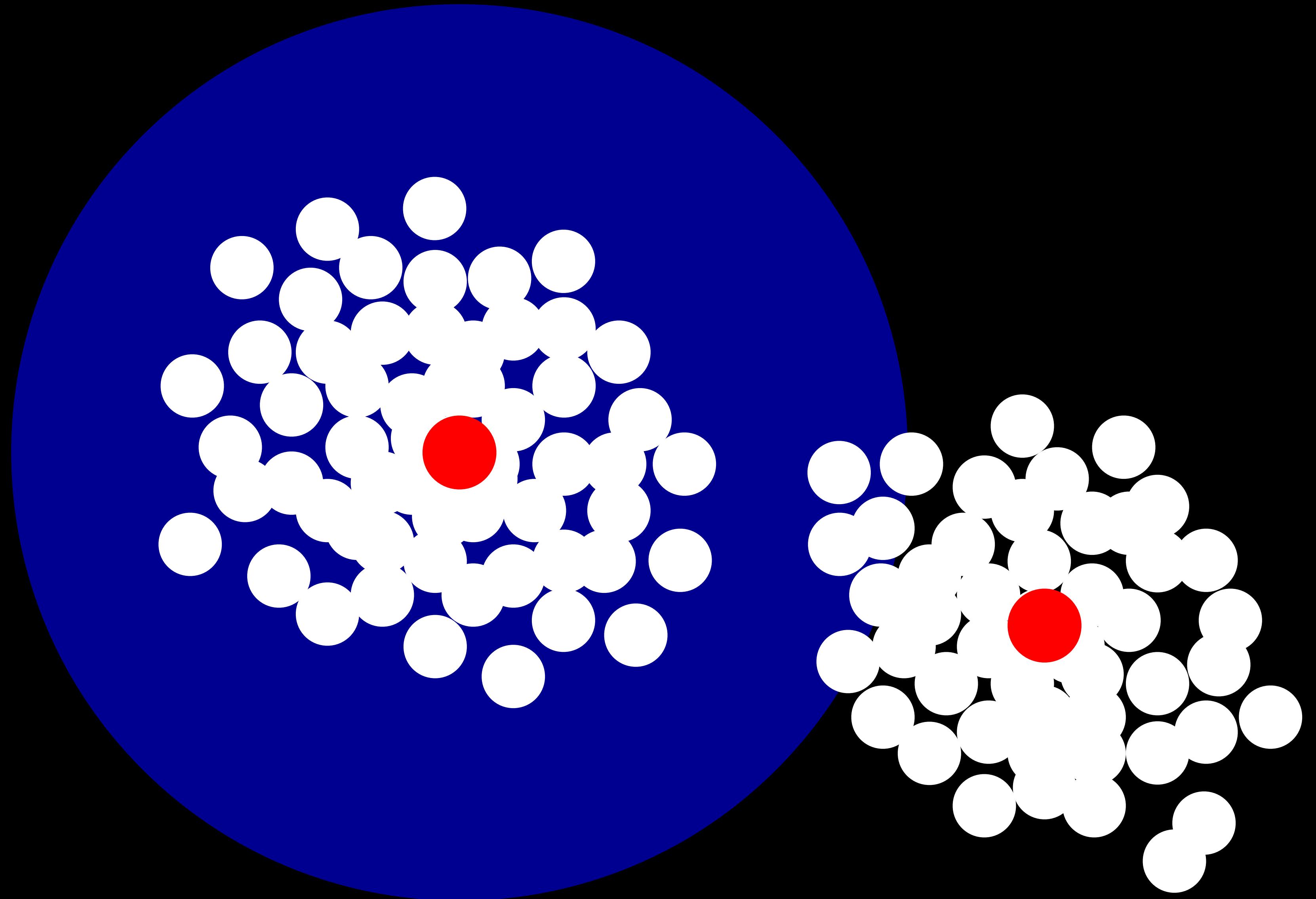


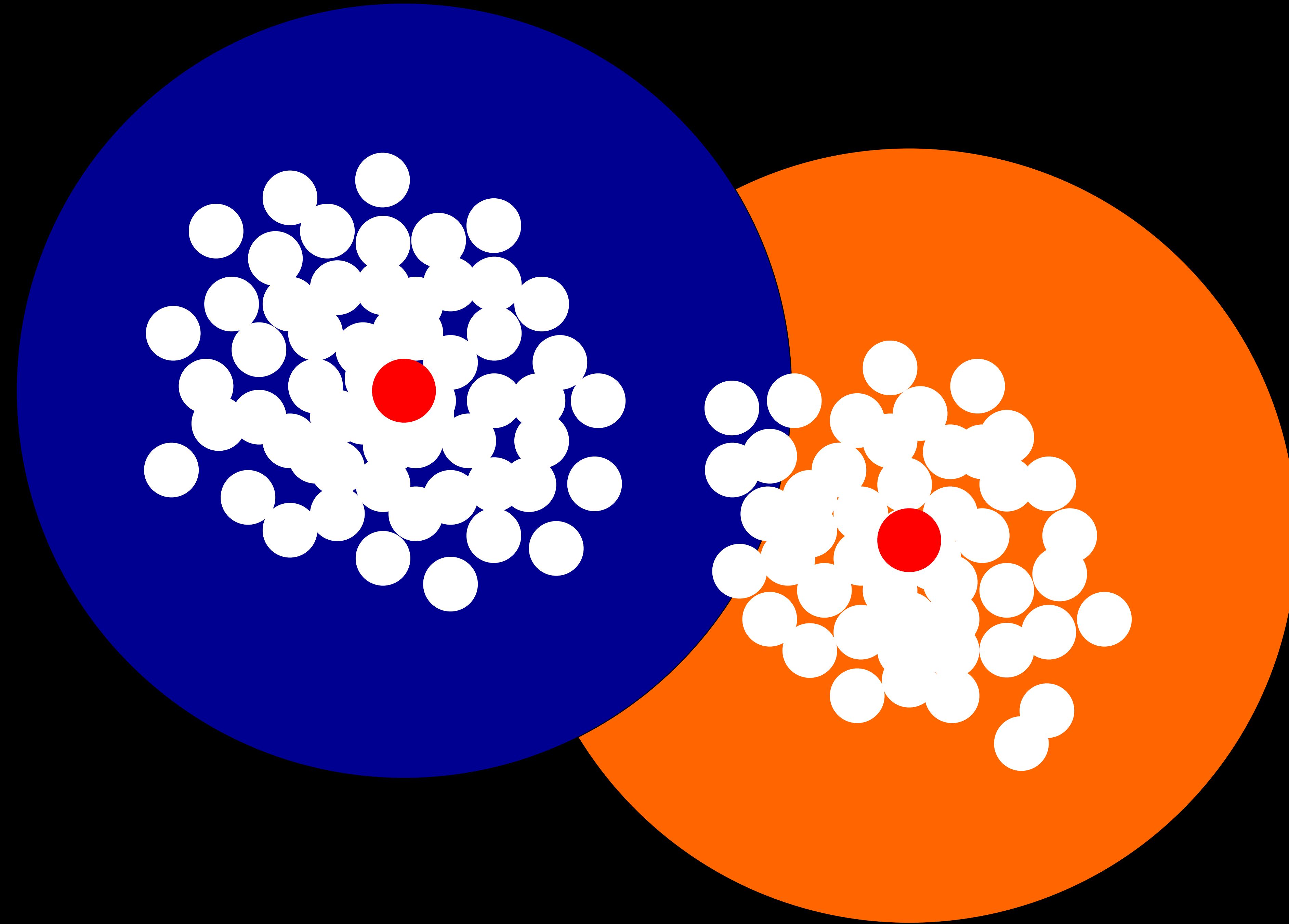


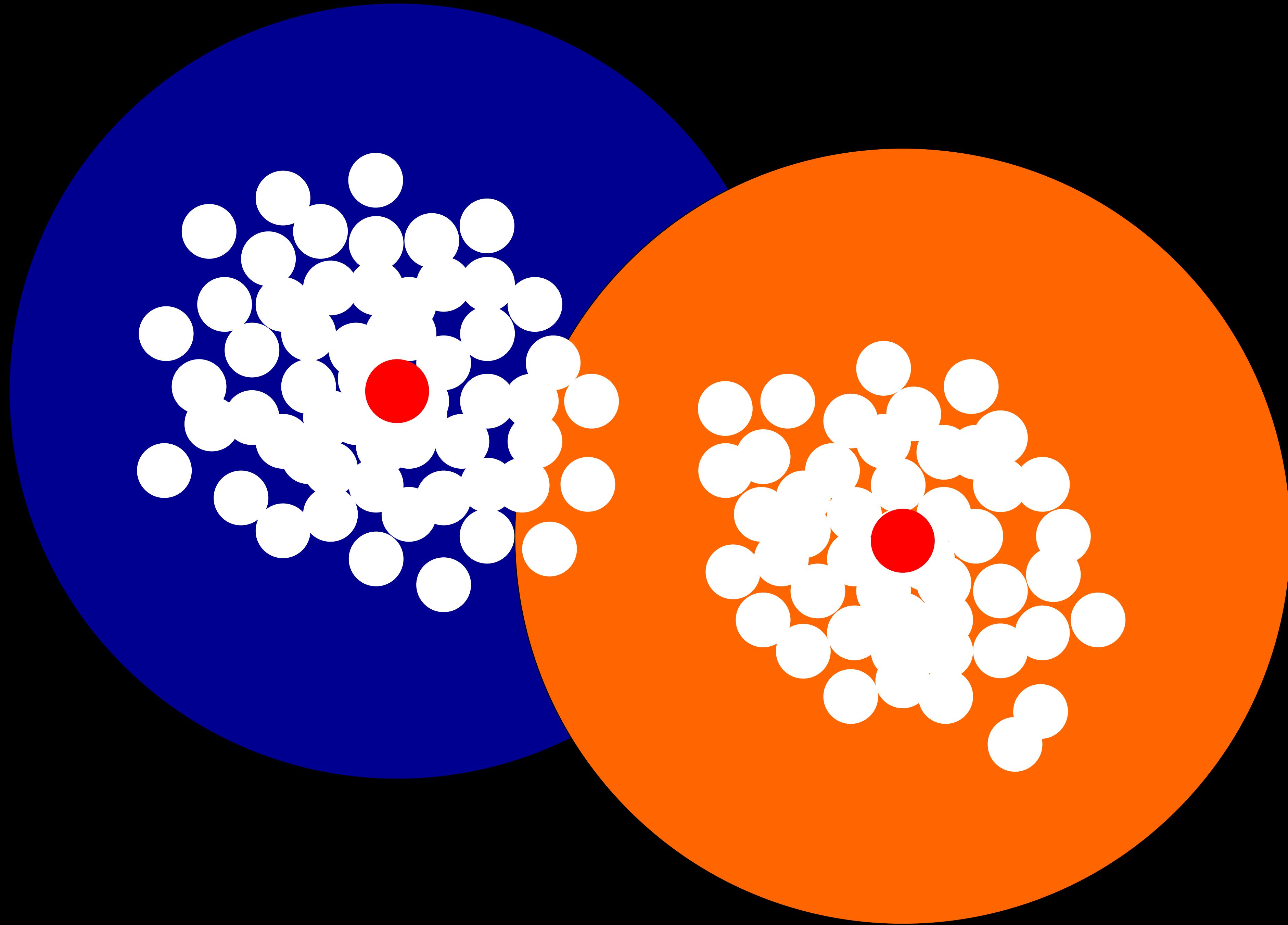












97%

INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Oct. 1994, p. 846-849  
0020-7713/94/\$04.00+0  
Copyright © 1994, International Union of Microbiological Societies

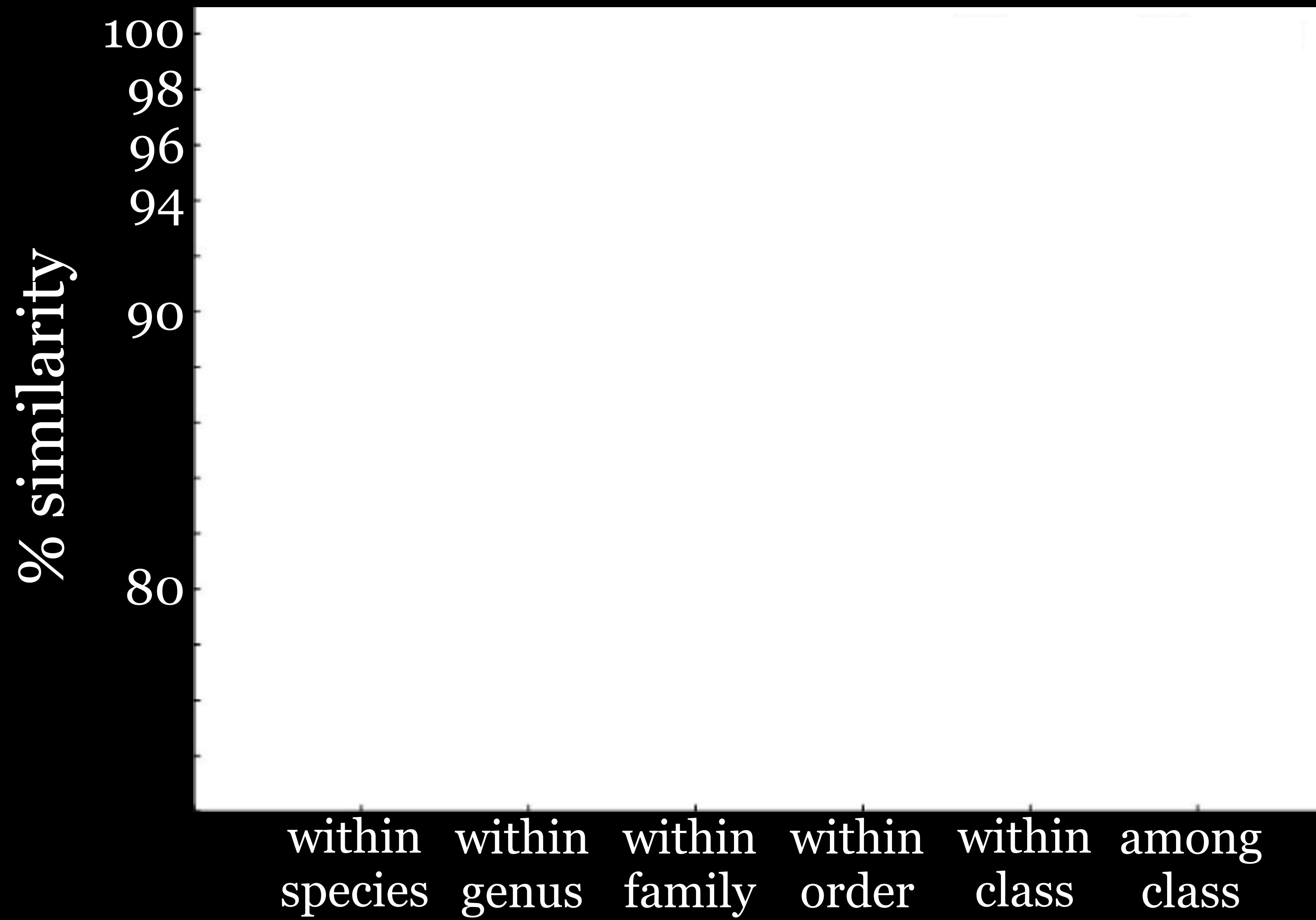
Vol. 44, No. 4

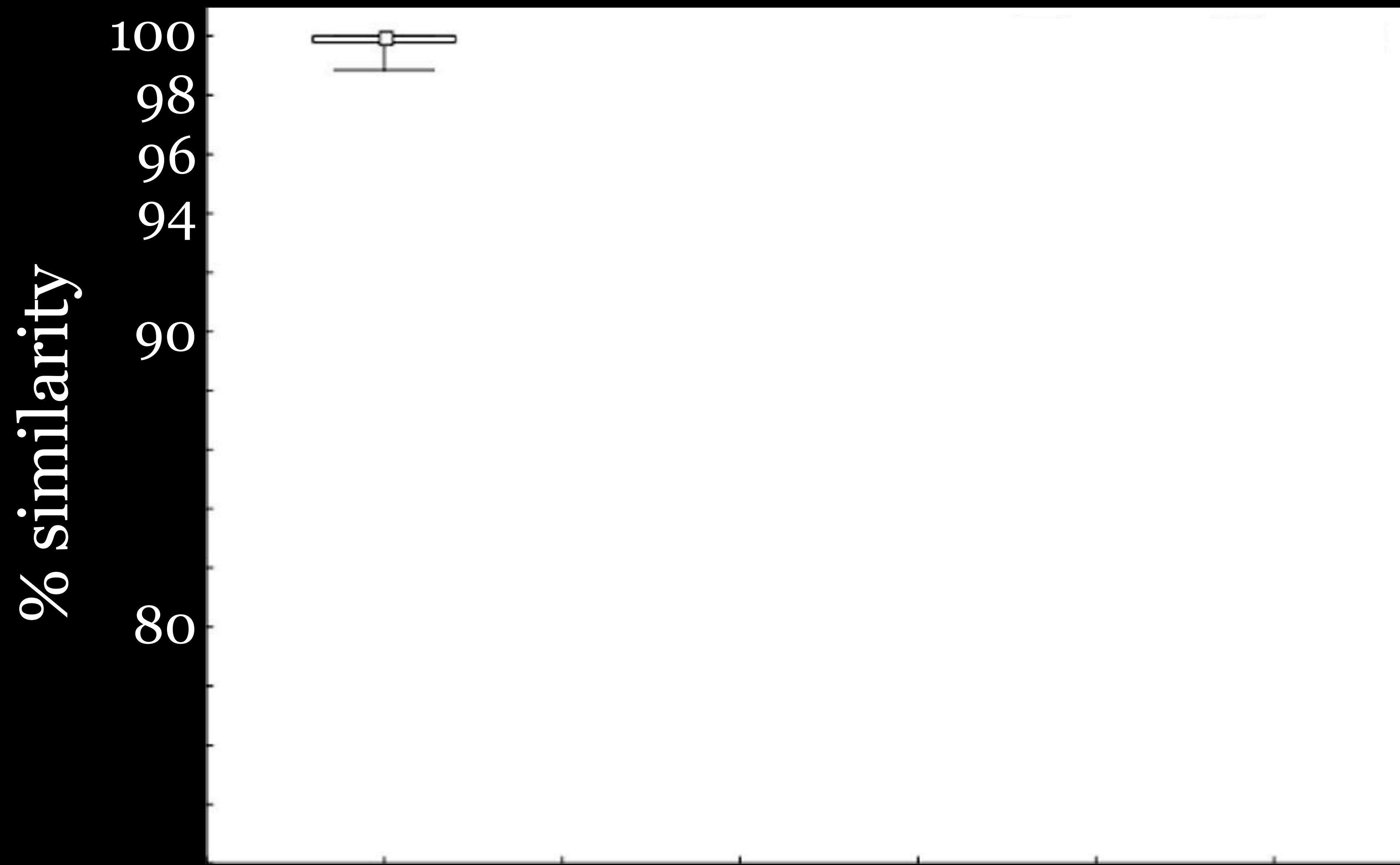
# Taxonomic Note: A Place for DNA-DNA Reassociation and 16S rRNA Sequence Analysis in the Present Species Definition in Bacteriology

E. STACKEBRANDT<sup>1</sup>\* AND B. M. GOEBEL<sup>2</sup>

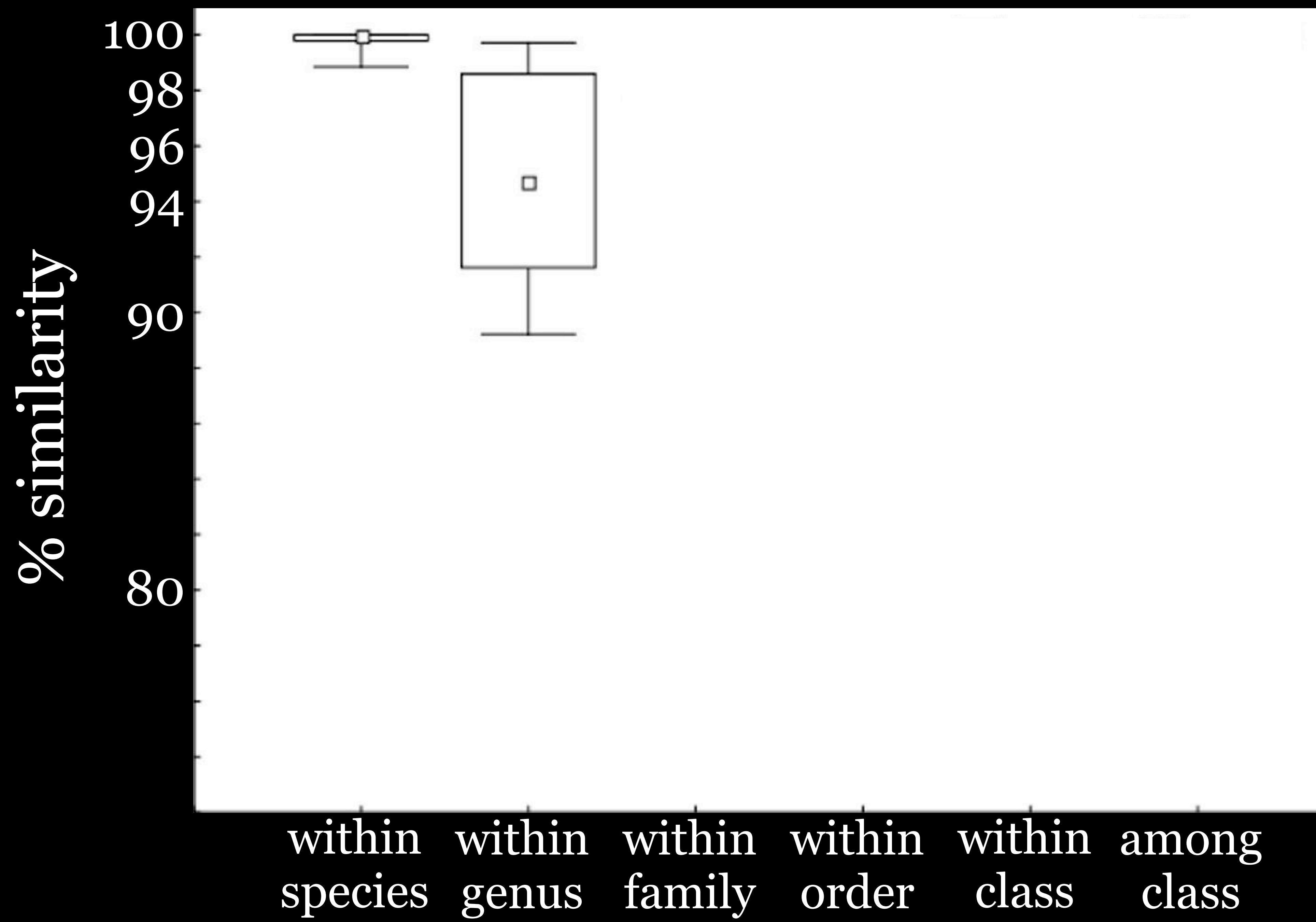
# **Delimiting operational taxonomic units for assessing ciliate environmental diversity using small-subunit rRNA gene sequences**

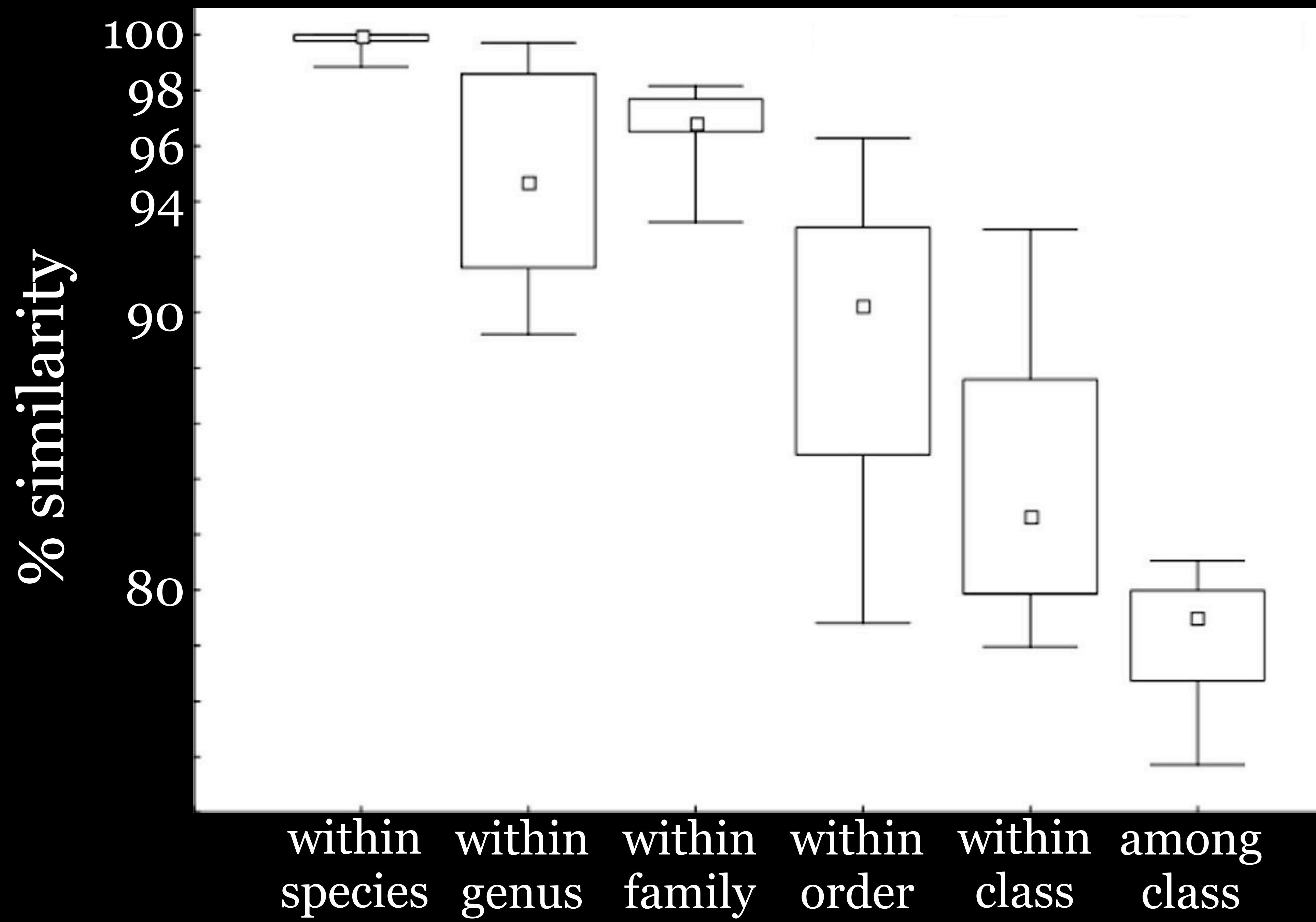
**Markus Nebel,<sup>1</sup> Cornelia Pfabel,<sup>2†</sup> Alexandra Stock,<sup>2</sup>  
Micah Dunthorn<sup>2</sup> and Thorsten Stoeck<sup>2\*</sup>**

