

Equating OTUs With Species

Micah Dunthorn



Natural History
Museum



OTUS

OTUs $\stackrel{?}{=}$ species

clustering as a step

clustering

= grouping of similar sequences

clustering

sampling,
storage



nucleotide
extraction



amplification,
sequencing

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



amplification,
sequencing

cleaning,
dereplication



clustering



more
cleaning



2017 8:1188

ARTICLE

program: LULU

DOI: 10.1038/s41467-017-01312-x

OPEN

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

Tobias Guldberg Frøslev ^{1,2}, Rasmus Kjøller¹, Hans Henrik Bruun ¹, Rasmus Ejrnæs³, Ane Kirstine Brunbjerg³, Carlotta Pietroni² & Anders Johannes Hansen²

sampling,
storage



nucleotide
extraction



amplification,
sequencing

cleaning,
dereplication



clustering



more
cleaning

taxonomic assignments,
phylogenetic placements



α, β diversities,
co-occurrences

what is a species

1997 in "Species: The units of biodiversity"