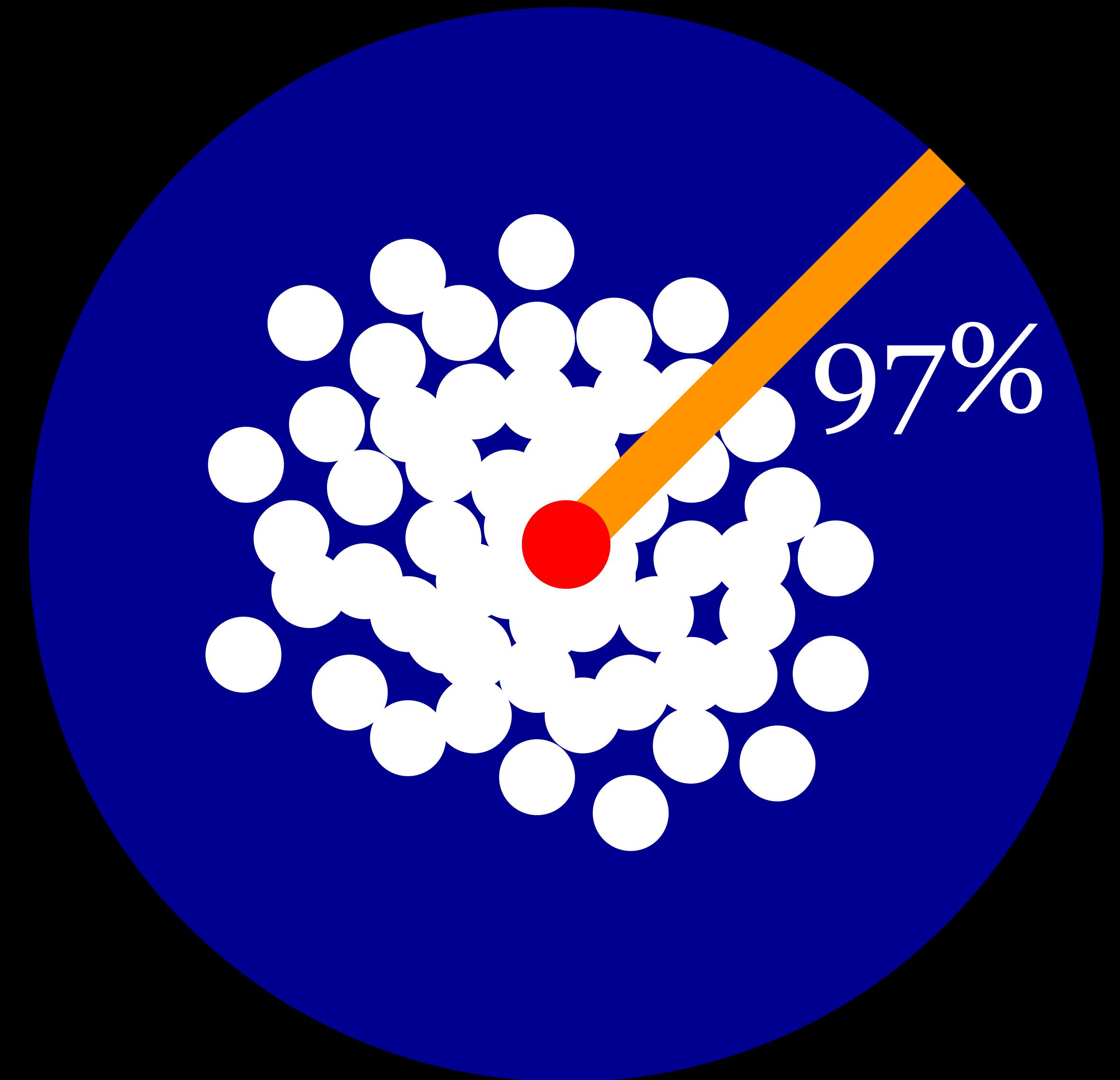


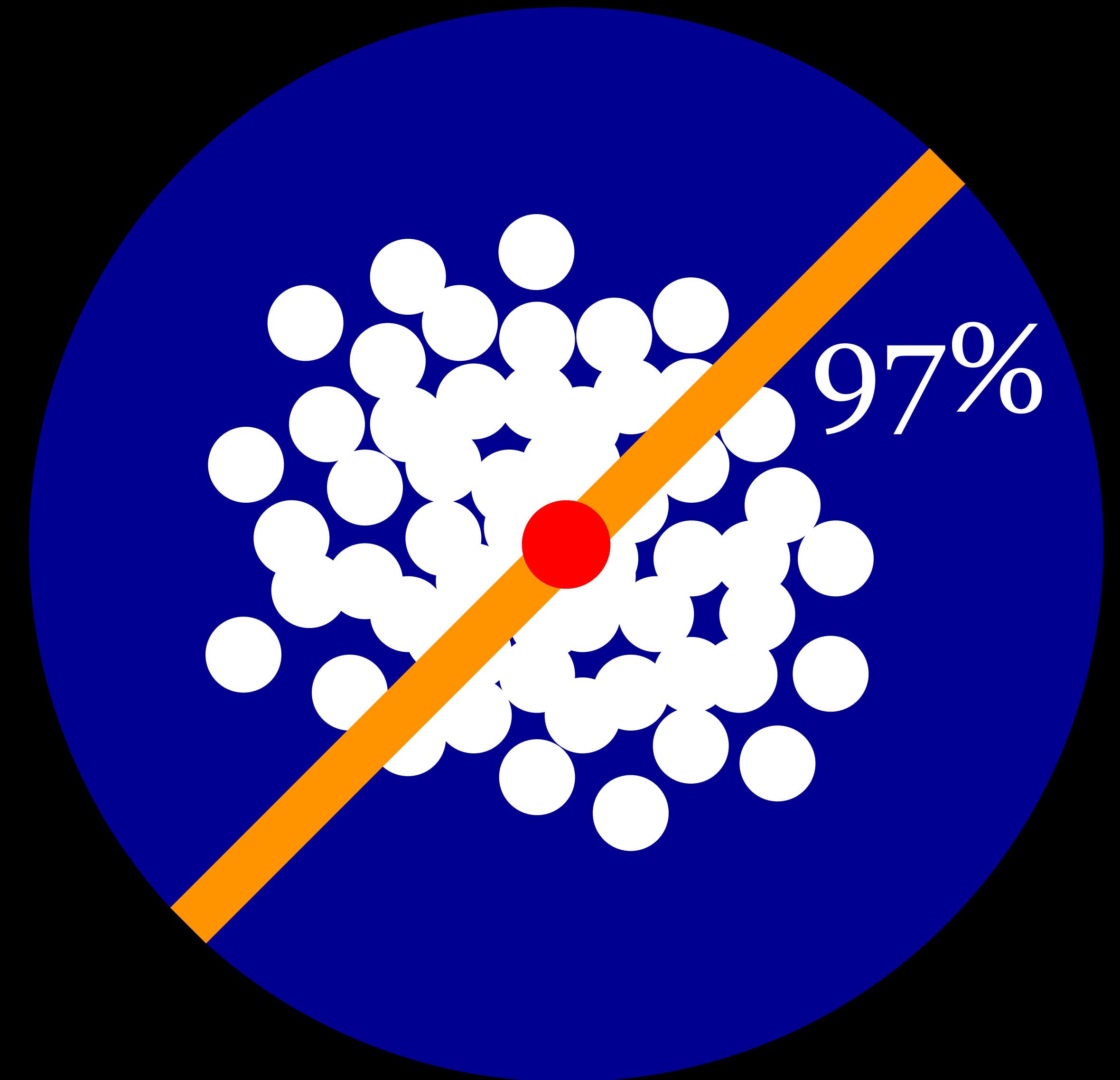


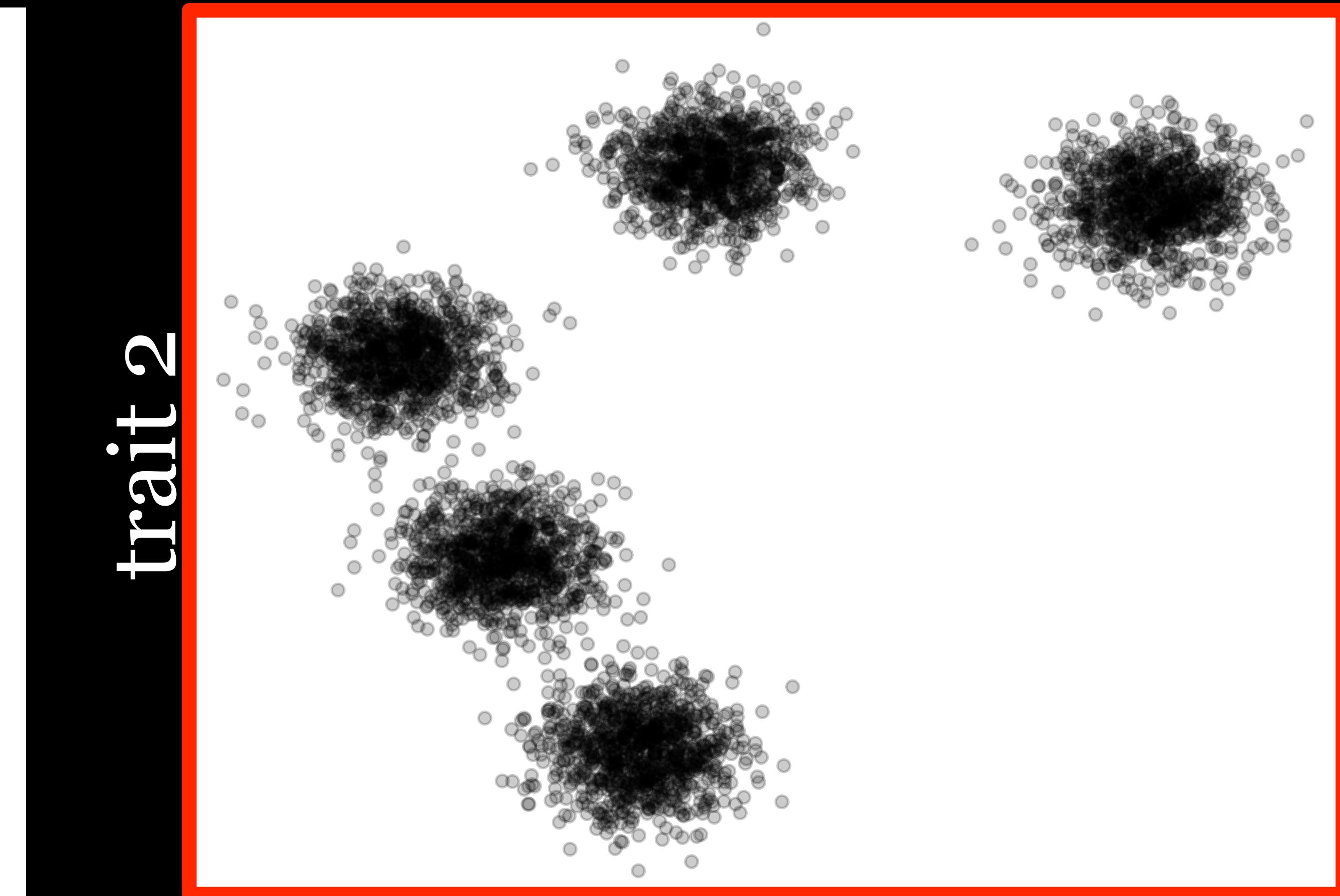
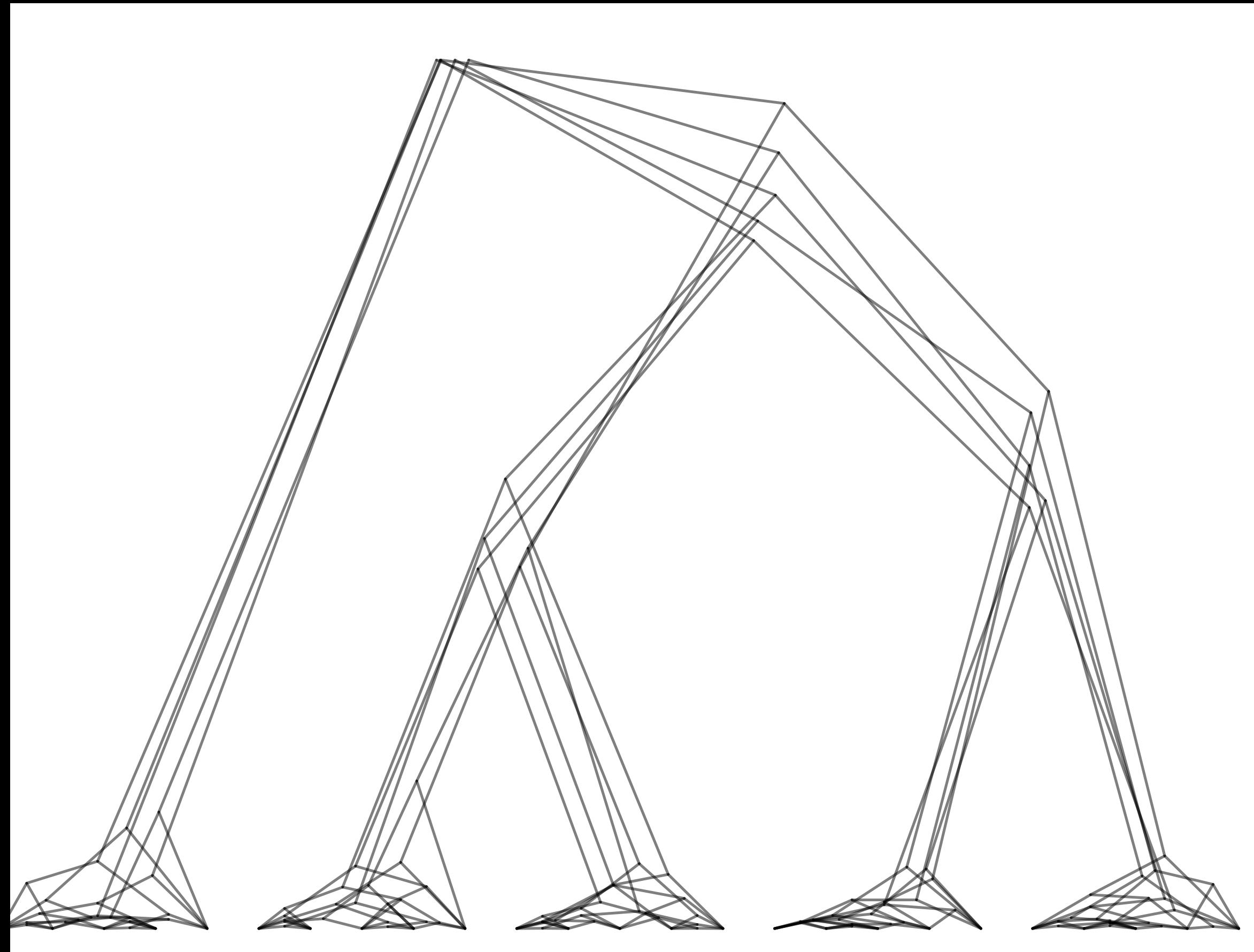
within within within within within among  
species genus family order class class











pairwise  
comparisons

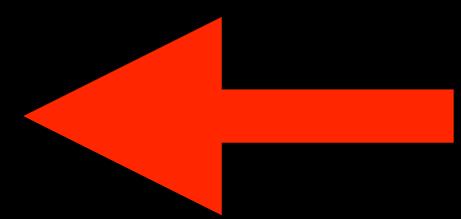
phylogenetic  
comparisons

global clustering  
thresholds

local clustering  
thresholds

VSEARCH/  
Mothur  
  
DADA

Swarm



multi-rate  
PTP

BRIEF COMMUNICATIONS

2016 13:581-583

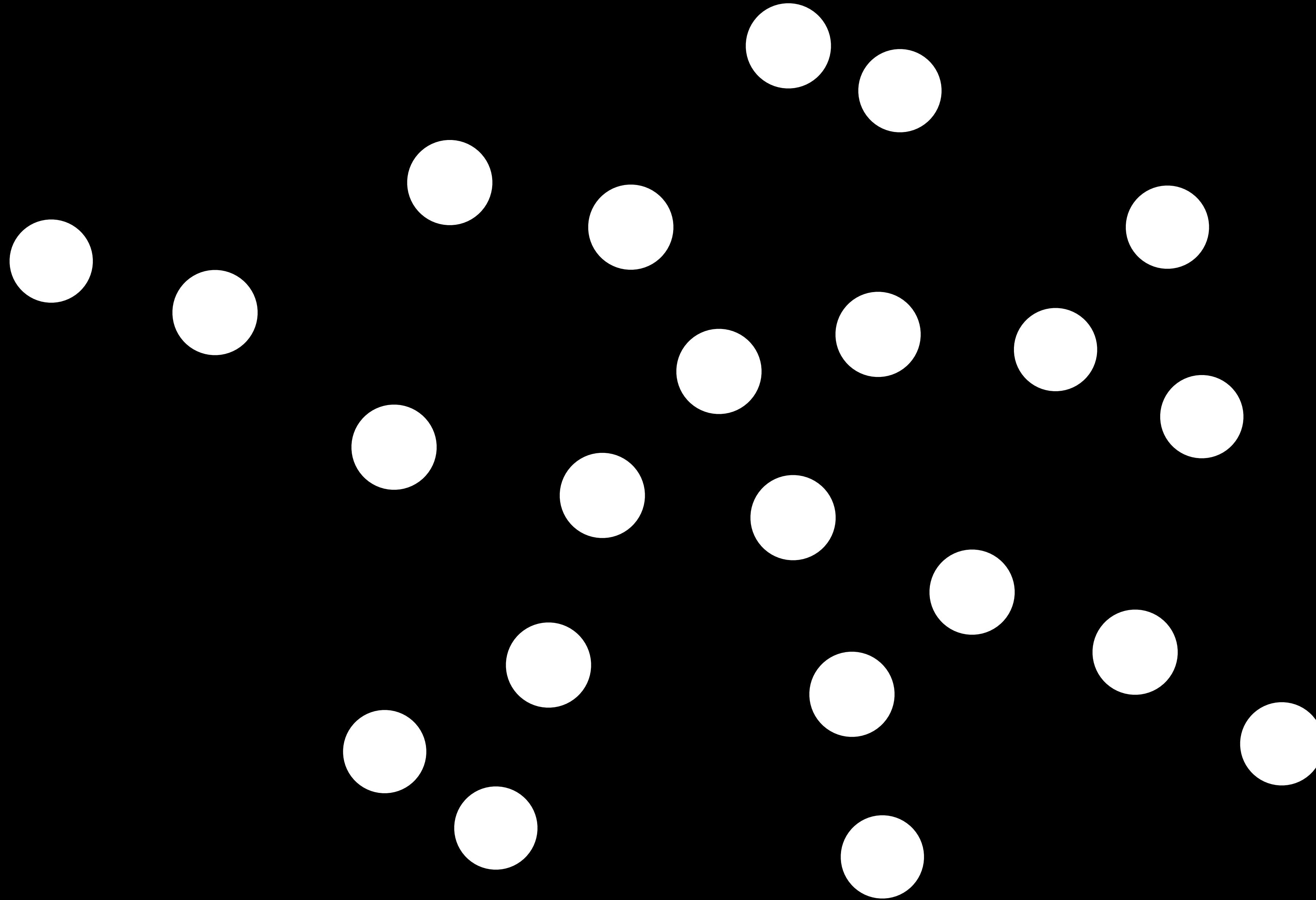
# DADA2: High-resolution sample inference from Illumina amplicon data

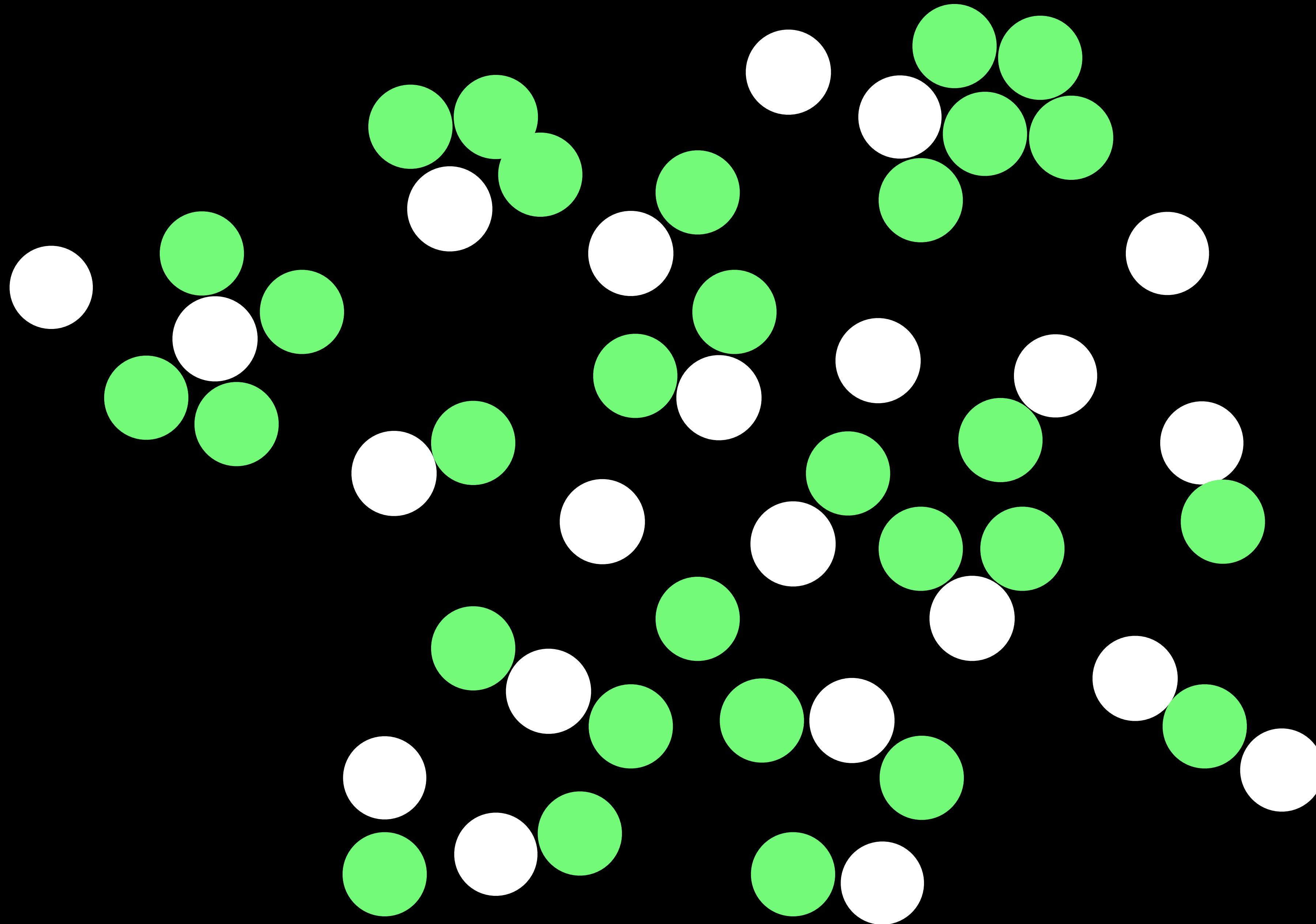
Benjamin J Callahan<sup>1</sup>, Paul J McMurdie<sup>2</sup>,  
Michael J Rosen<sup>3</sup>, Andrew W Han<sup>2</sup>, Amy Jo A Johnson<sup>2</sup> &  
Susan P Holmes<sup>1</sup>

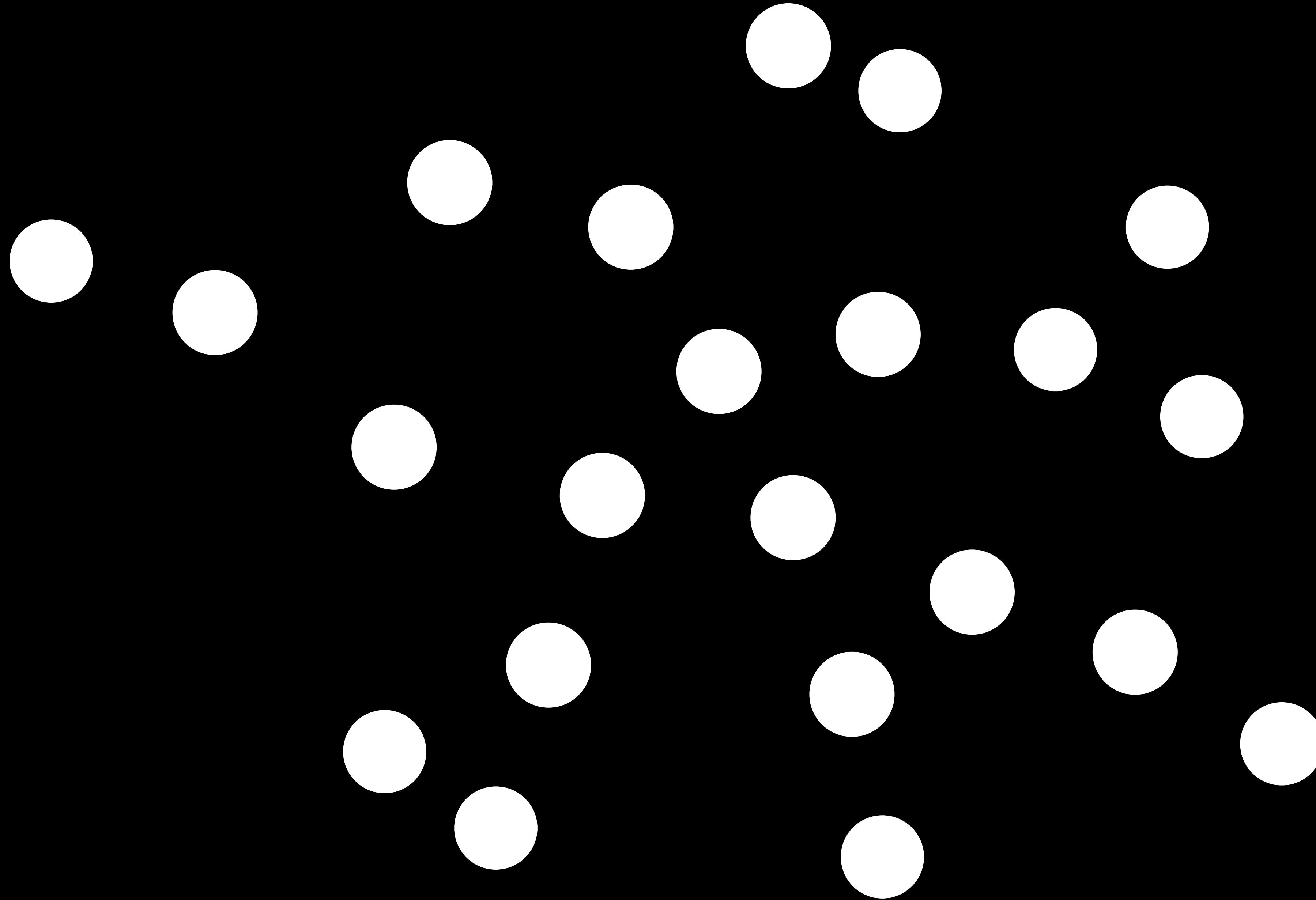
Nature Methods

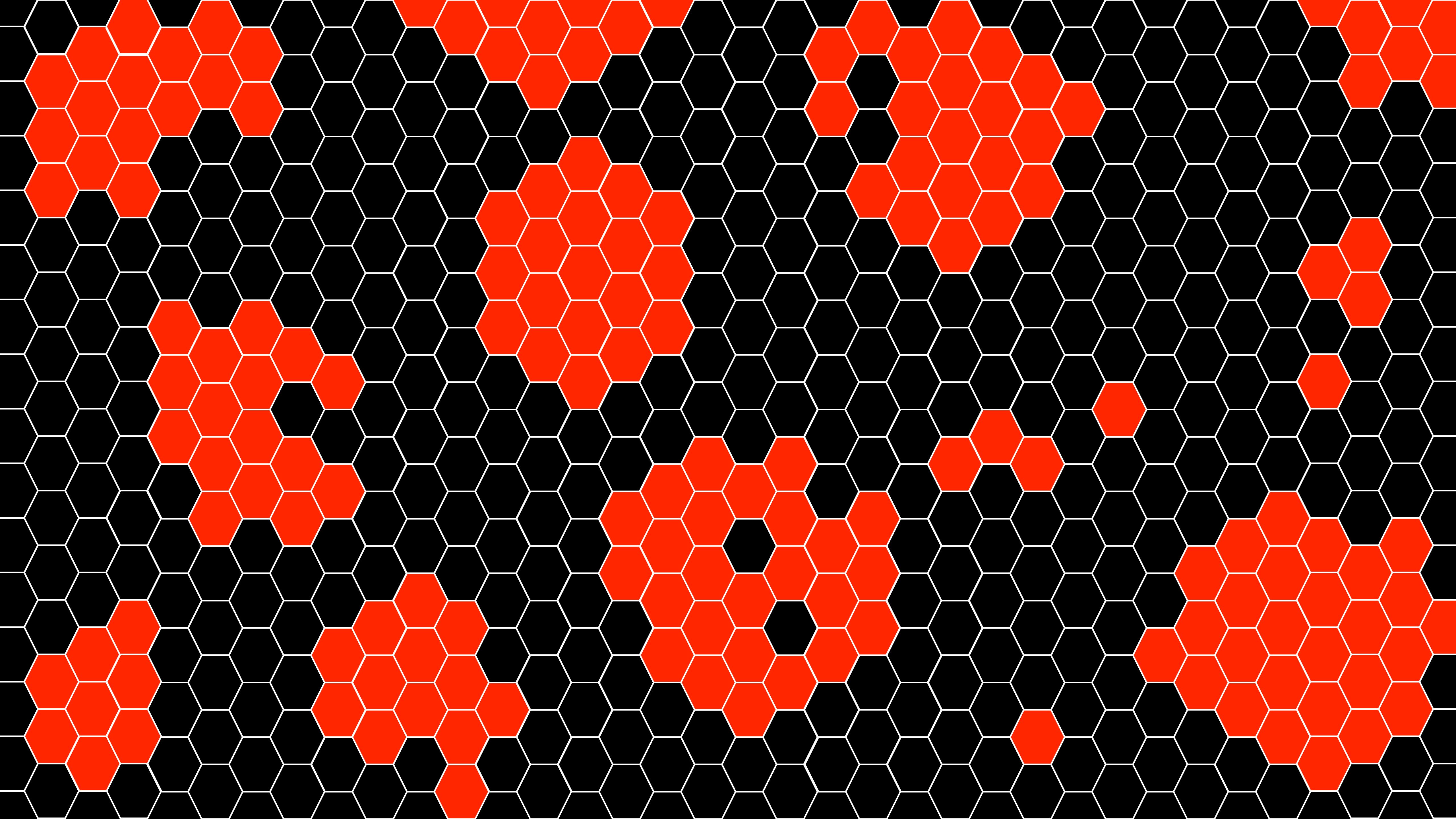
# amplicon sequence variants (ASVs)

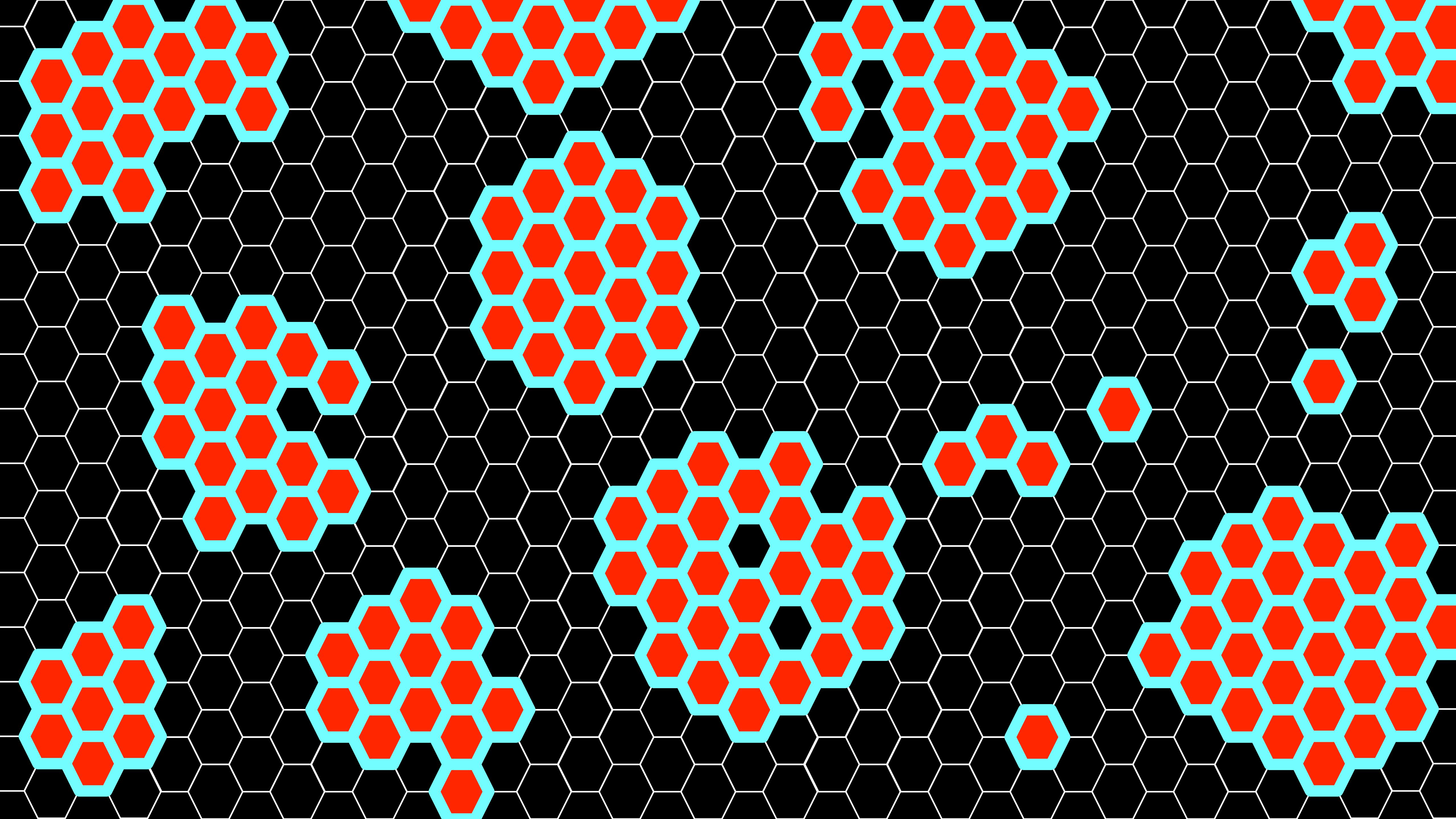
...clustering by another name









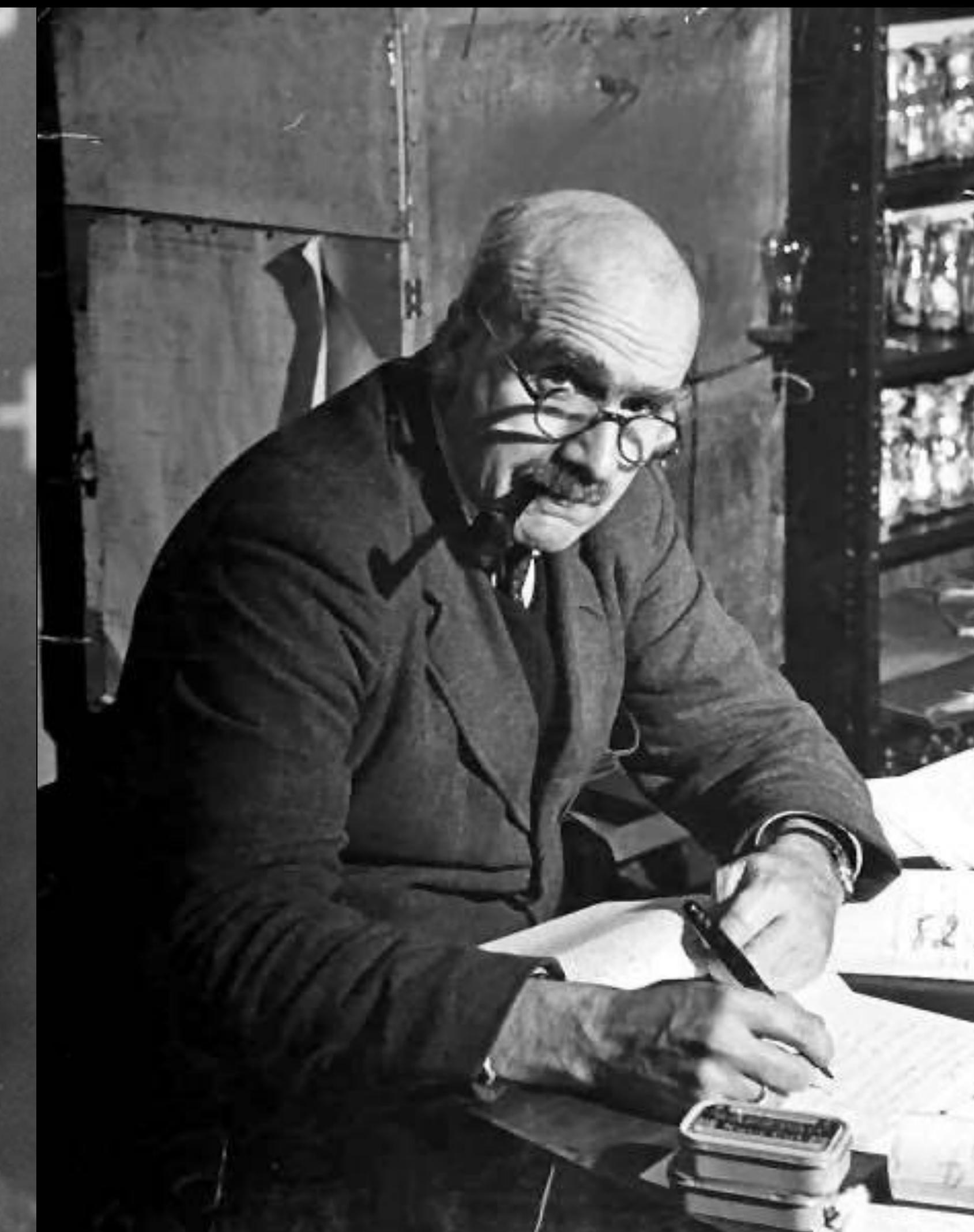


100%

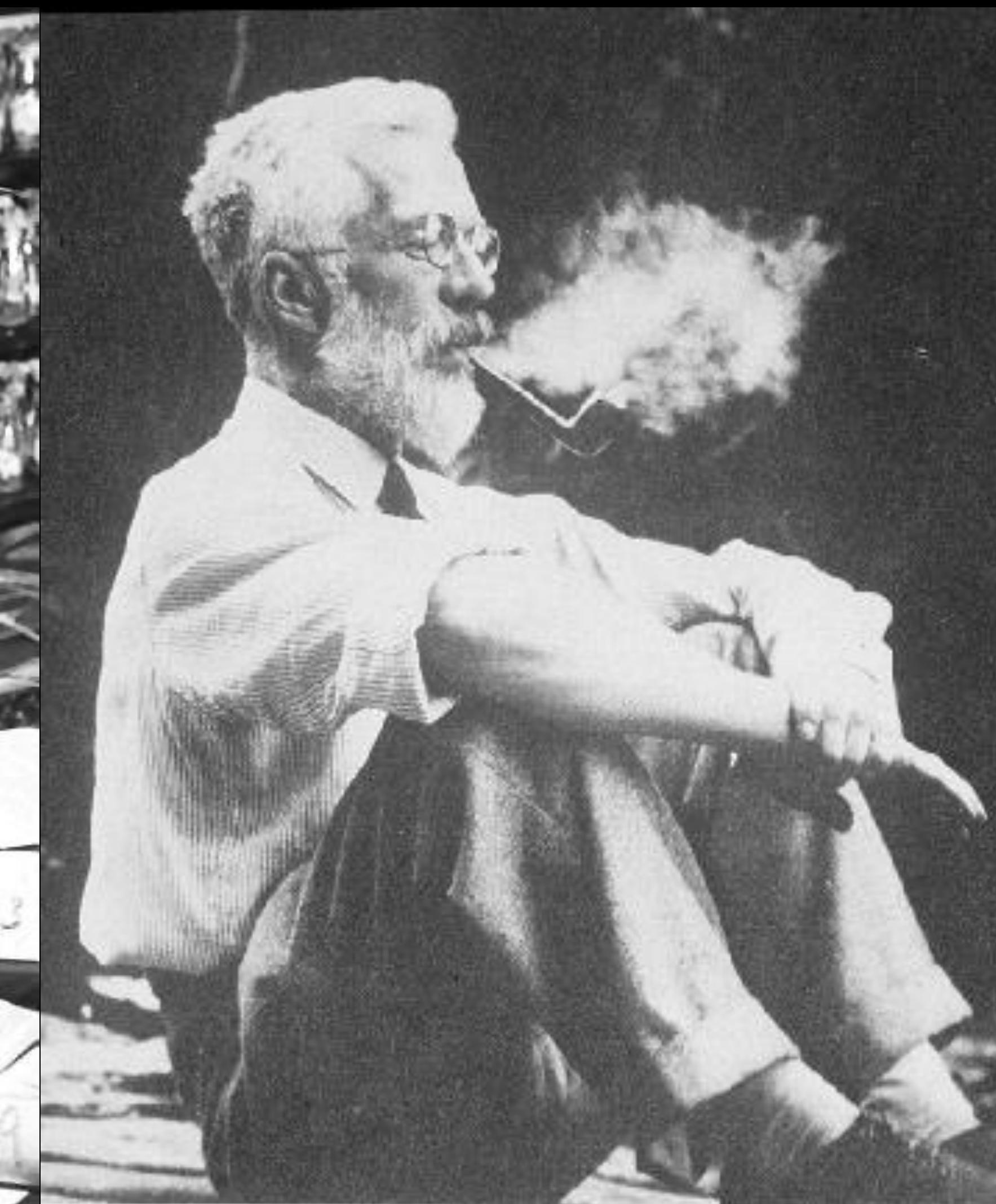
# population genetics doesn't matter



Sewall Wright



J.B.S. Haldane



Ronald Fisher

sampling,  
storage



nucleotide  
extraction



amplification,  
sequencing

cleaning,  
dereplication



clustering



more  
cleaning

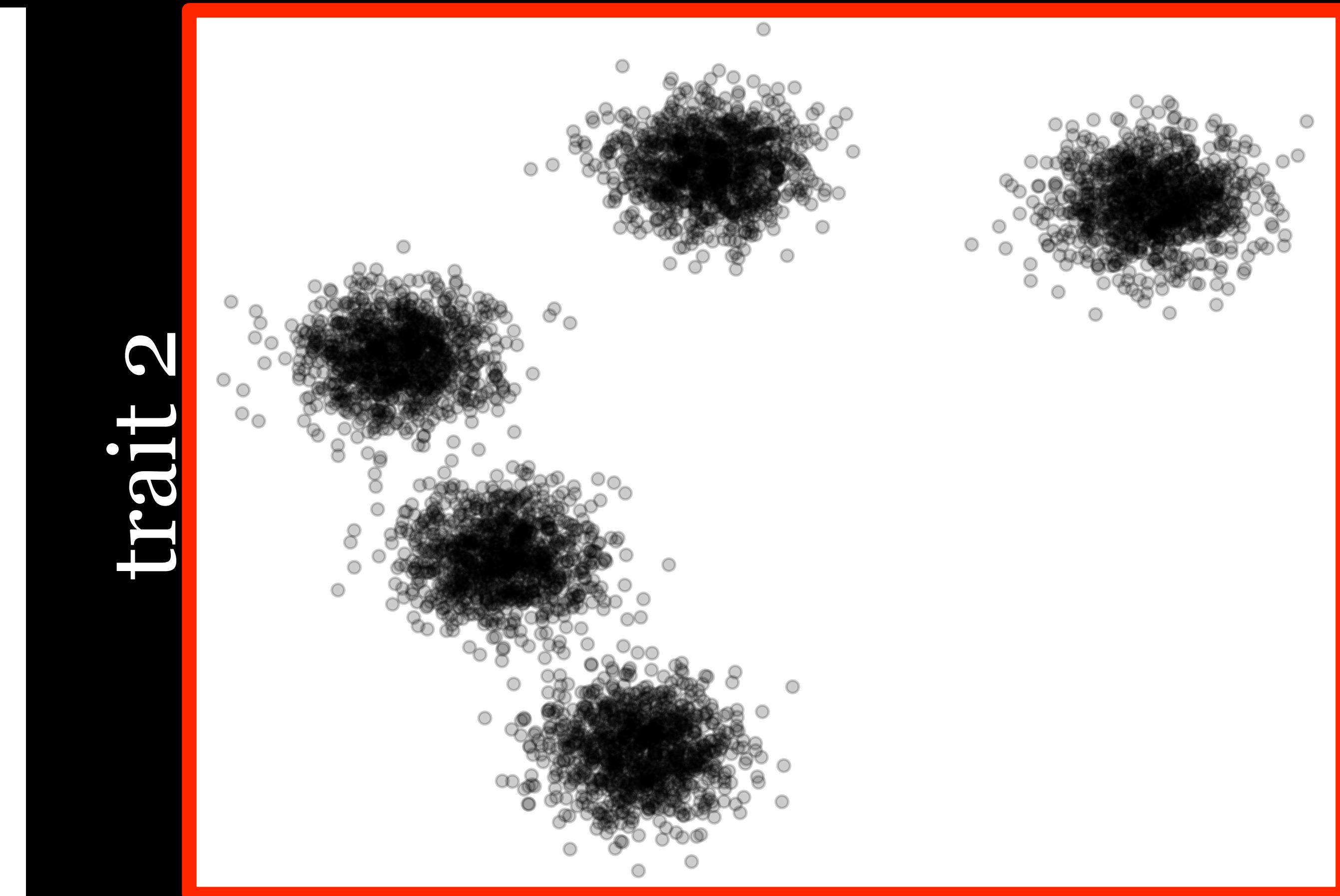
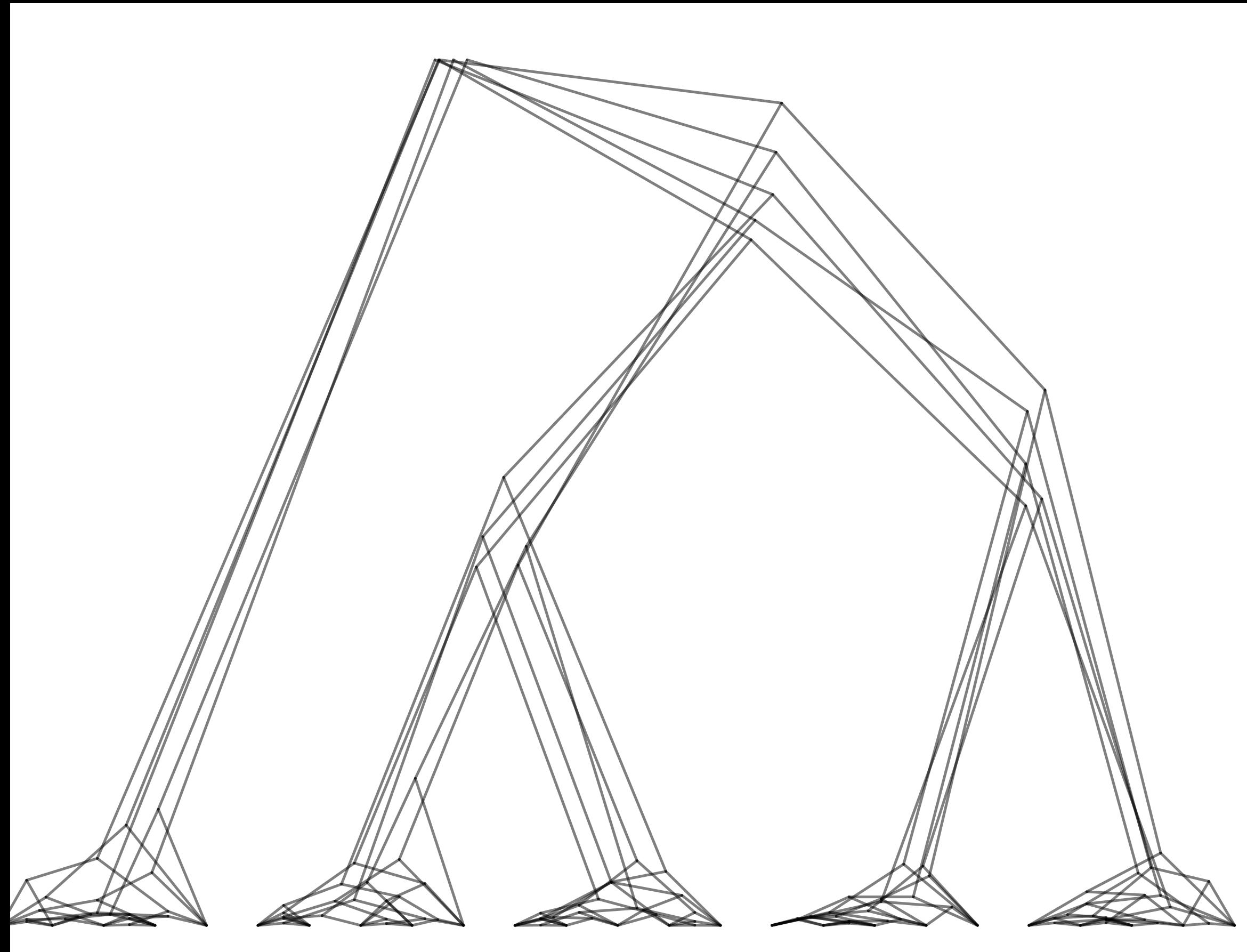
taxonomic  
assignments



phylogenetic  
placements



$\alpha, \beta$  diversities,  
co-occurrences



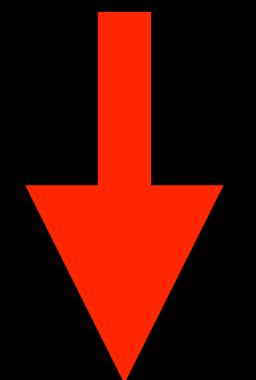
global clustering  
thresholds

local clustering  
thresholds

pairwise  
comparisons

phylogenetic  
comparisons

VSEARCH/  
Mothur  
DADA



Swarm

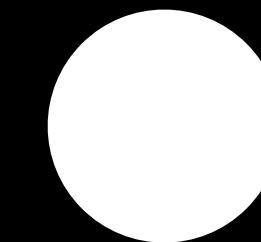
multi-rate  
PTP

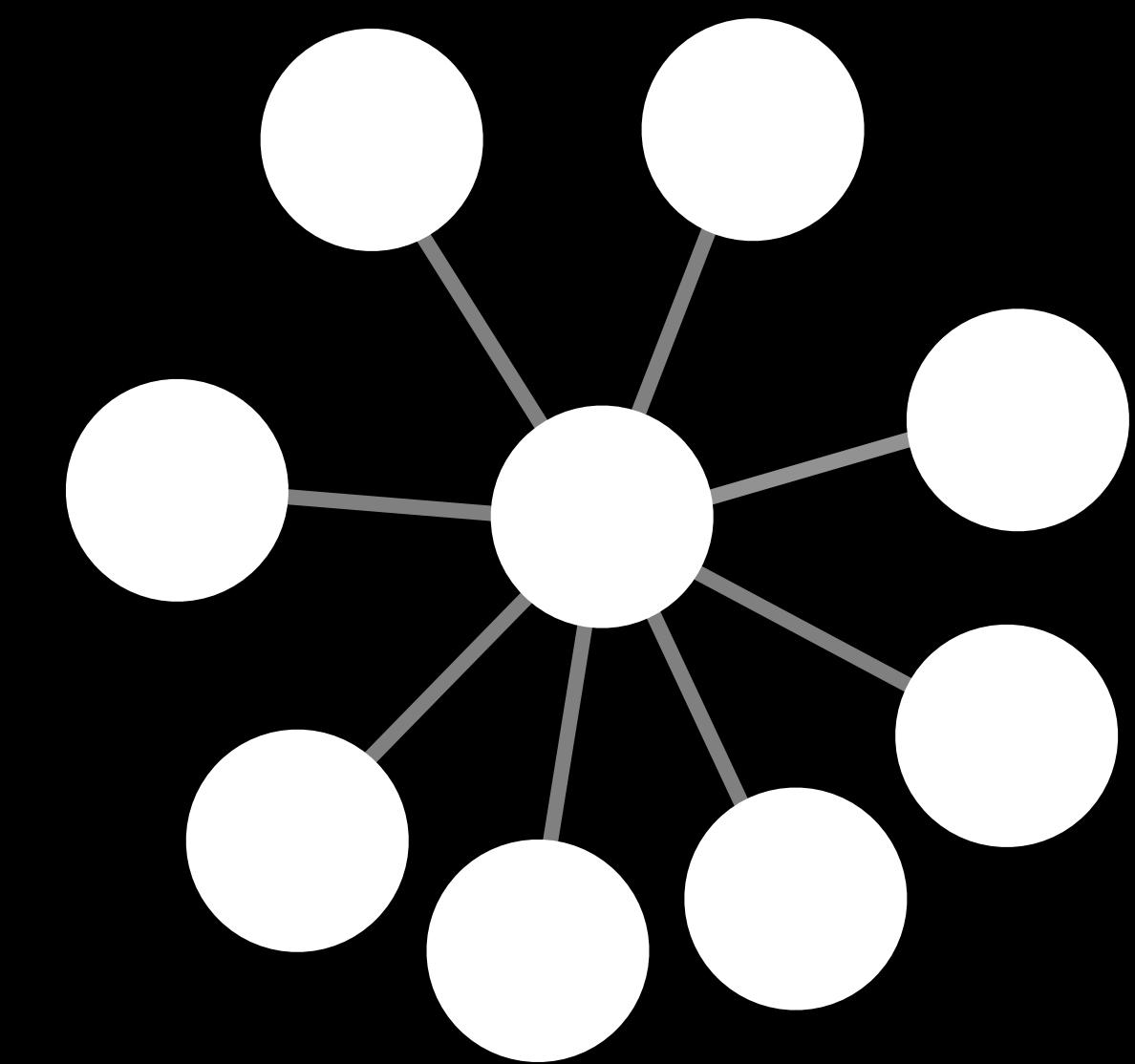
2015 3:e1420

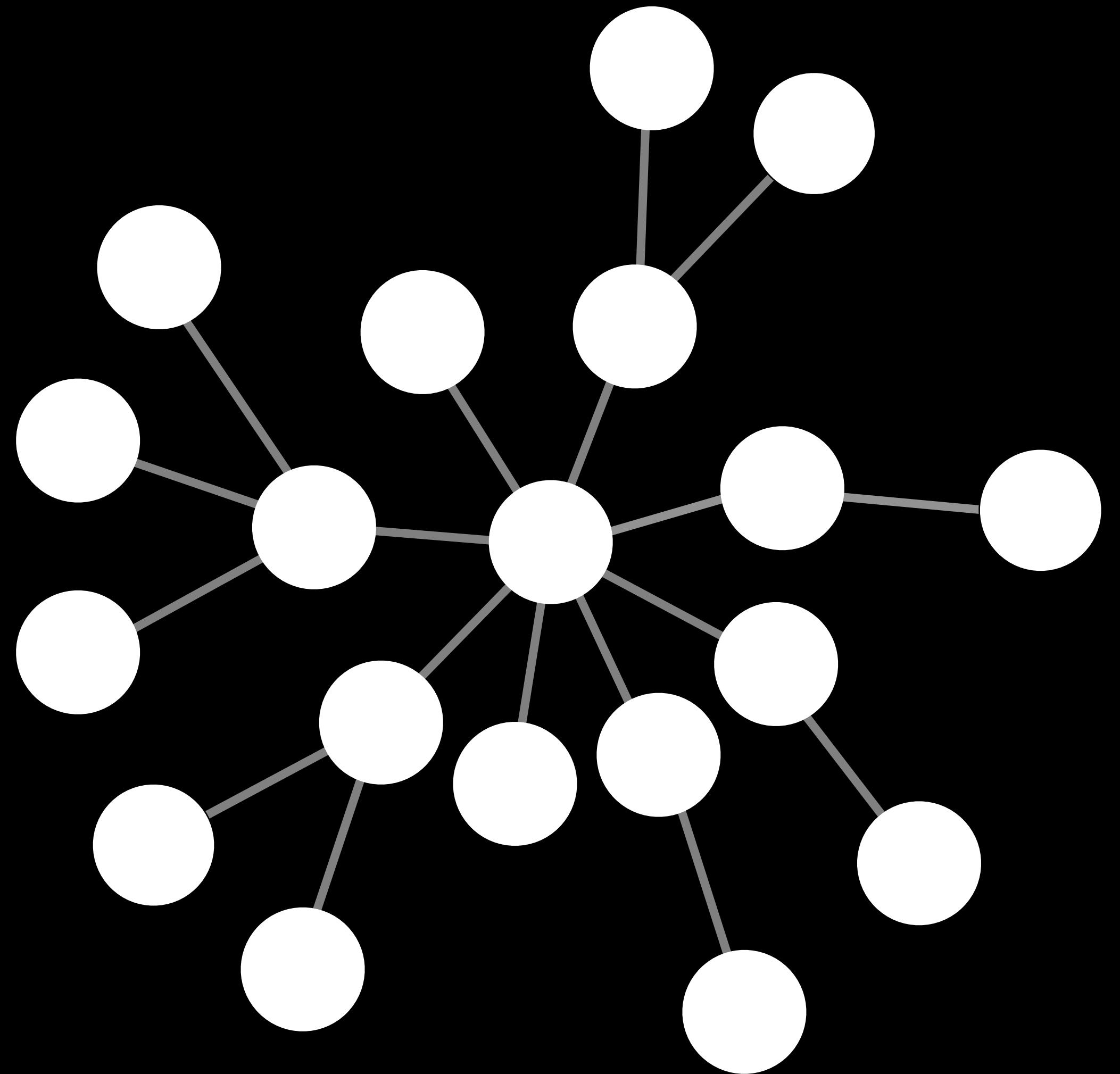
PeerJ

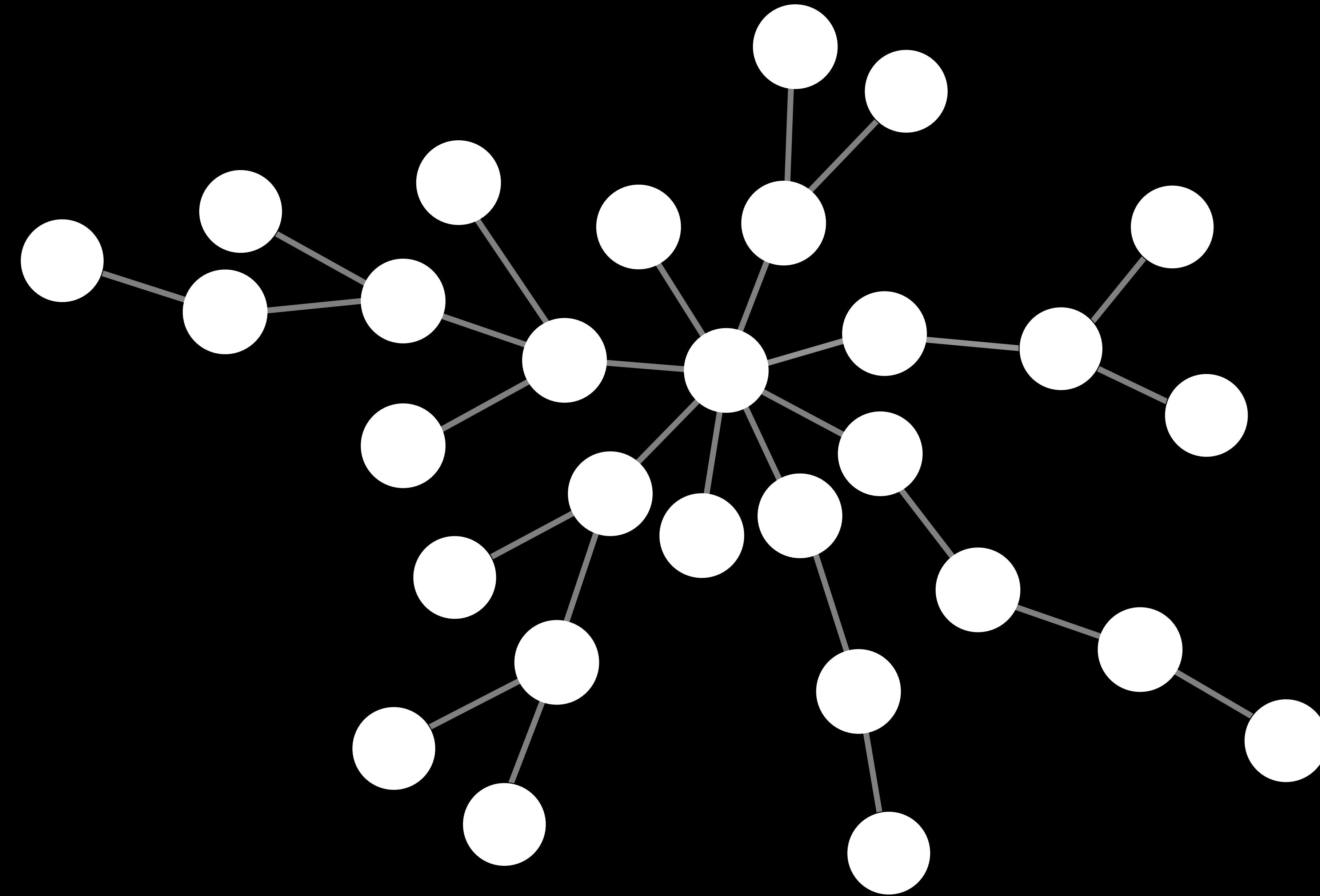
# Swarm v2: highly-scalable and high-resolution amplicon clustering

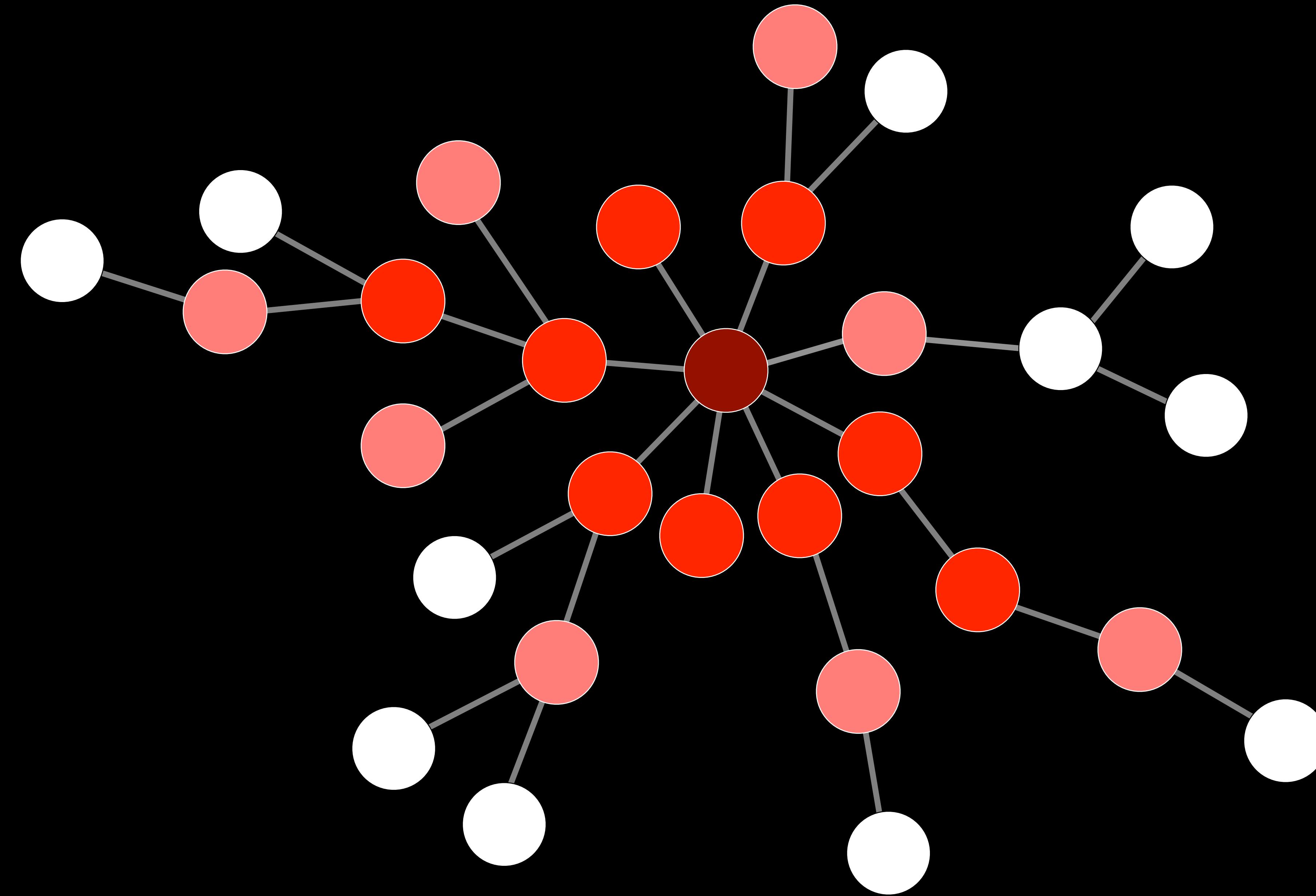
Frédéric Mahé<sup>1</sup>, Torbjørn Rognes<sup>2,3</sup>, Christopher Quince<sup>4</sup>,  
Colomban de Vargas<sup>5,6</sup> and Micah Dunthorn<sup>1</sup>

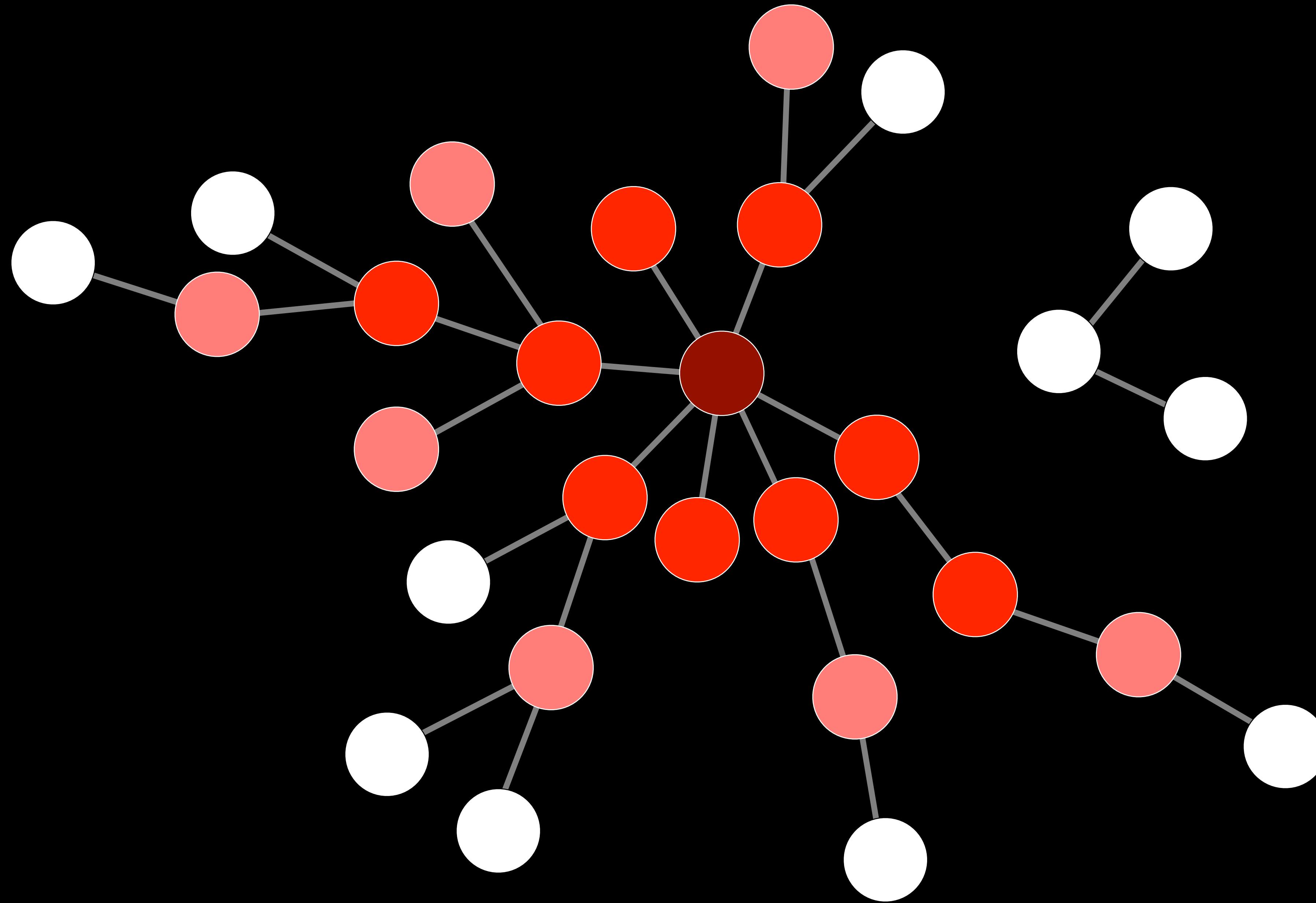


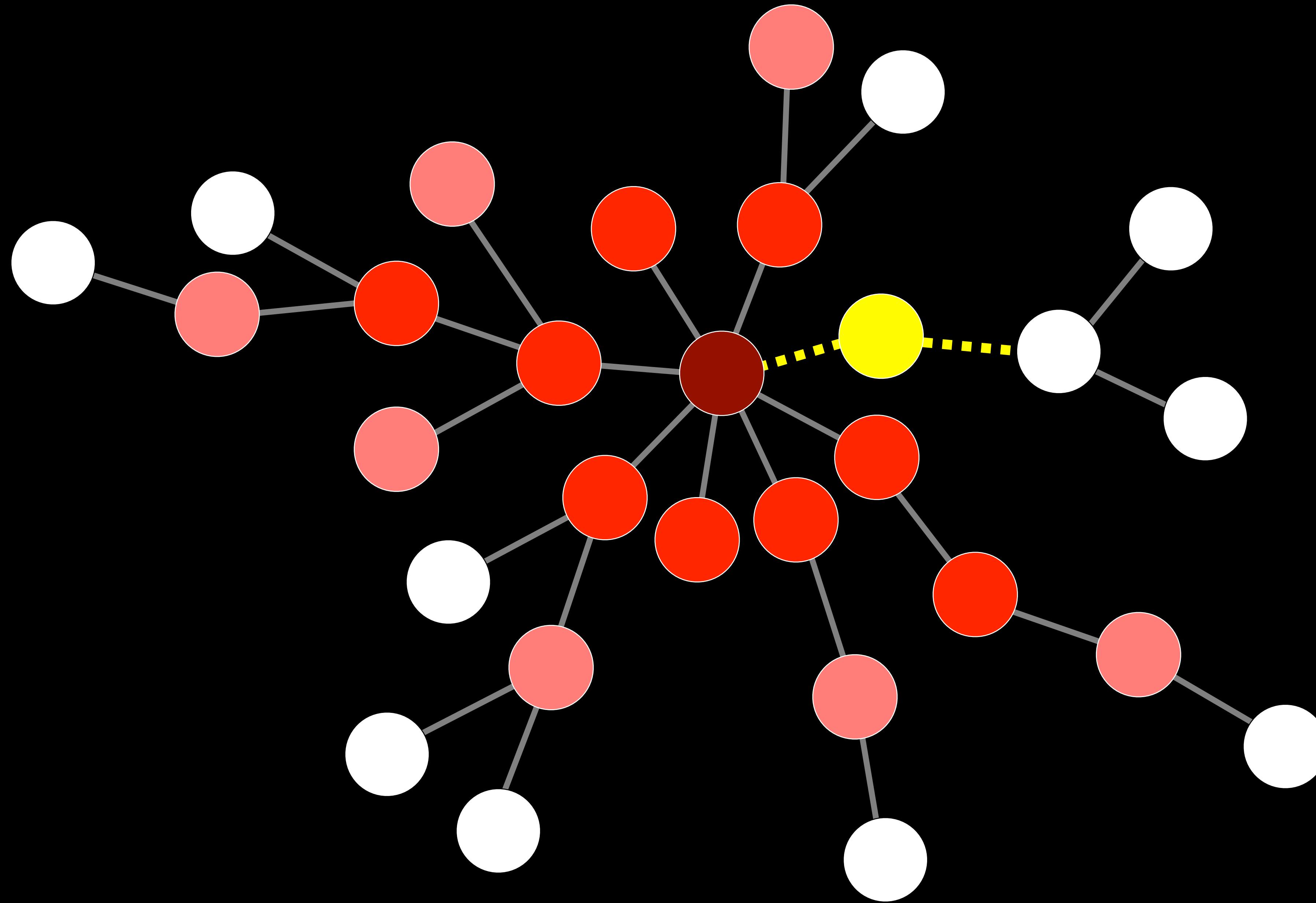




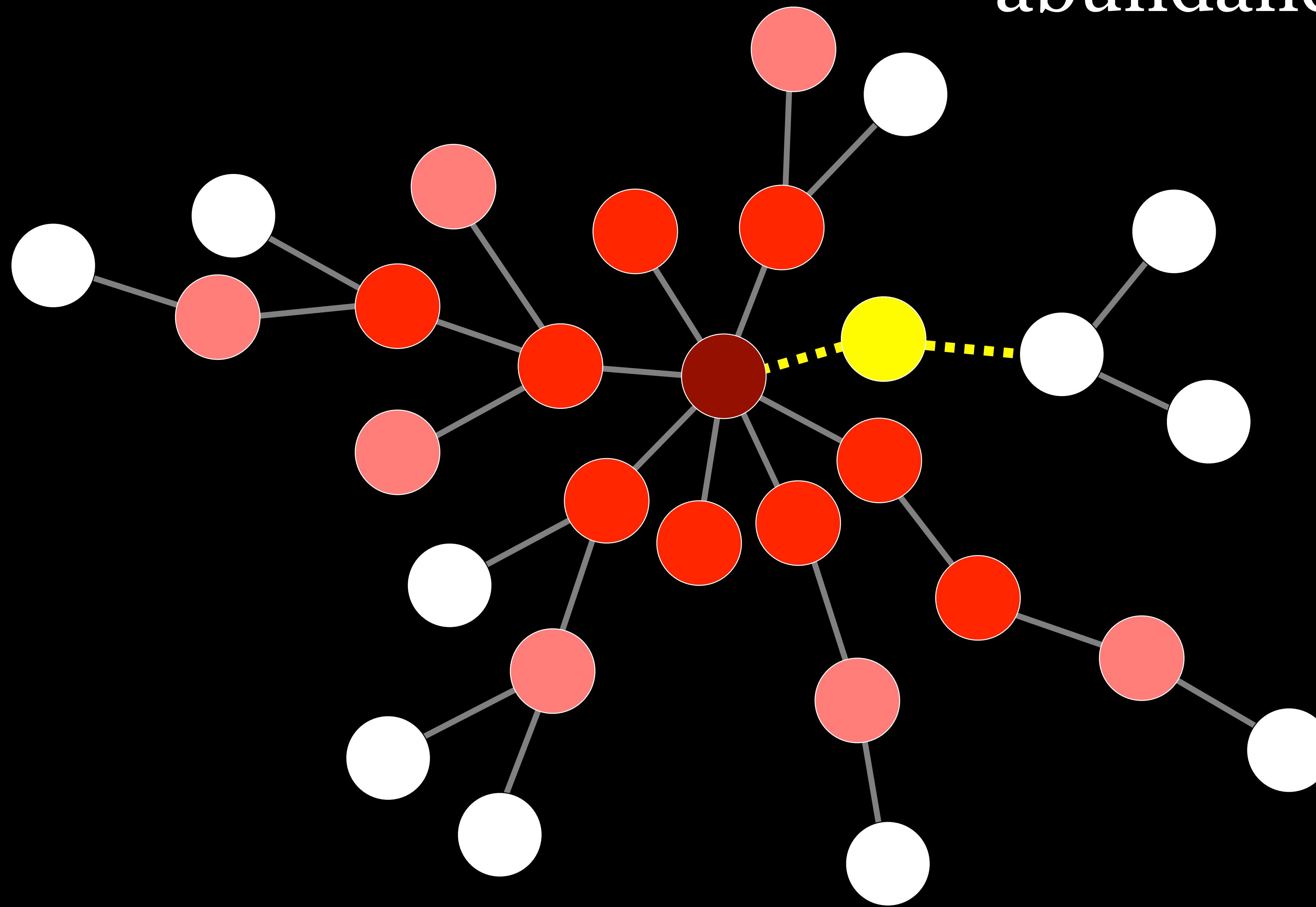




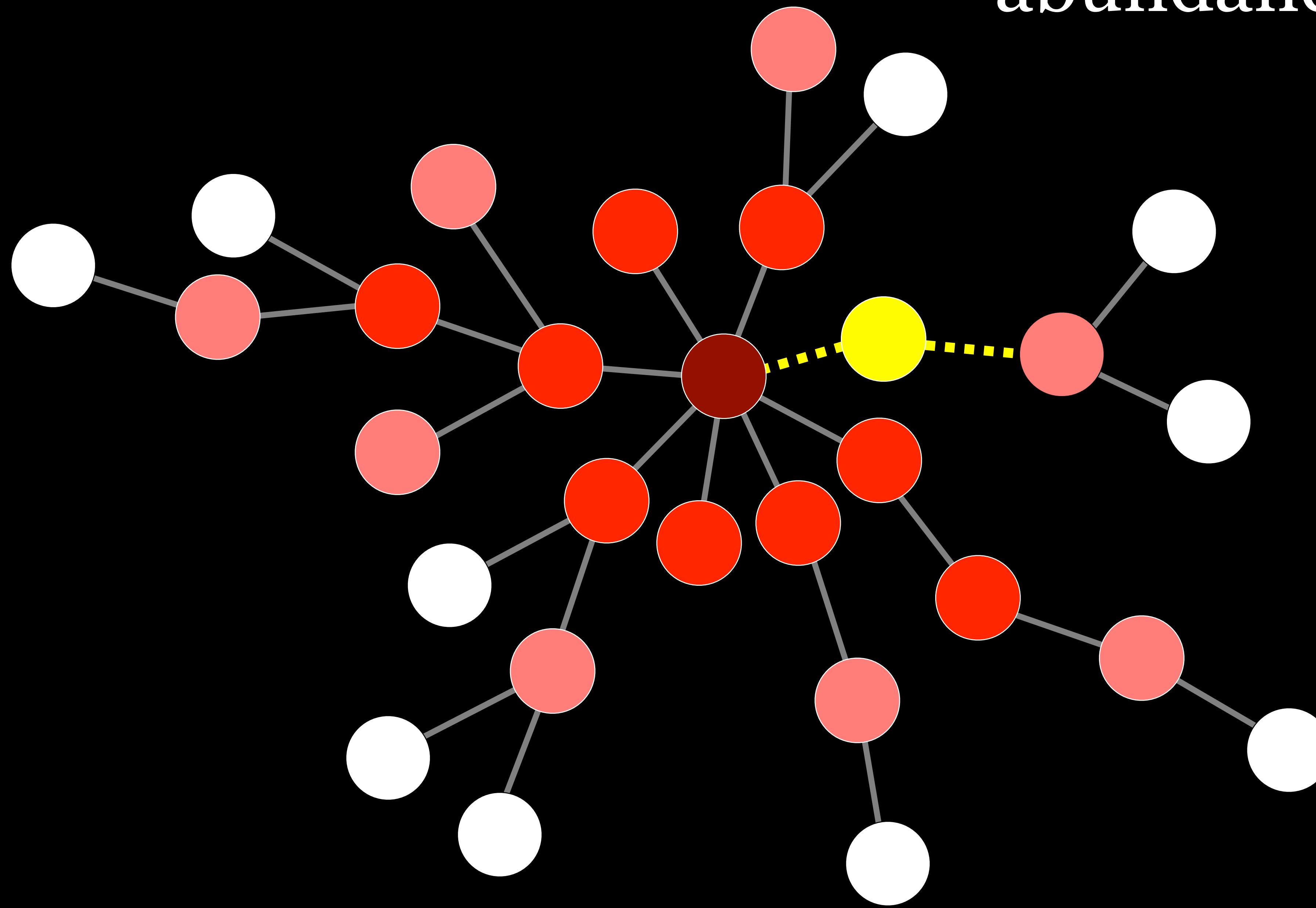




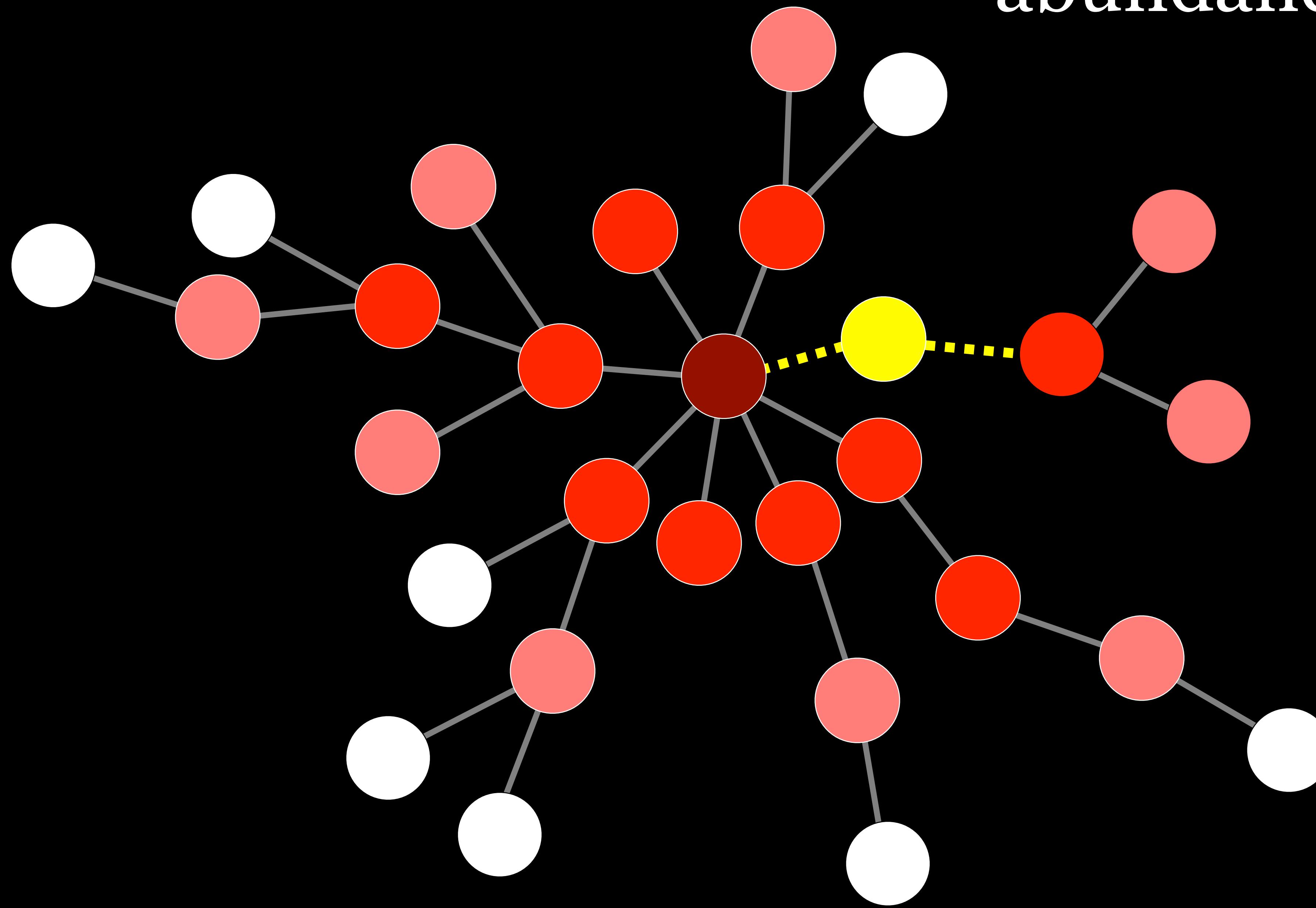
abundance = 3

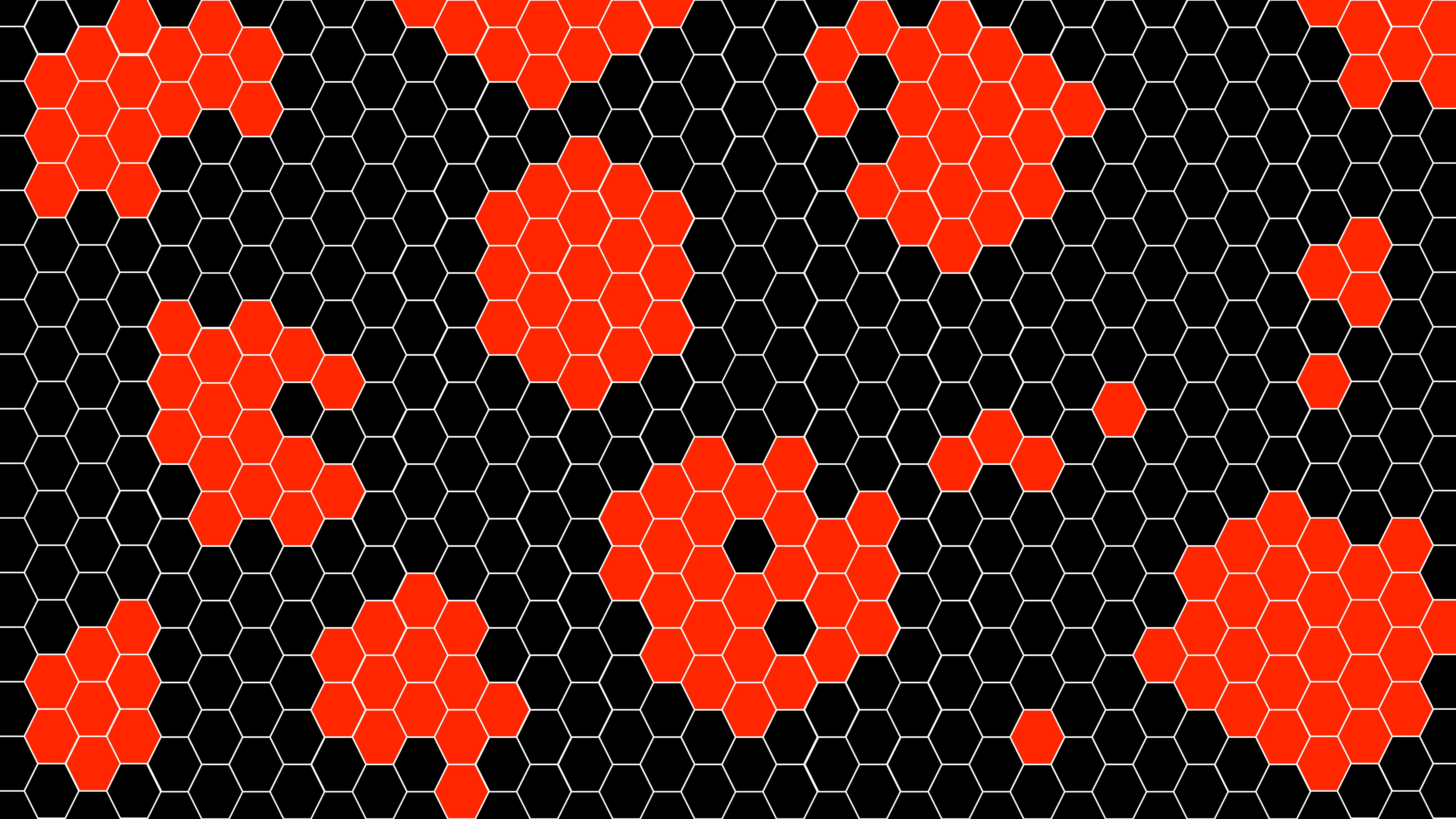


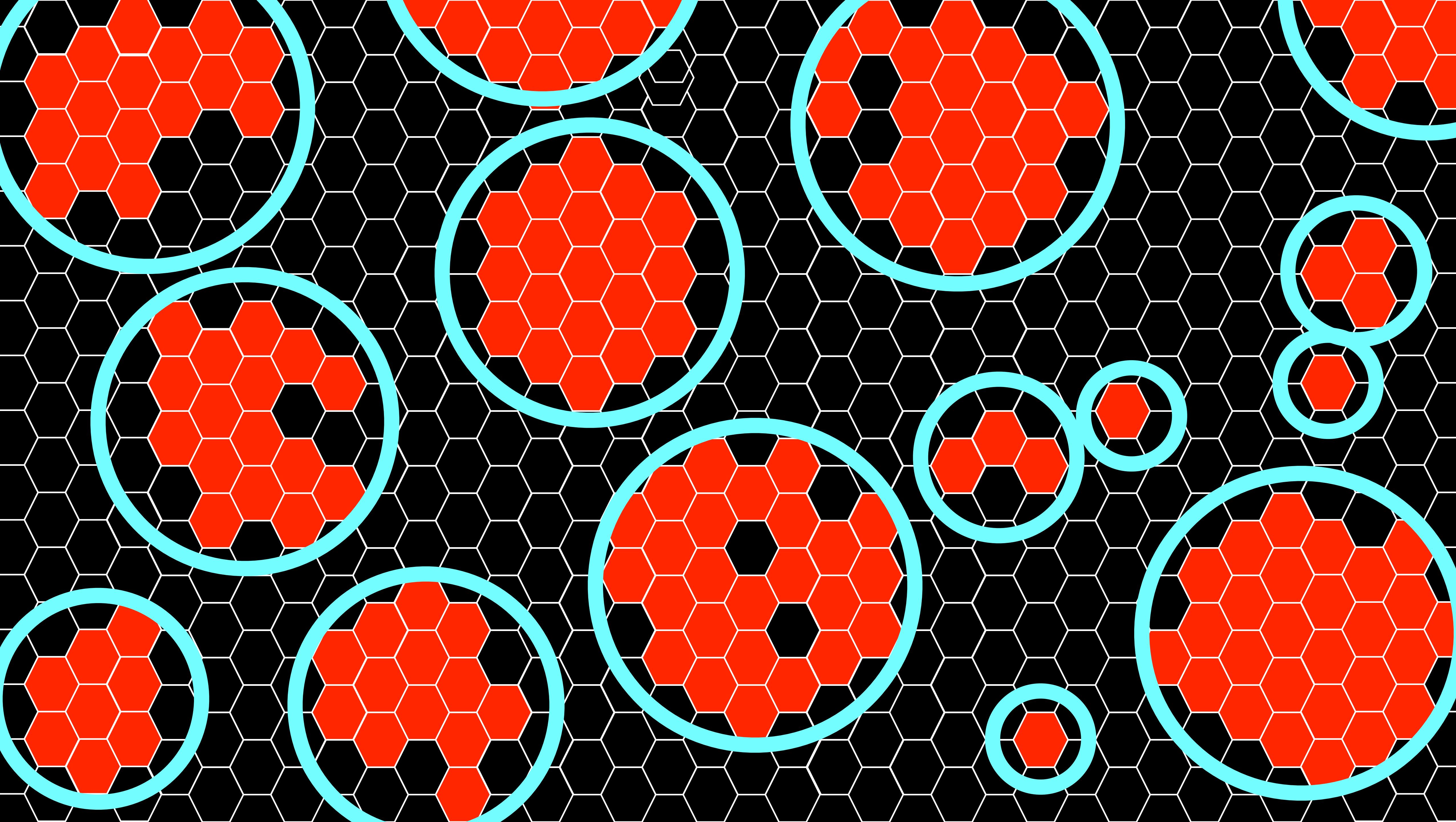
abundance = 100

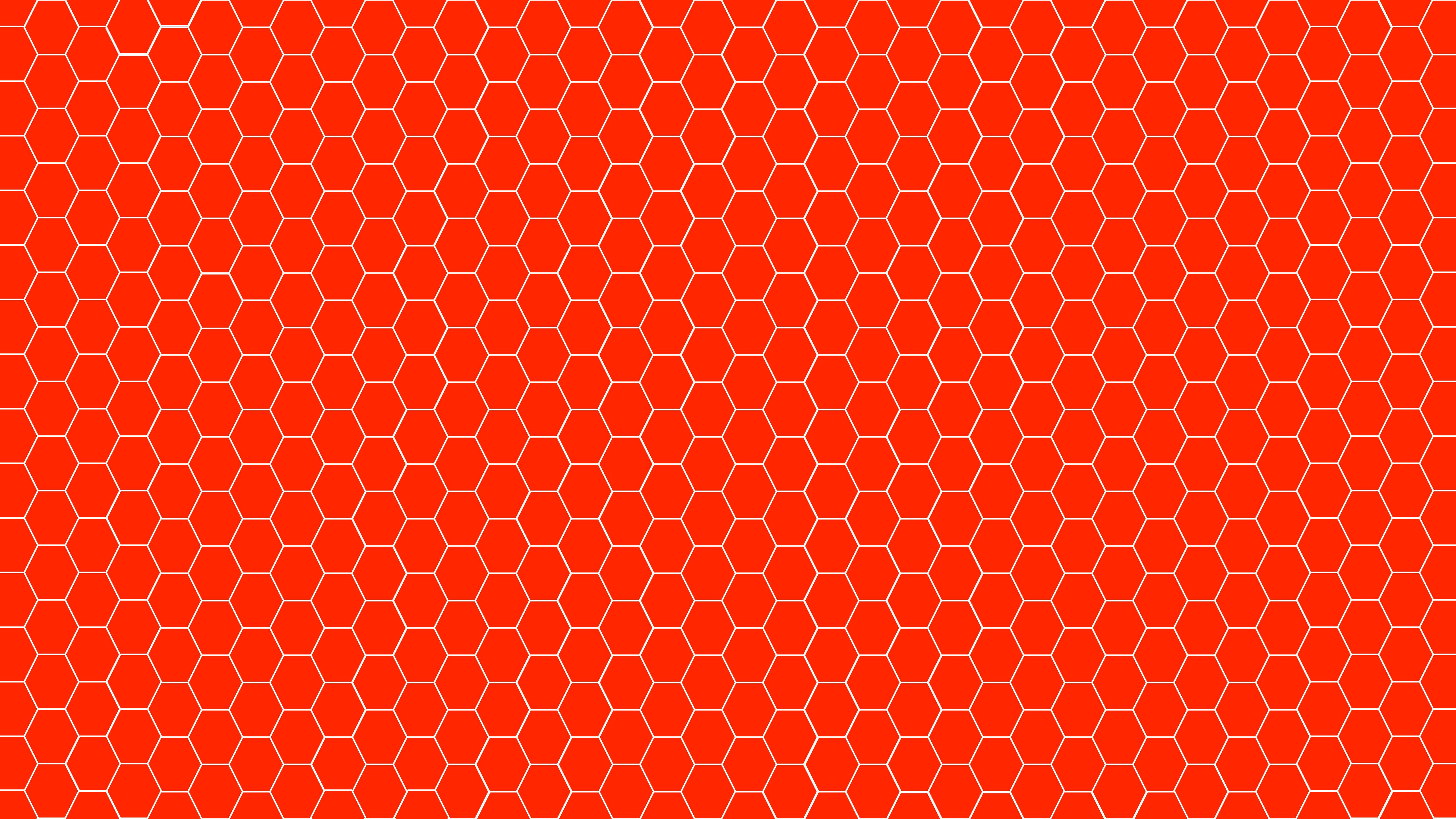


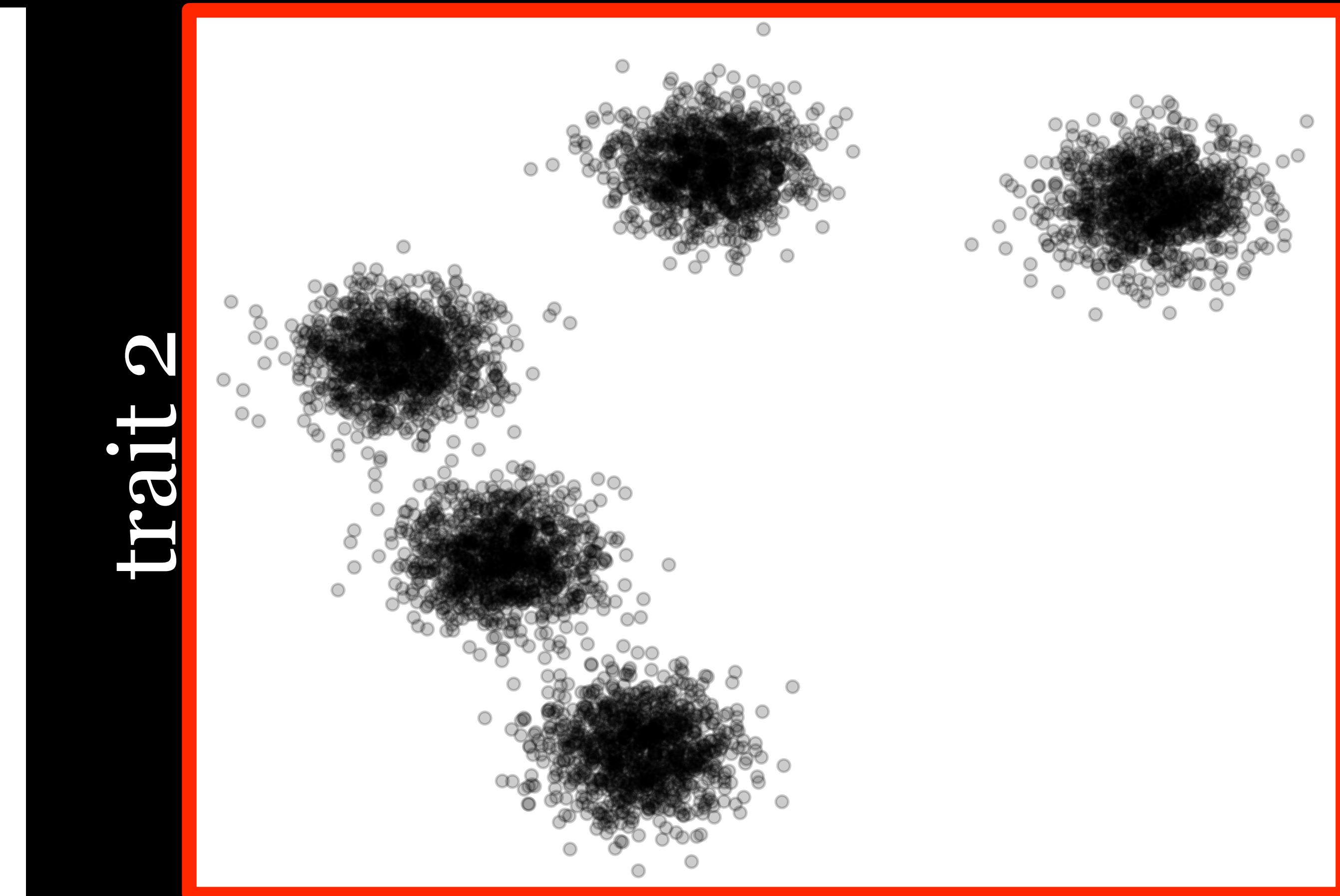
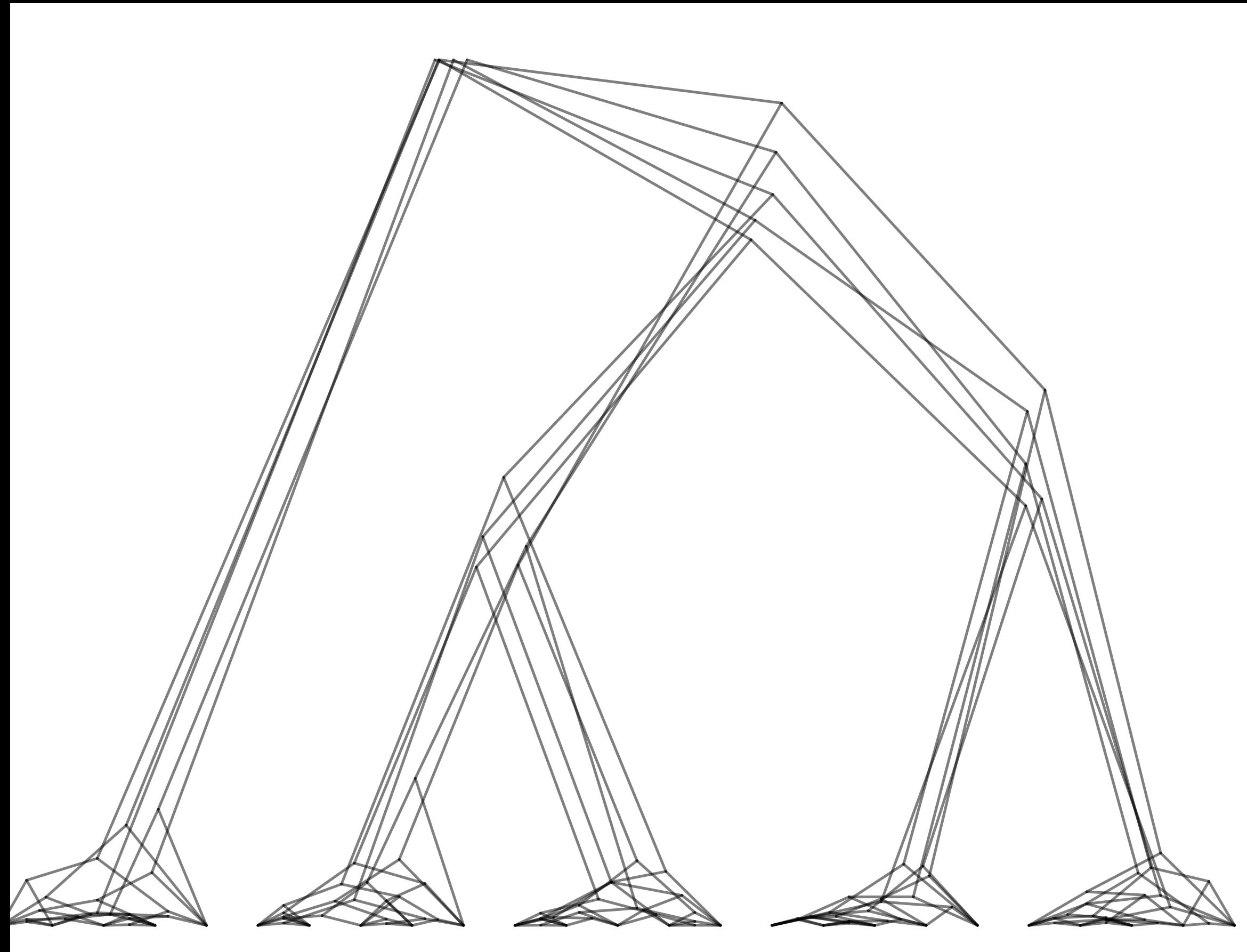
abundance = 1000











global clustering  
thresholds

local clustering  
thresholds

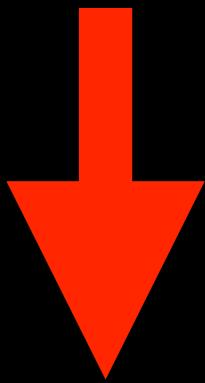
pairwise  
comparisons

phylogenetic  
comparisons

VSEARCH/  
Mothur  
DADA

Swarm

multi-rate  
PTP



*Bioinformatics*, 33(11), 2017, 1630–1638

doi: 10.1093/bioinformatics/btx025

Advance Access Publication Date: 20 January 2017

Original Paper

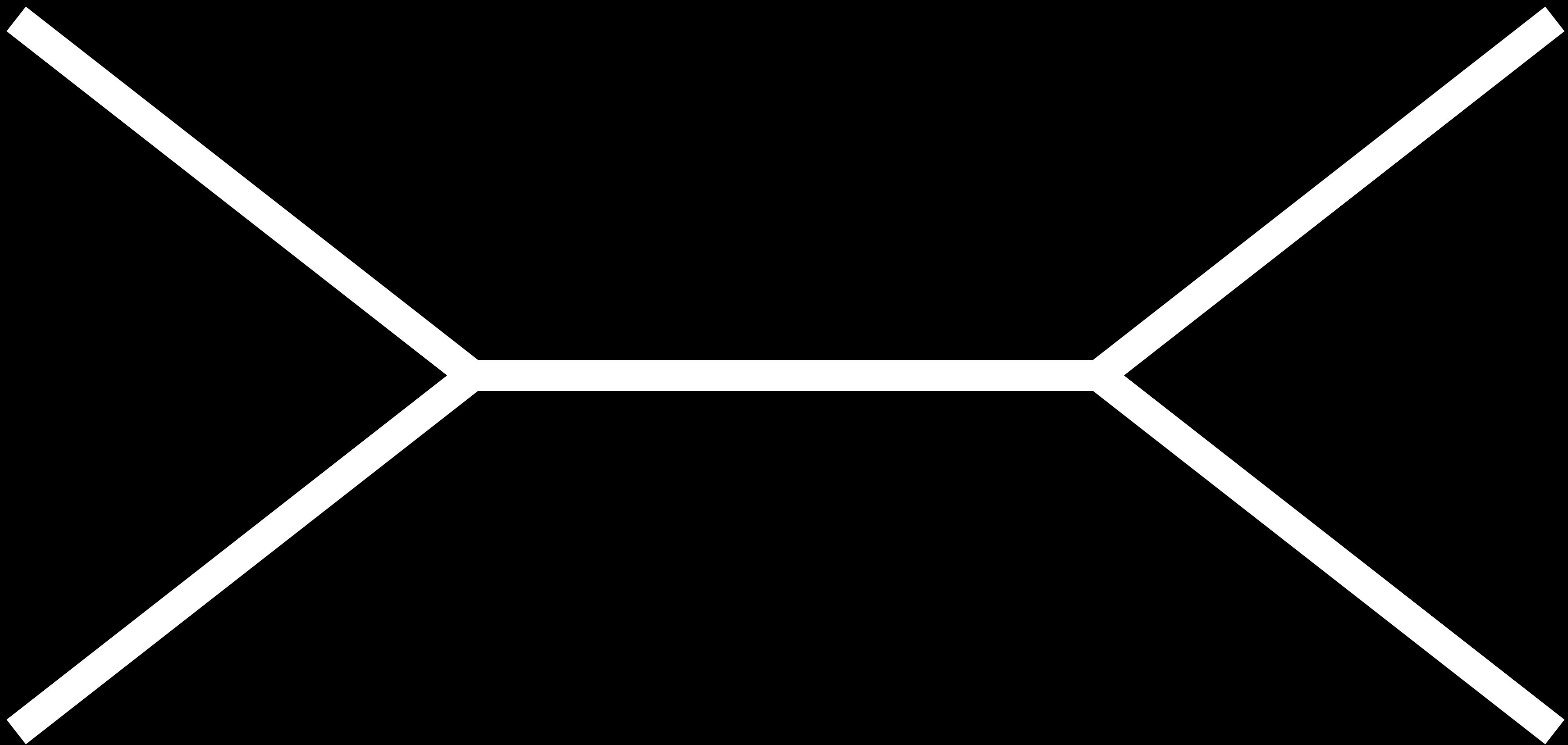
OXFORD

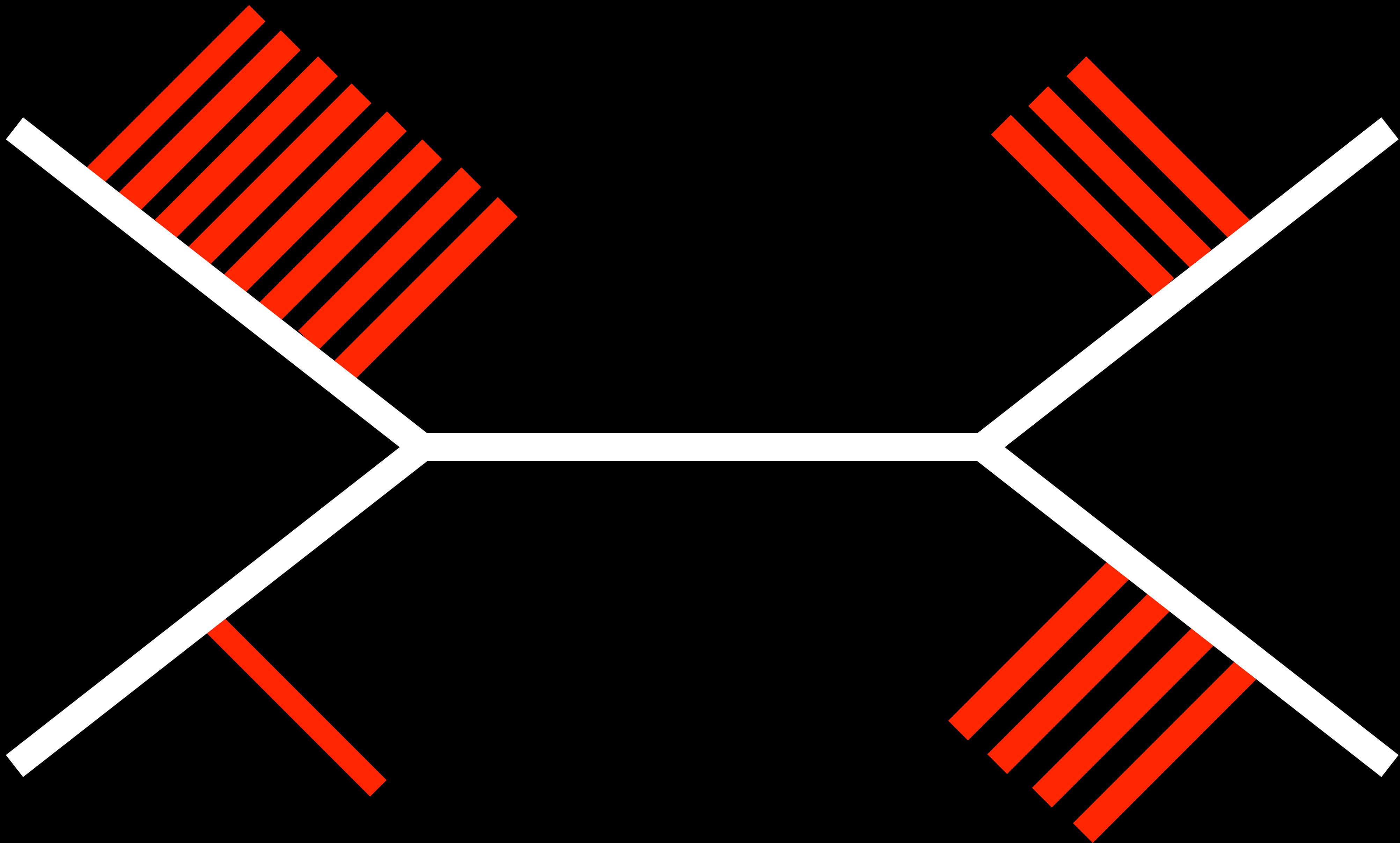
---

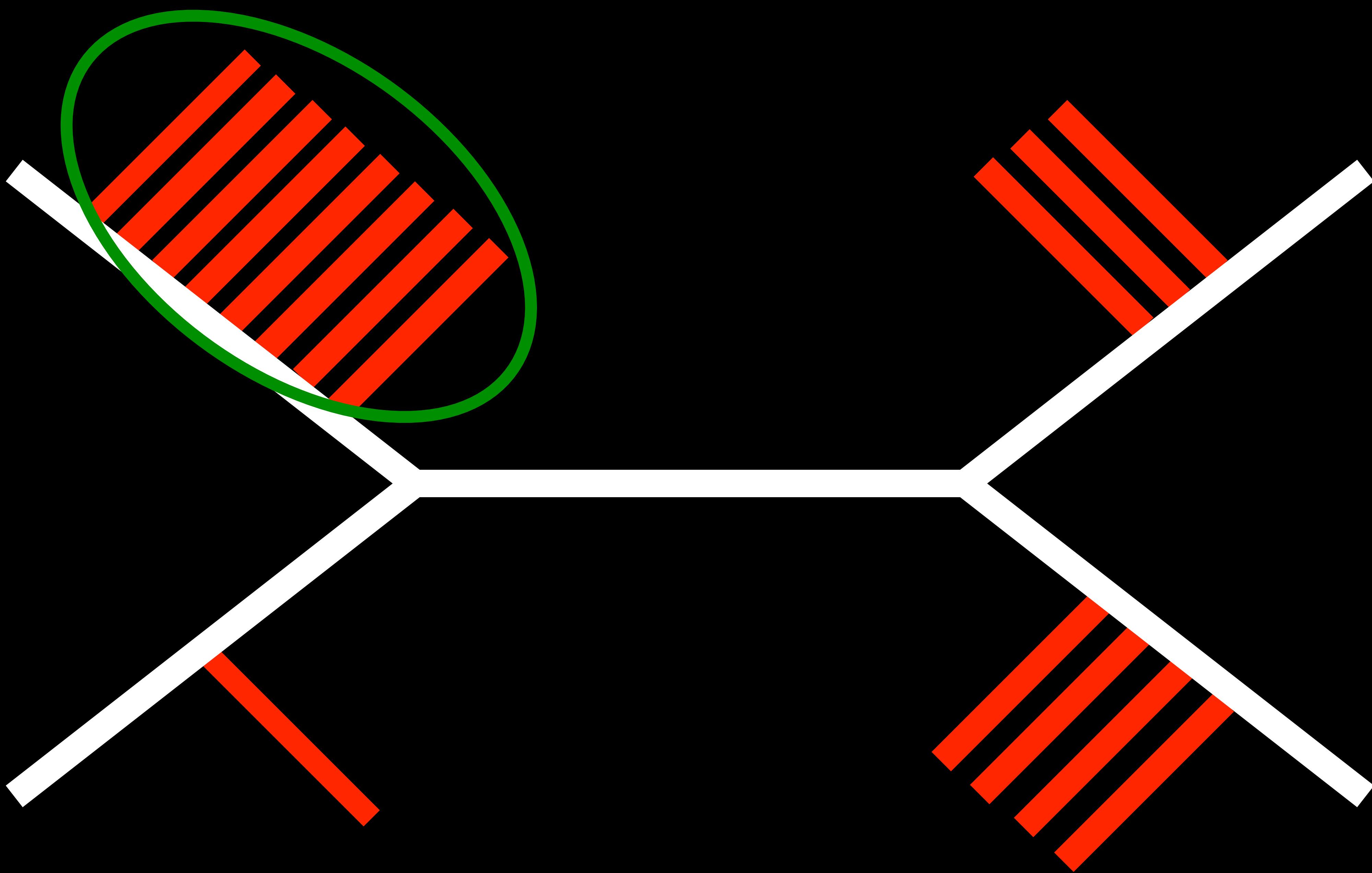
Phylogenetics

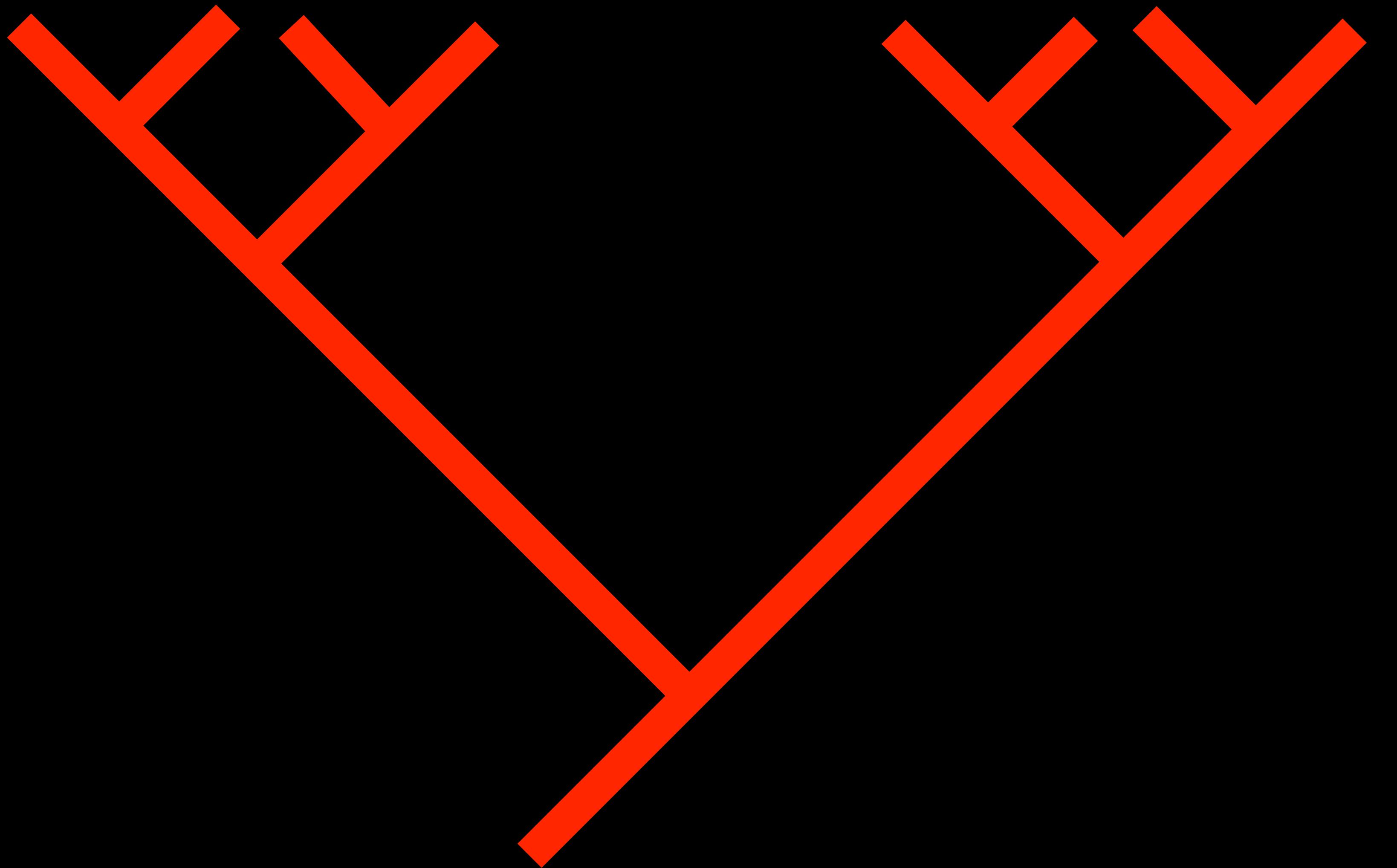
# **Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo**

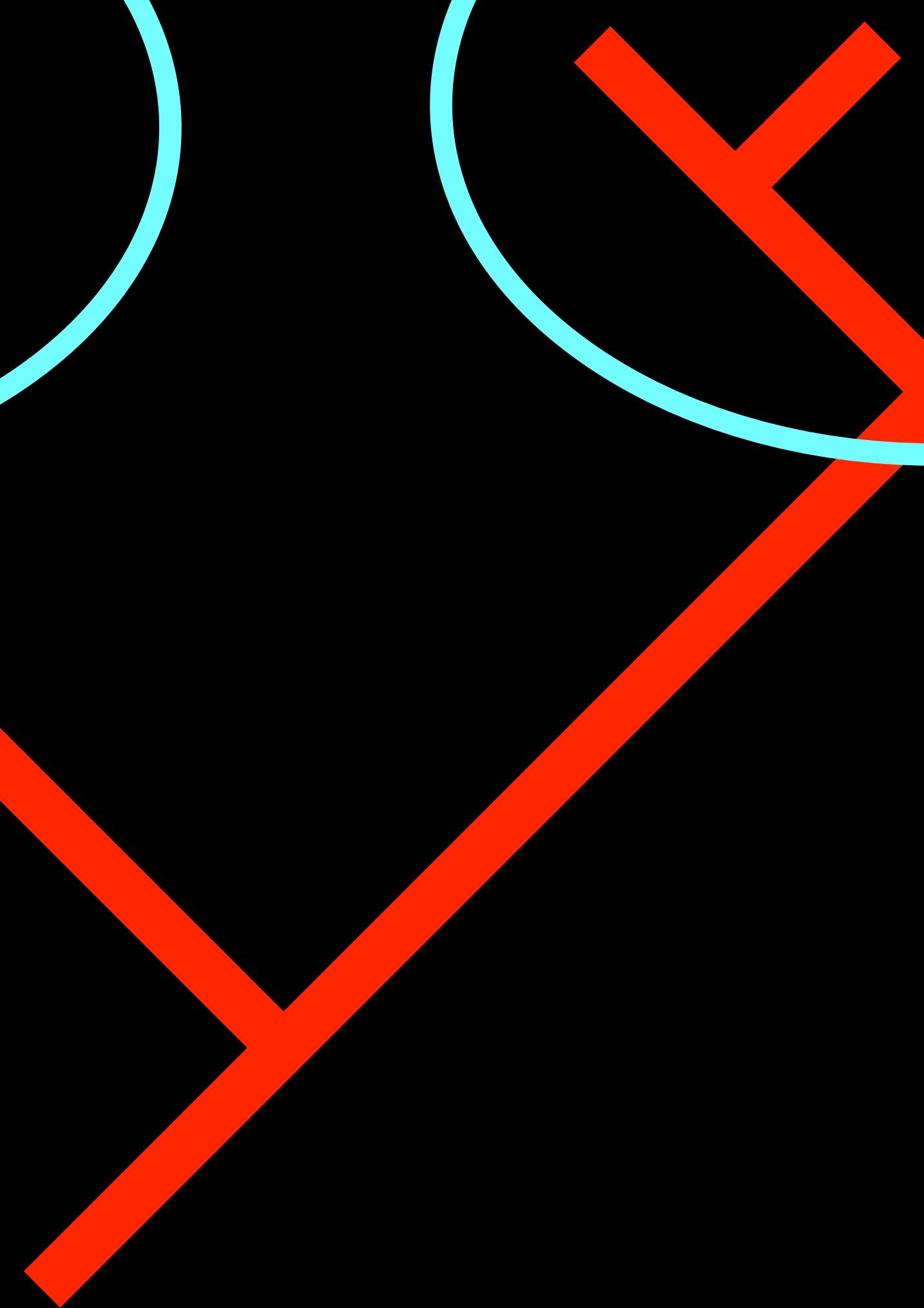
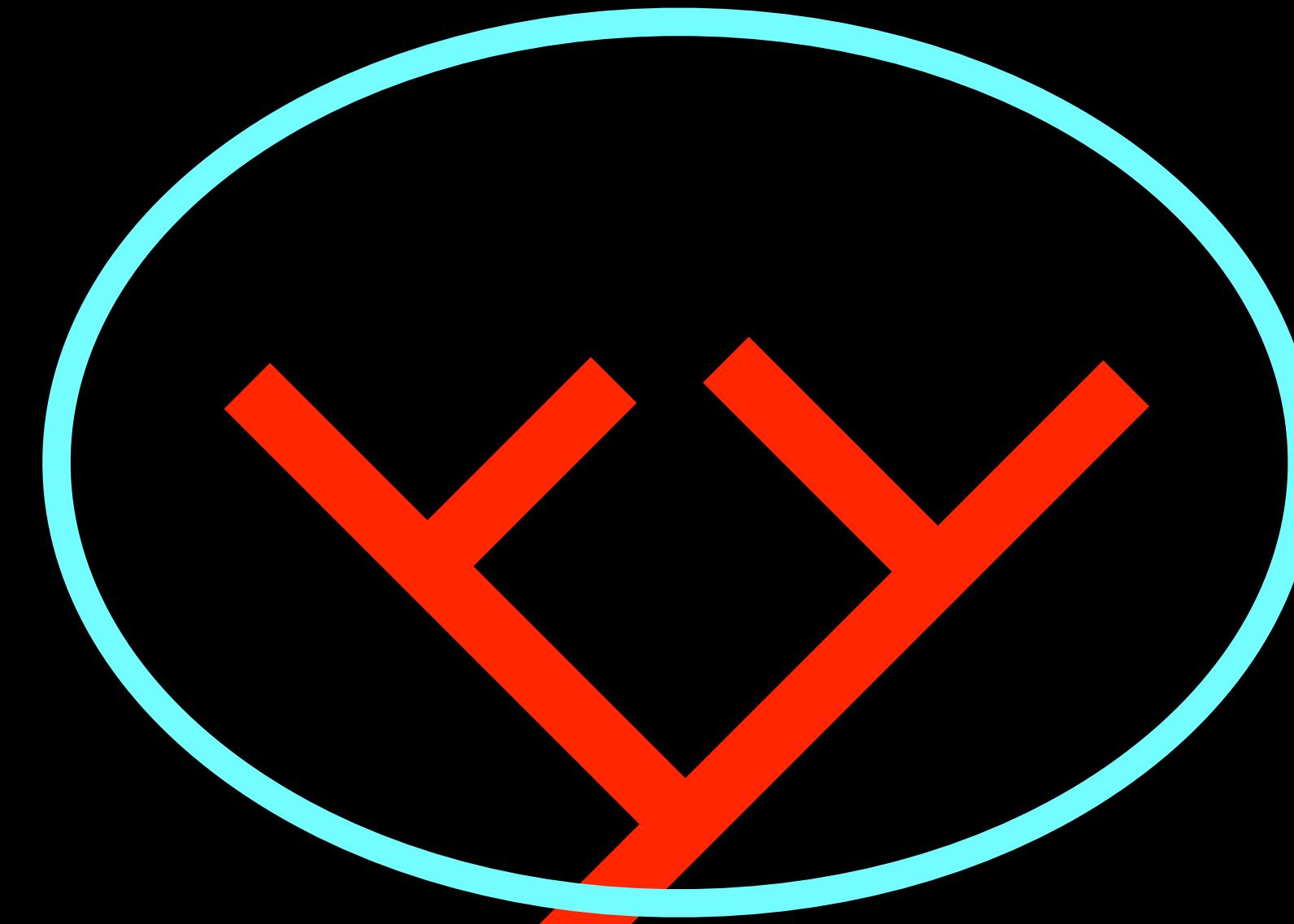
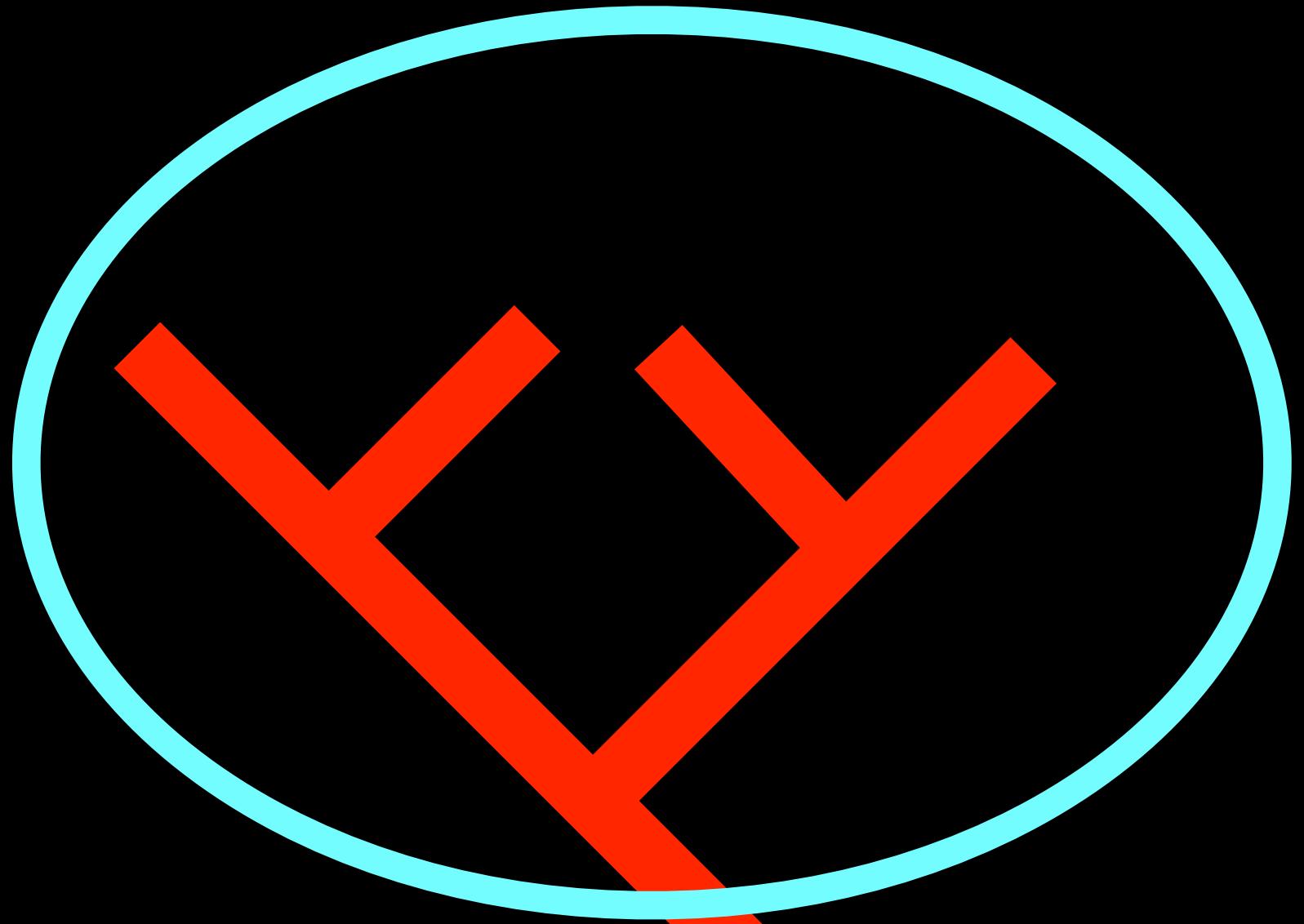
**P. Kapli<sup>1,\*</sup>, S. Lutteropp<sup>1,2</sup>, J. Zhang<sup>1</sup>, K. Kobert<sup>1</sup>, P. Pavlidis<sup>3</sup>,  
A. Stamatakis<sup>1,2,\*</sup> and T. Flouri<sup>1,2,\*</sup>**

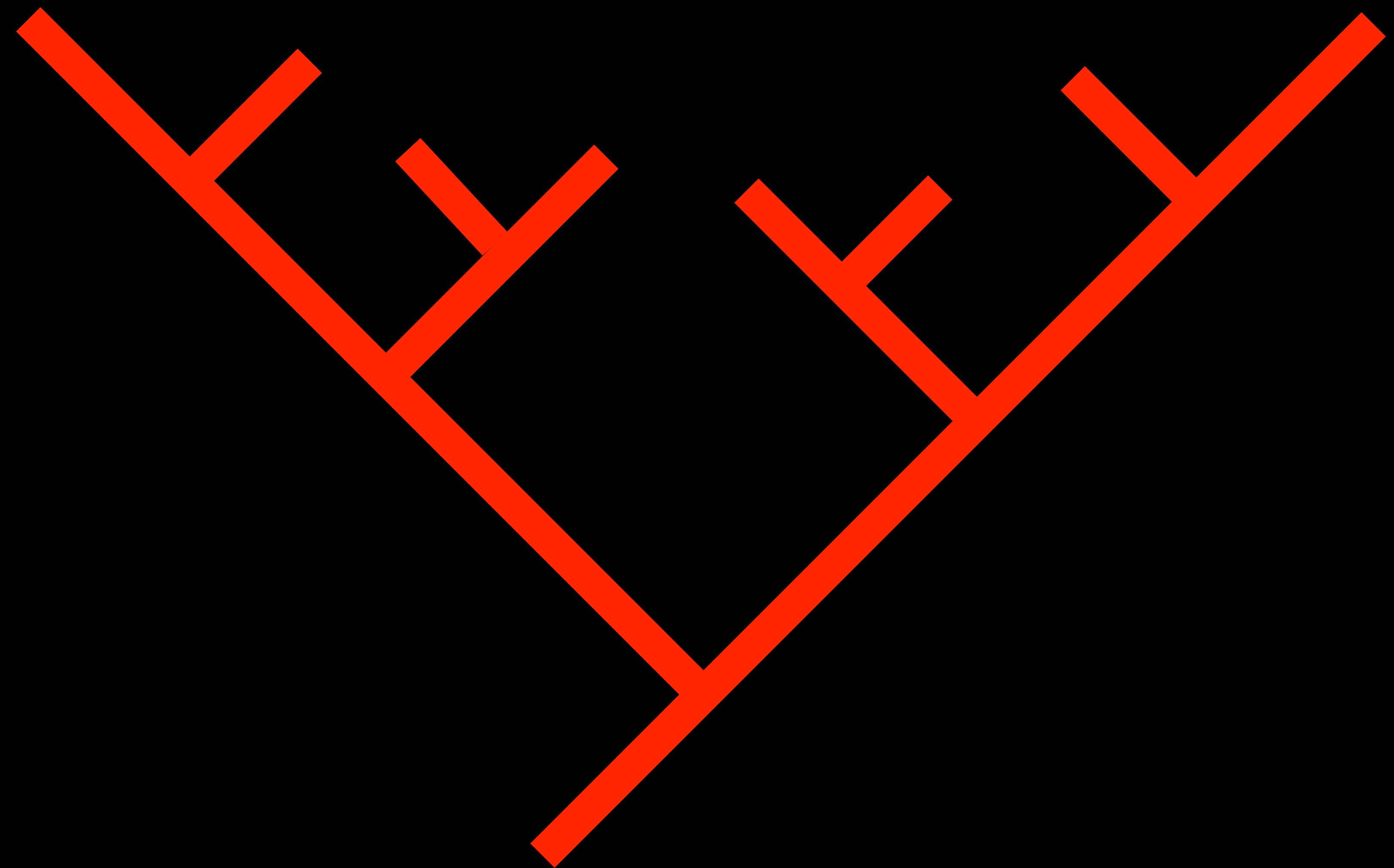


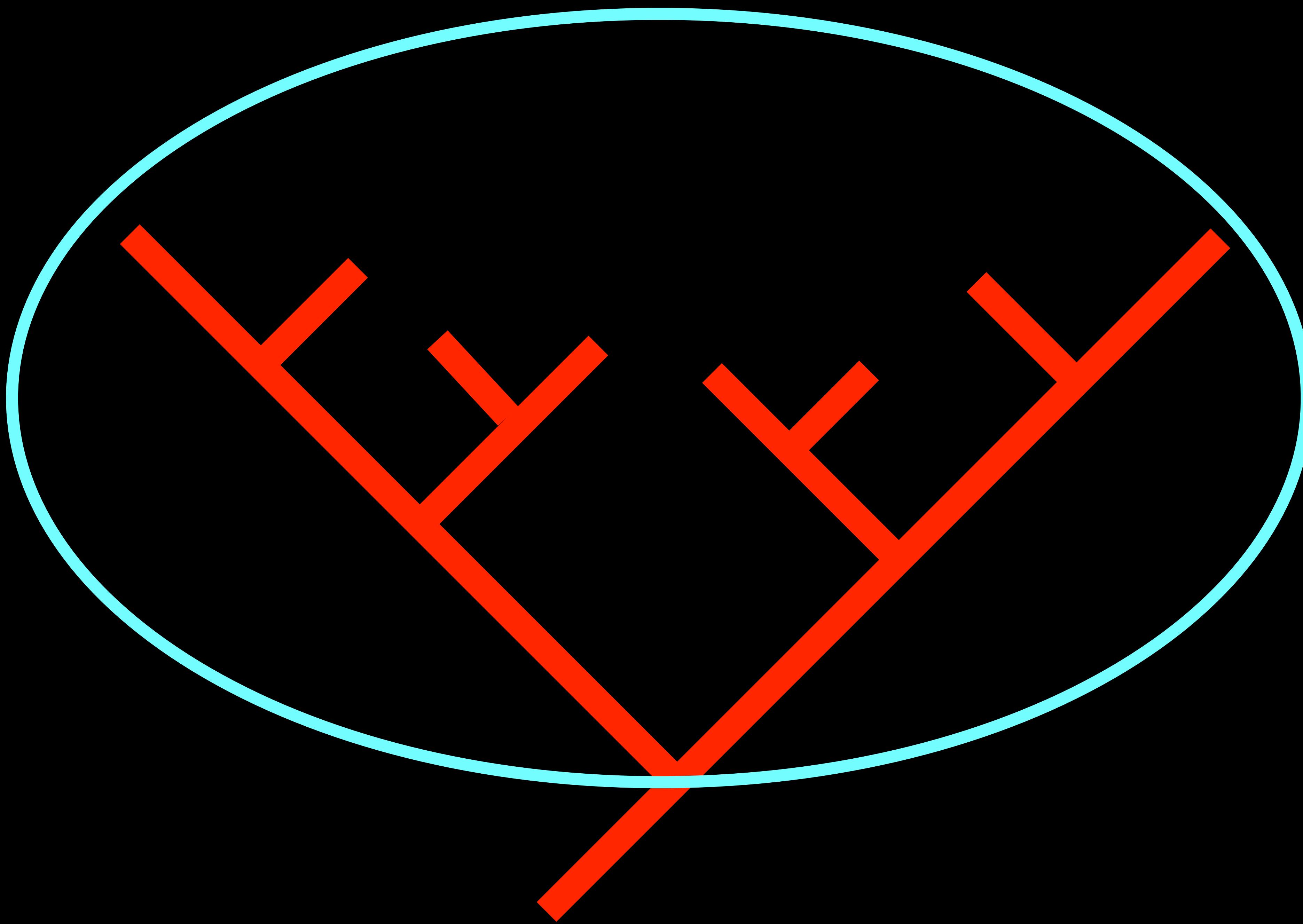


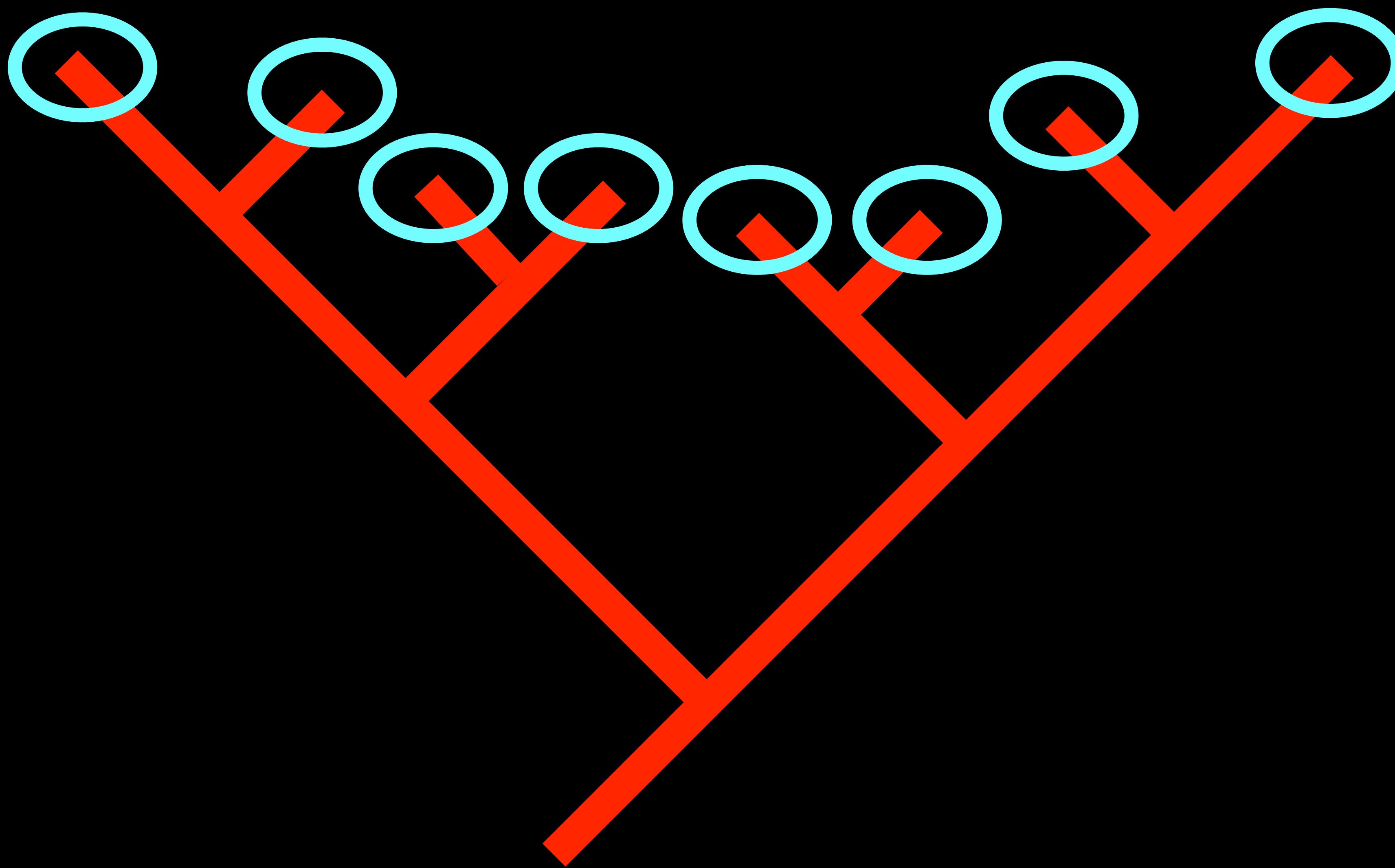




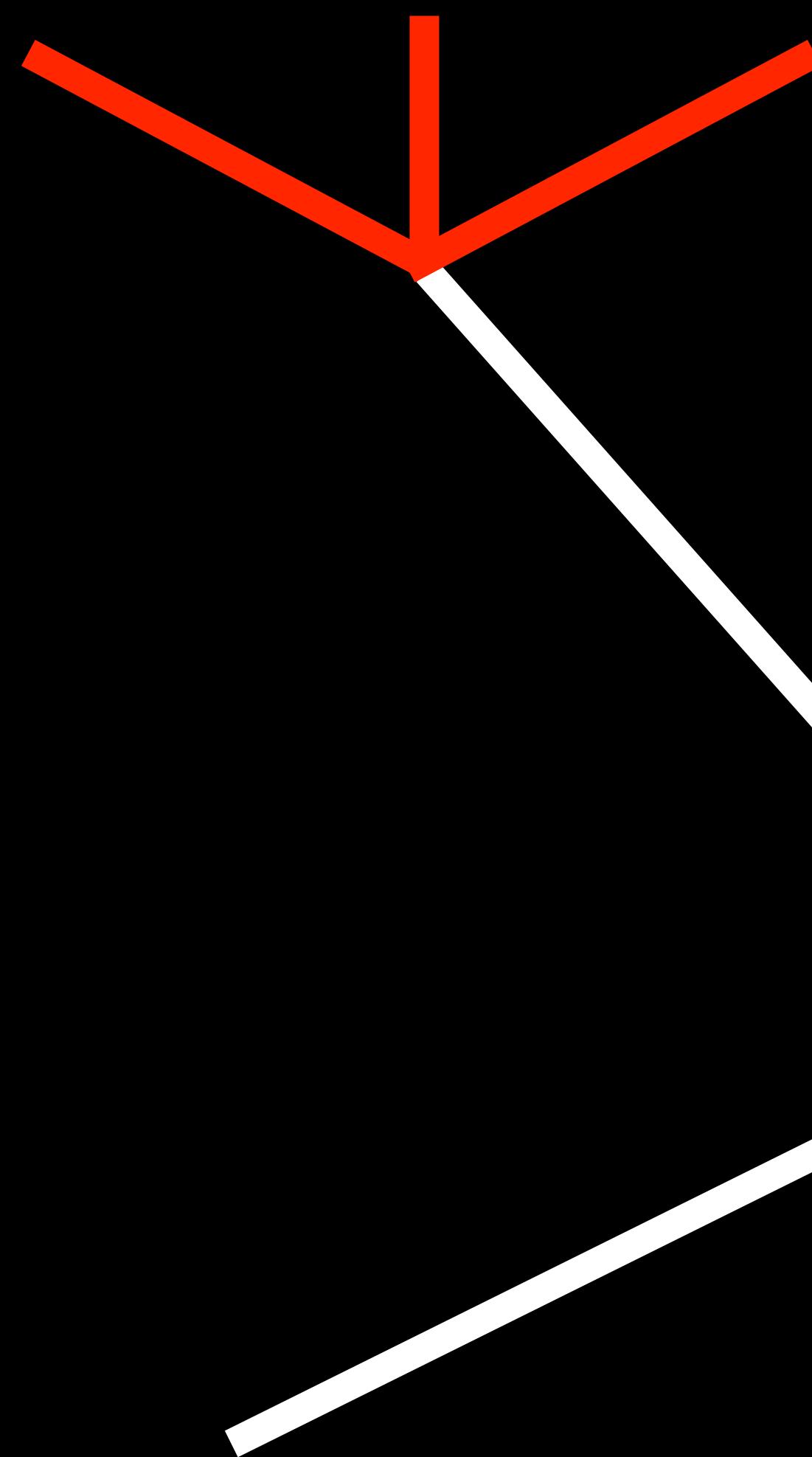




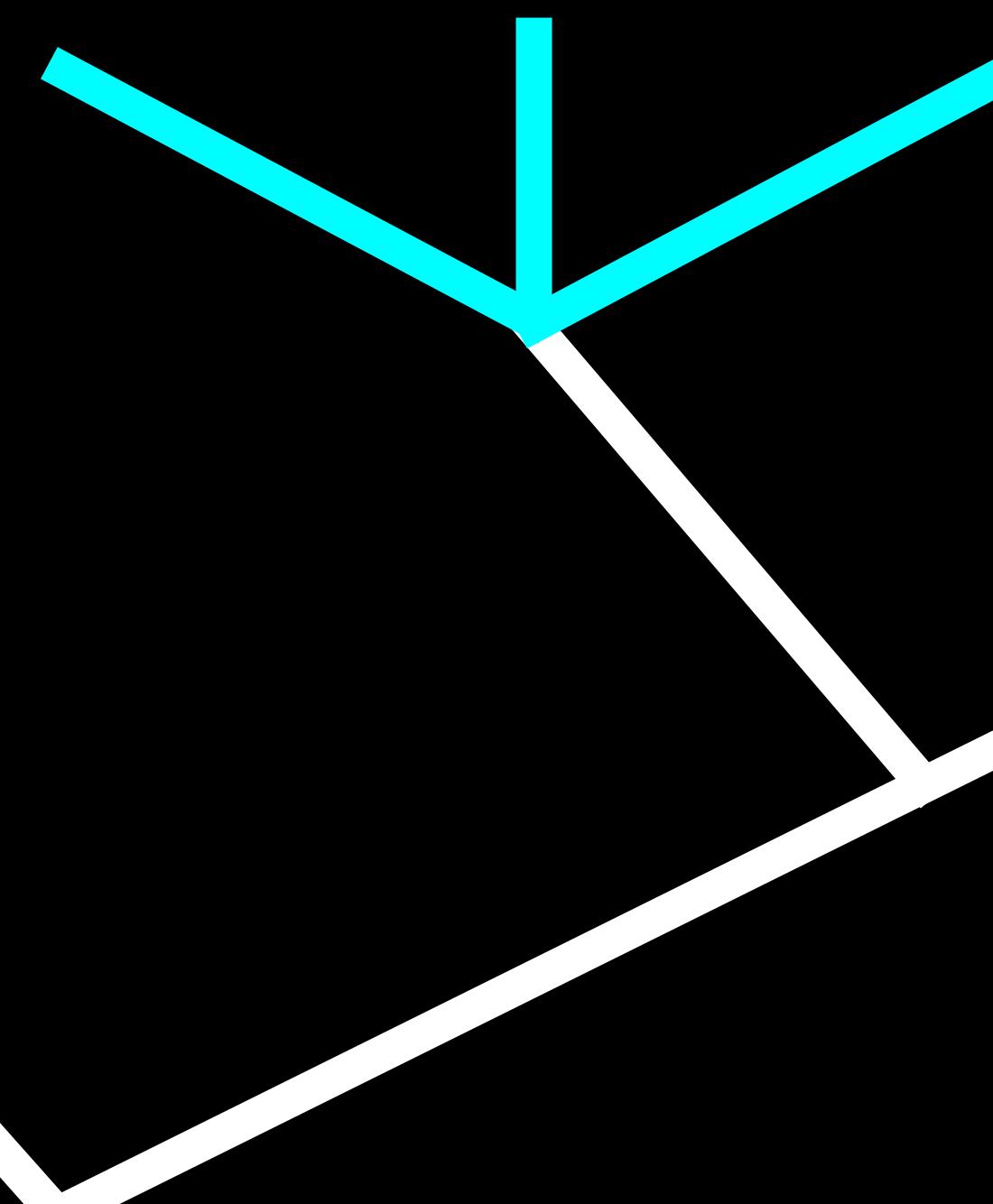




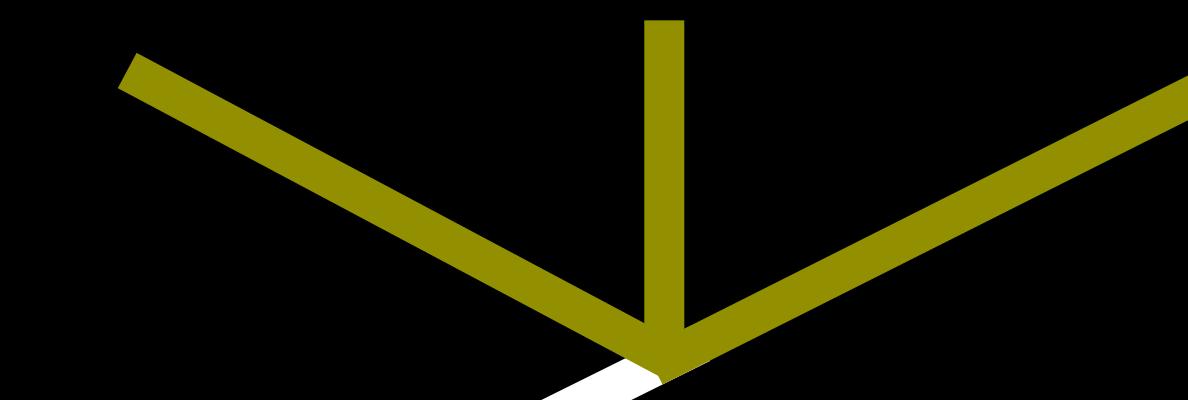
species 1

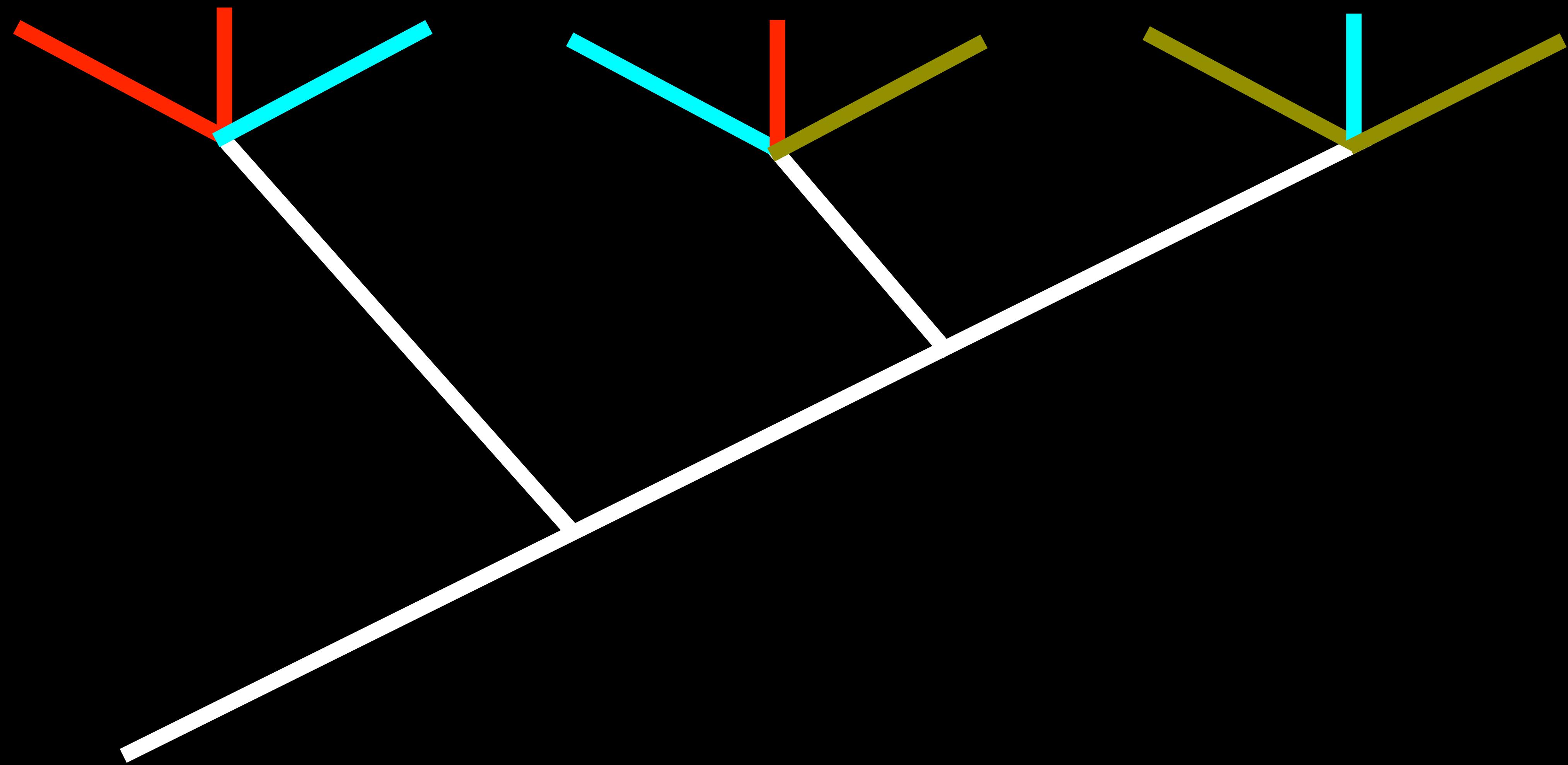


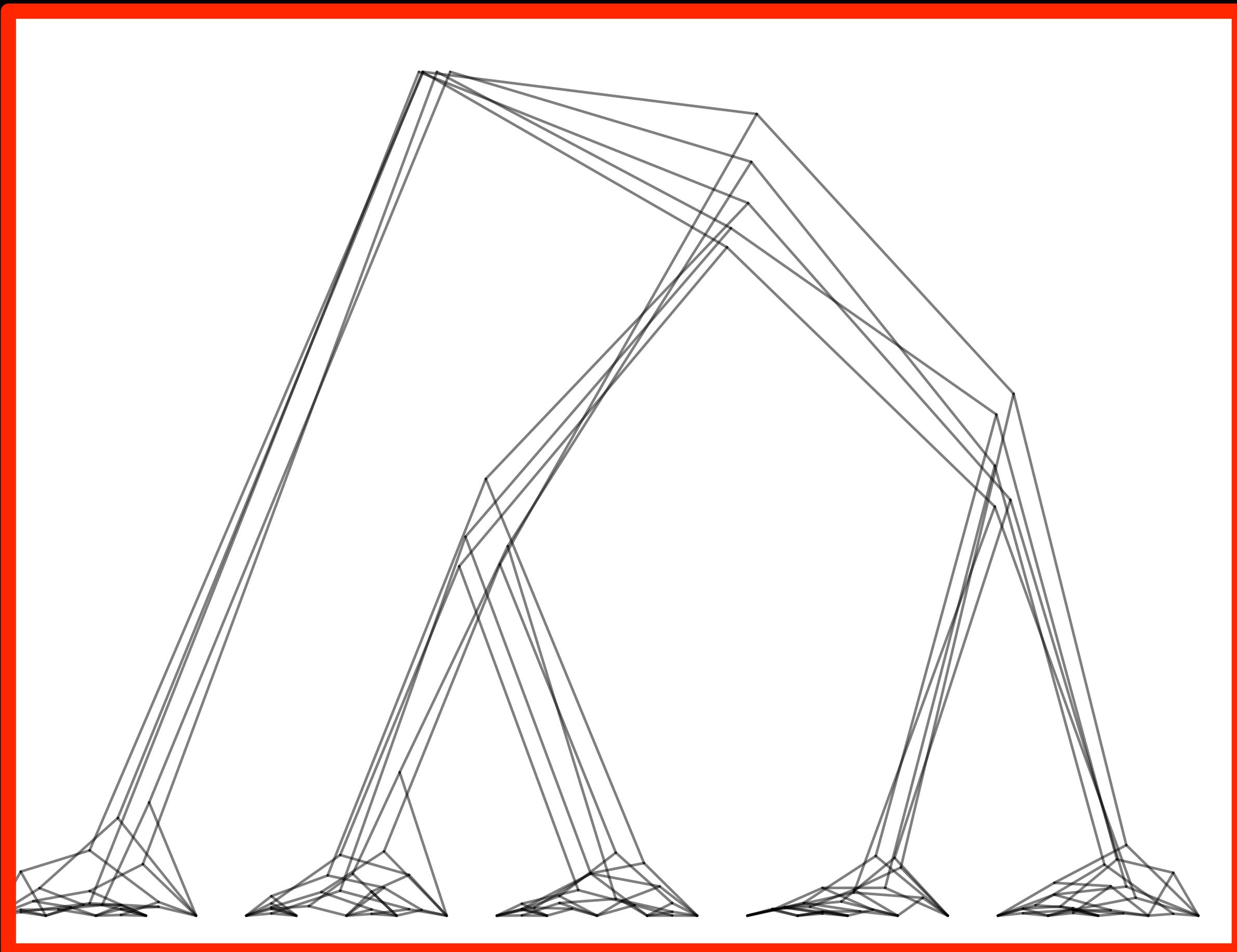
species 2



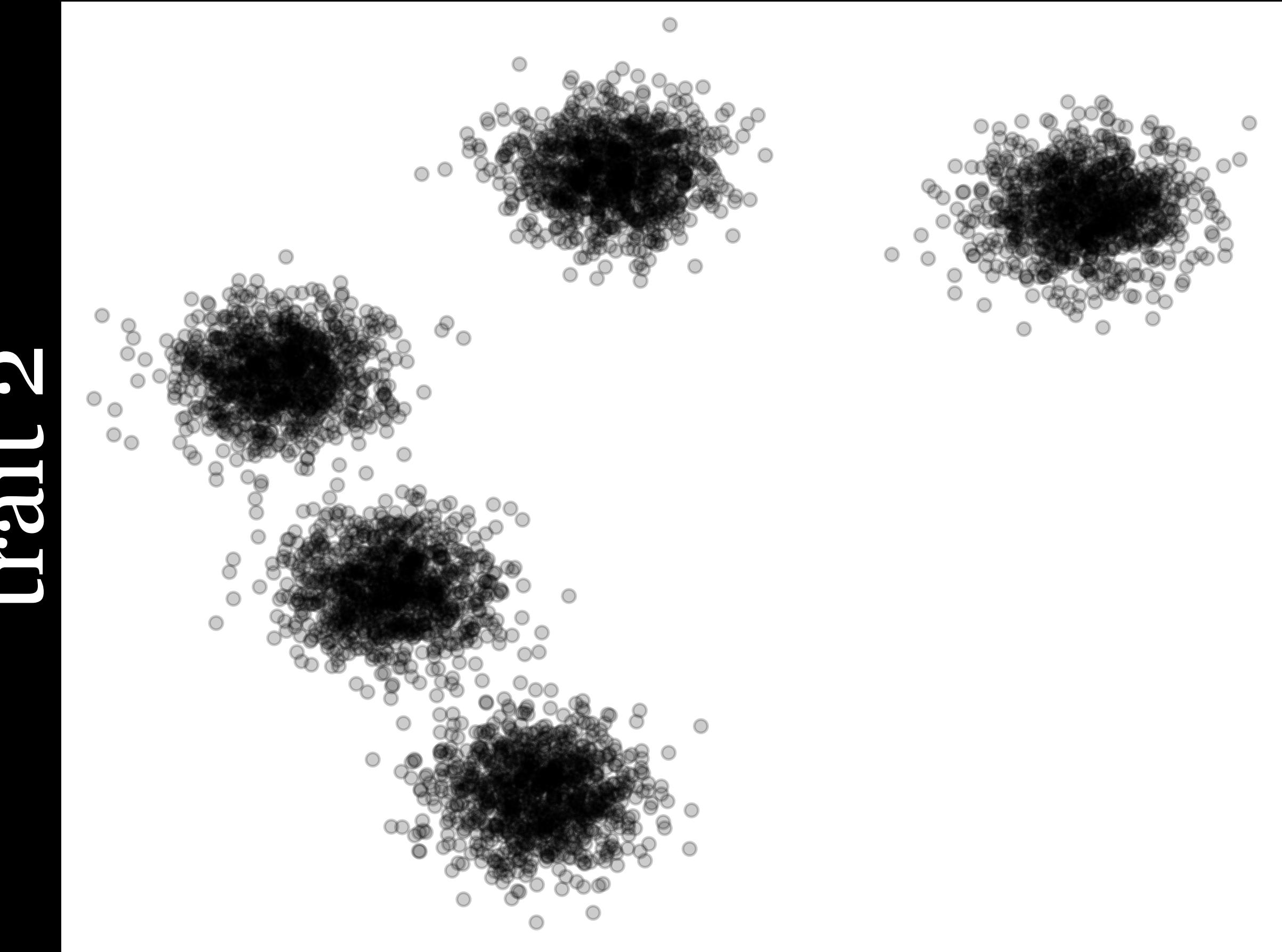
species 3







trait 2



trait 1

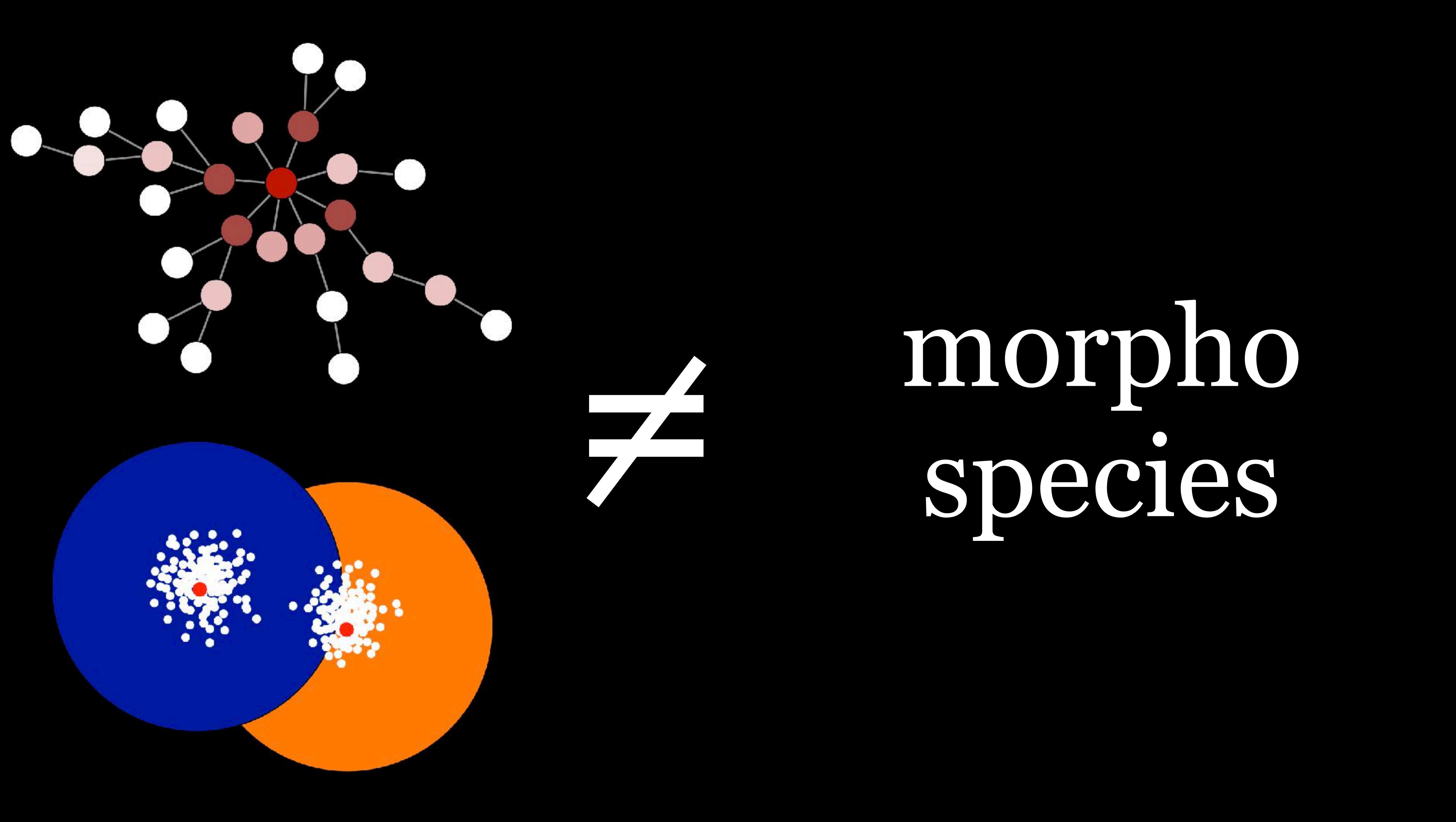
Where to now with OTUs...

operational taxonomic units

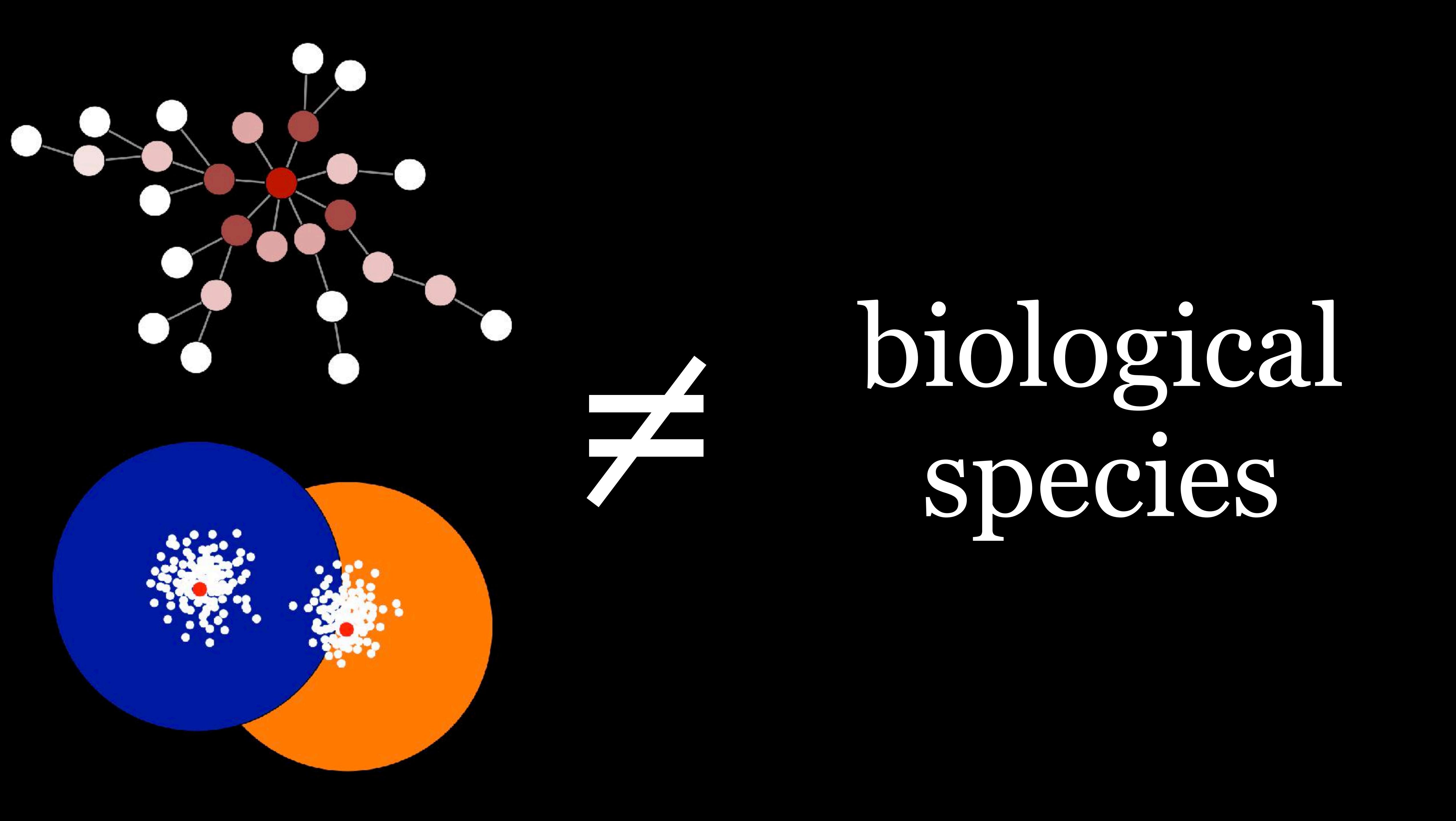
**operational taxonomic units**

are

**operational criteria for species delimitation**

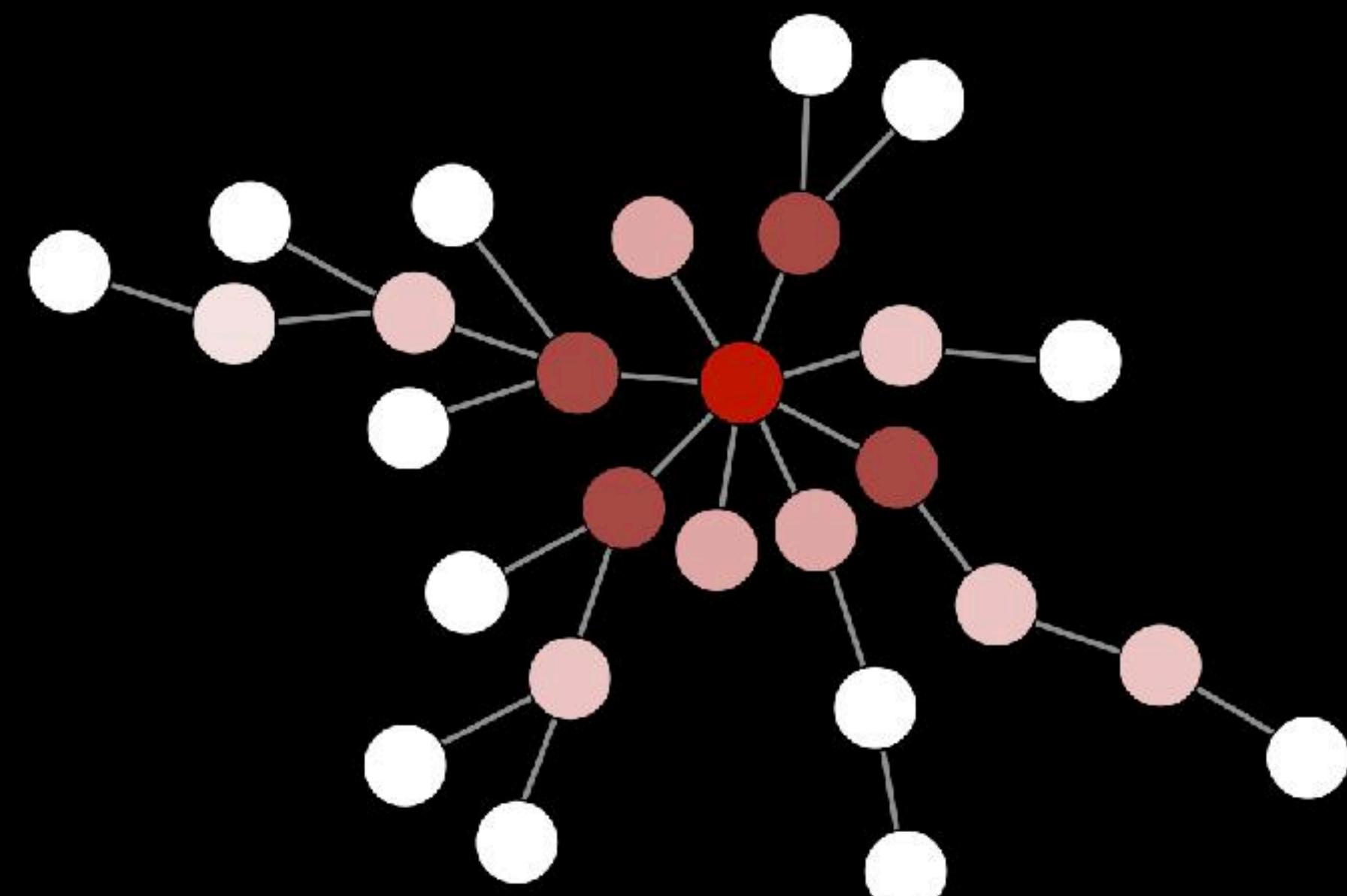
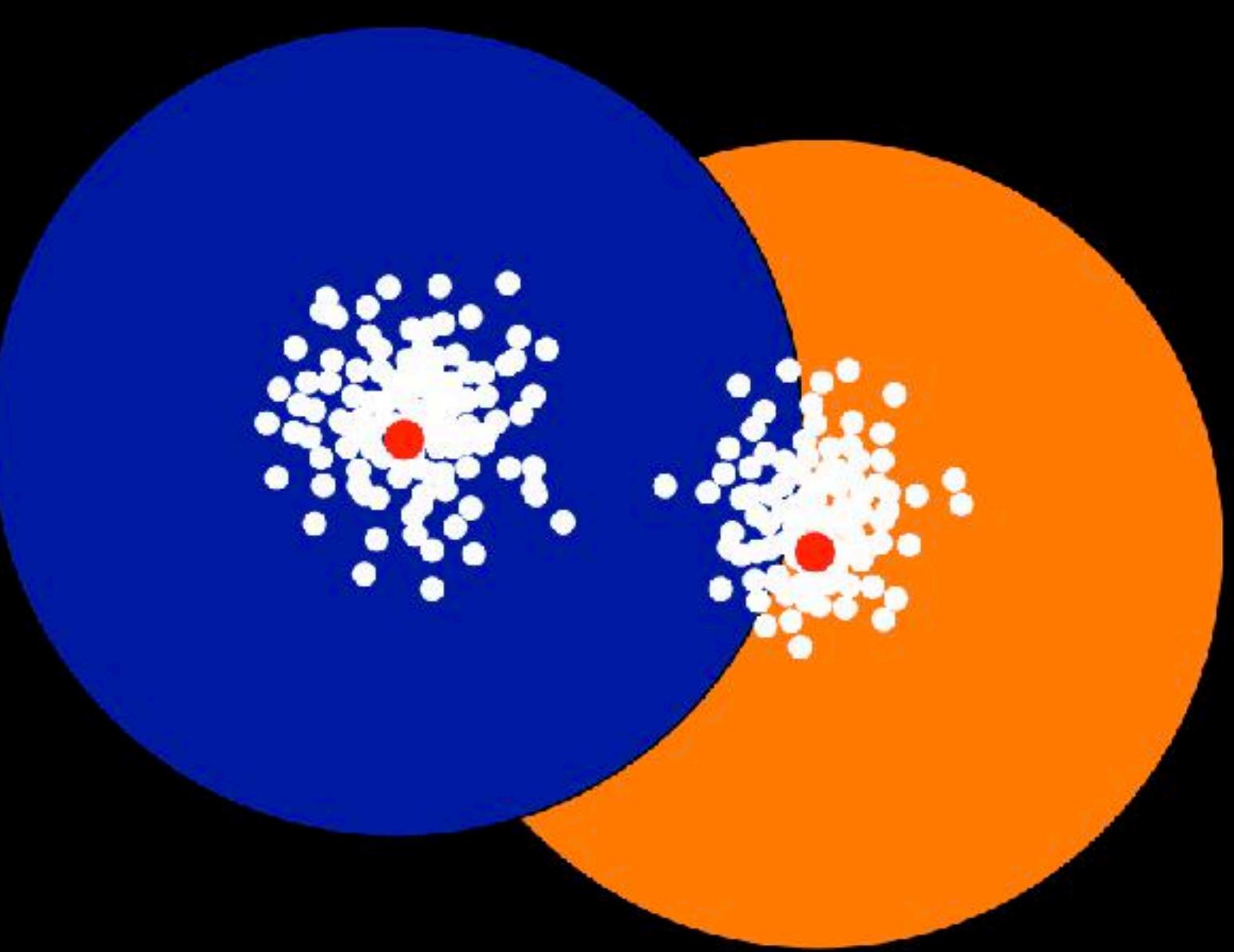
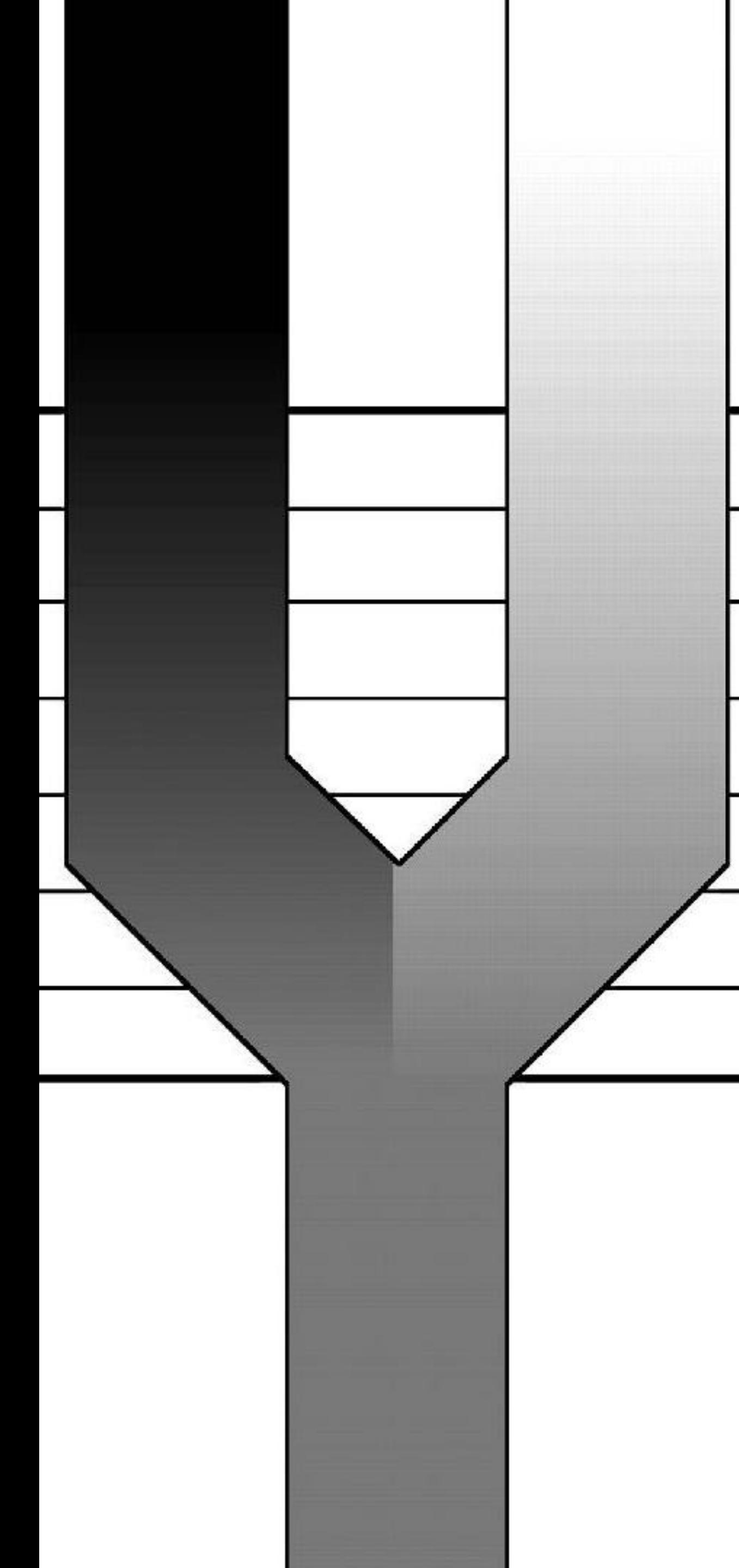


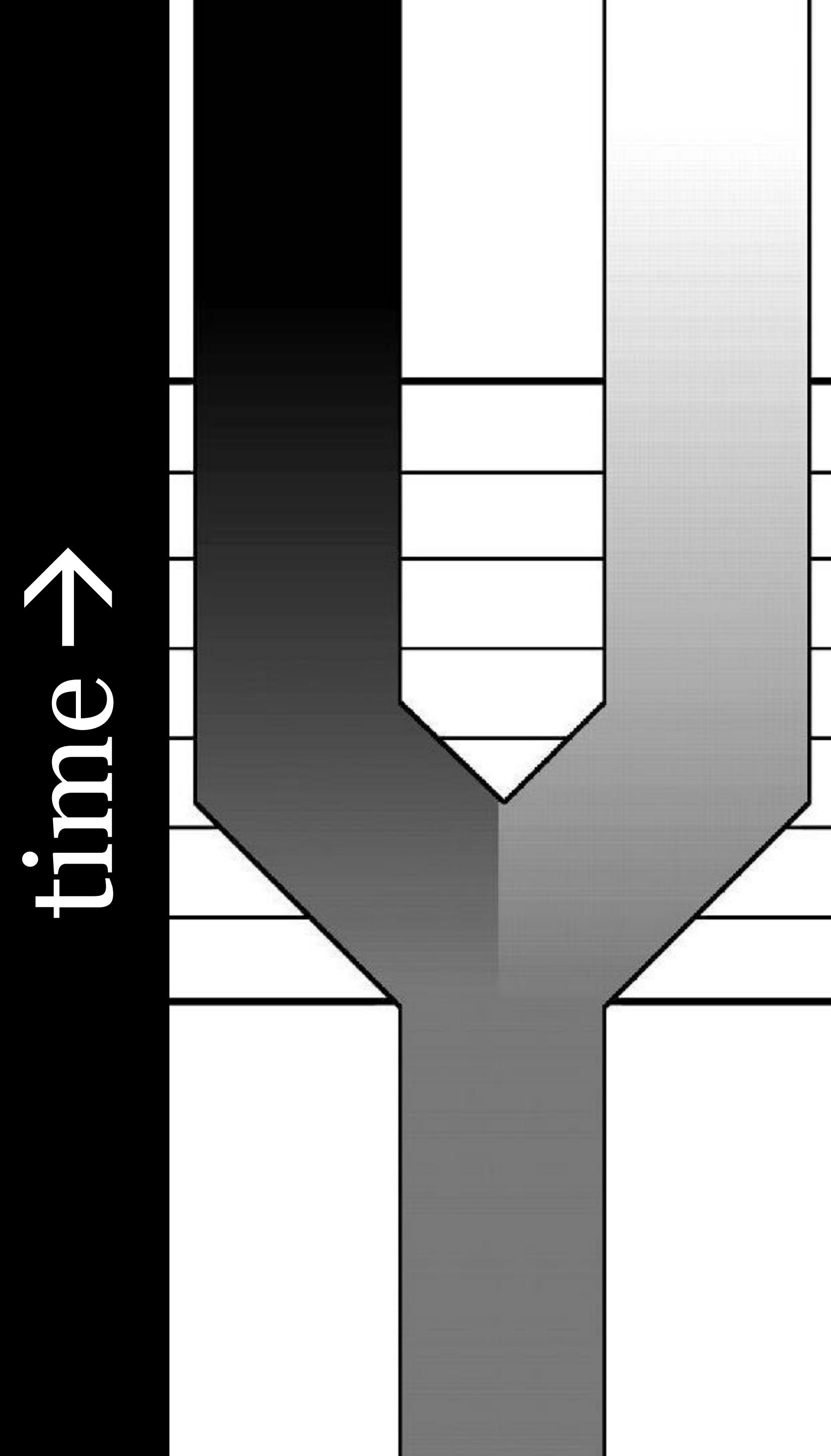
morpho  
species

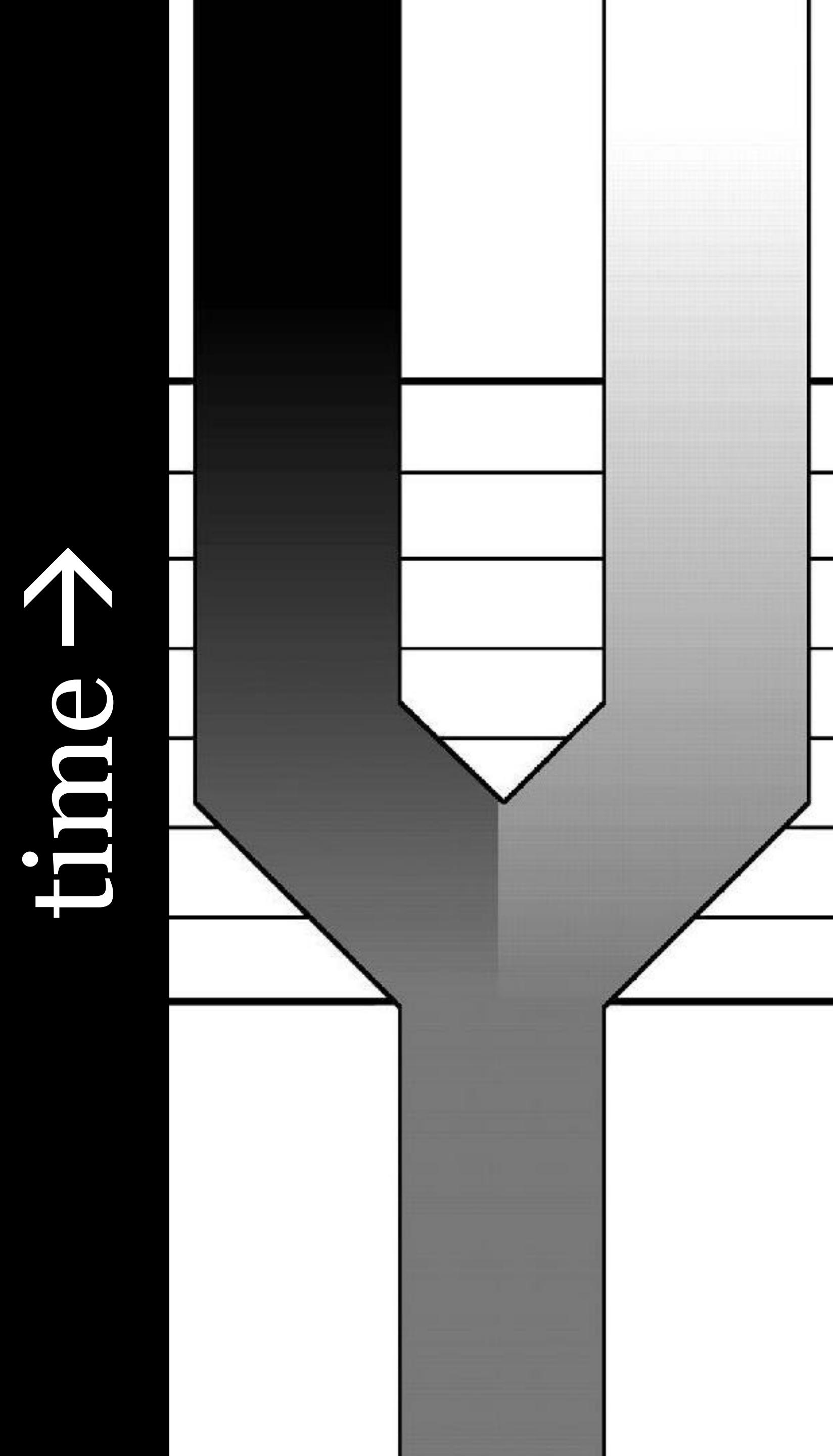


operational  
criteria

8  
7  
6  
5  
4  
3  
2  
1







operational criteria

Biological

Multi-rate PTP

Phylogenetic

Swarm

Morphological

DADA

operational criteria

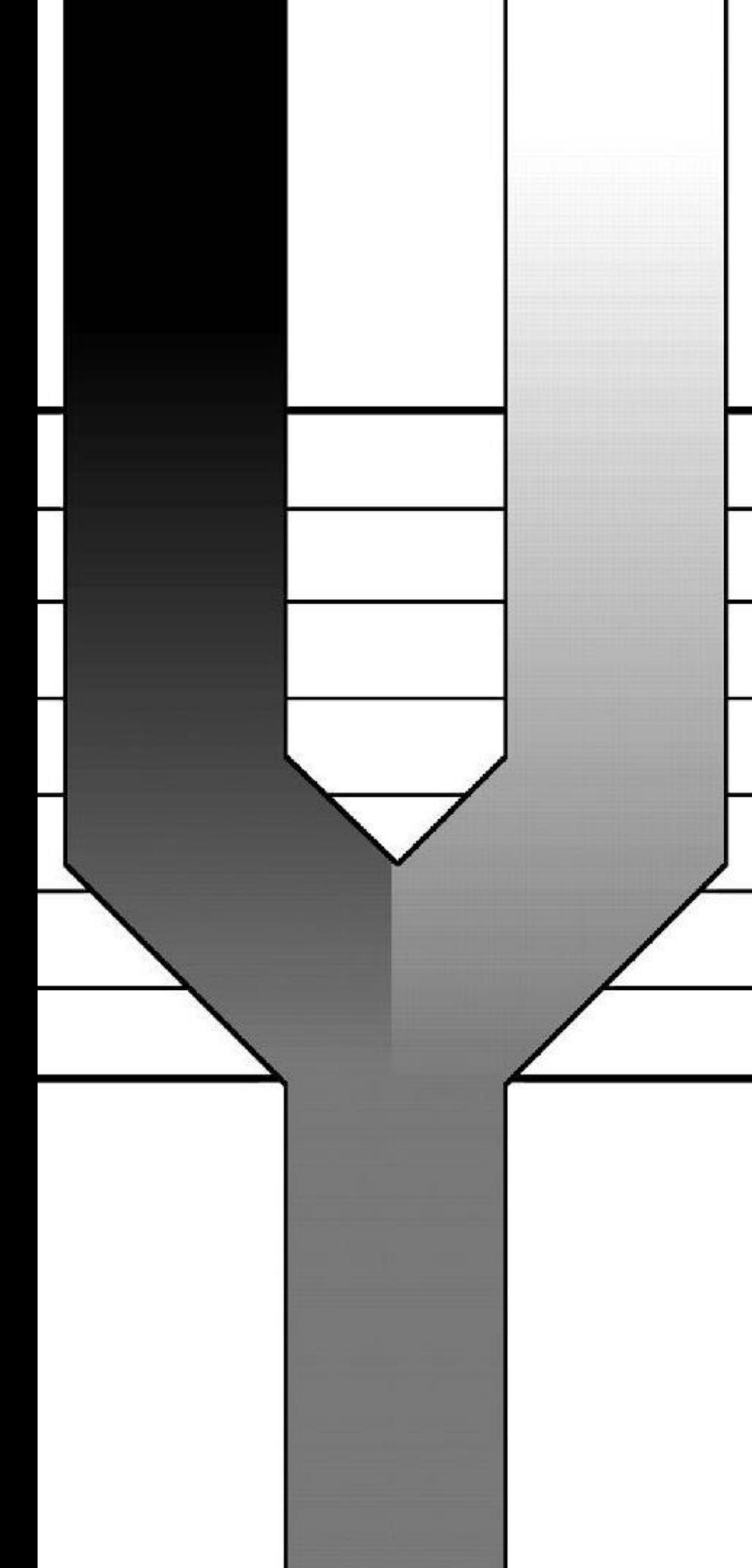
Biological

Multi-rate PTP  
Phylogenetic

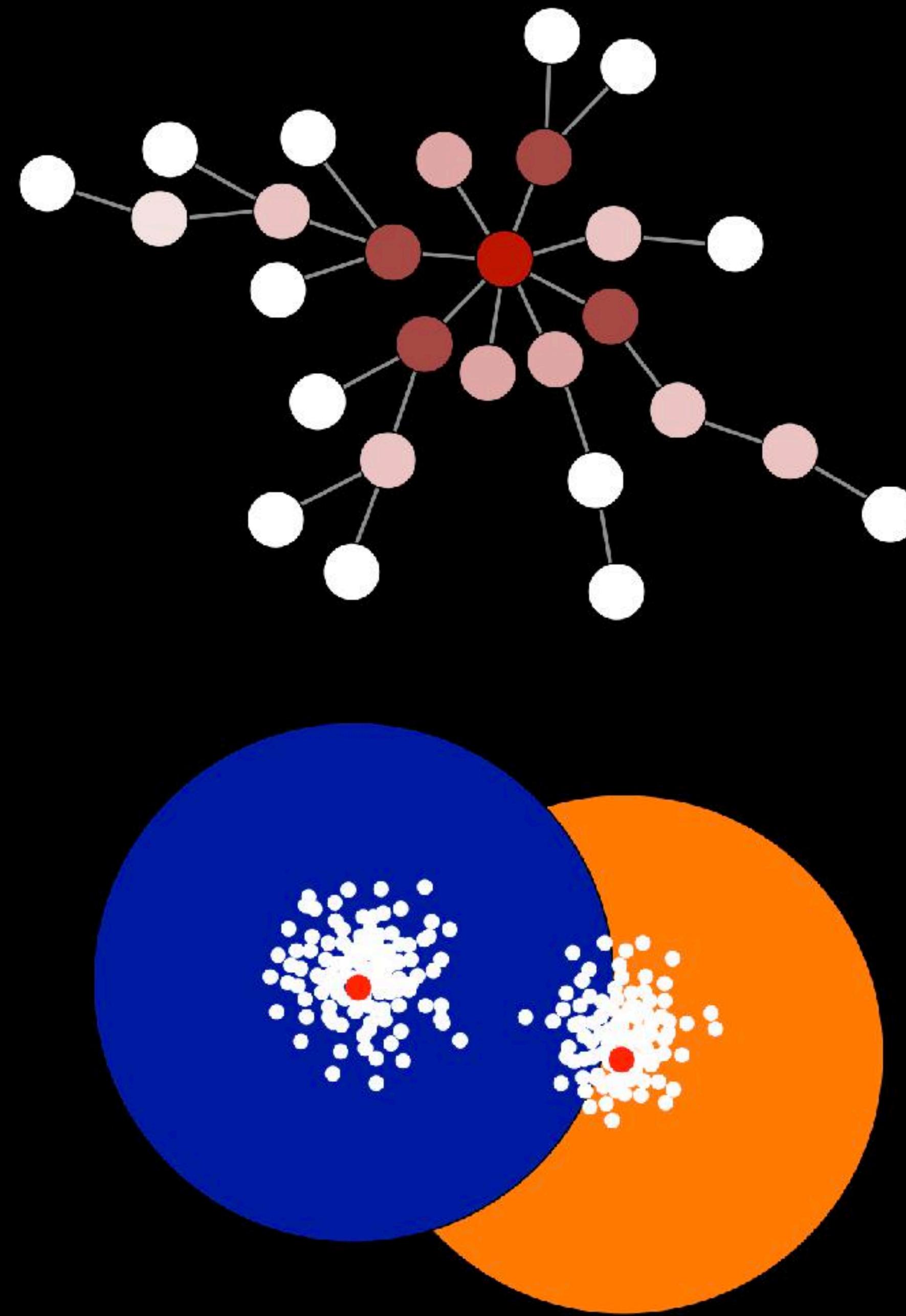
Swarm

Morphological  
DADA

time ↑



...in conclusion



?  
=

separately  
evolving  
metapopulation  
lineages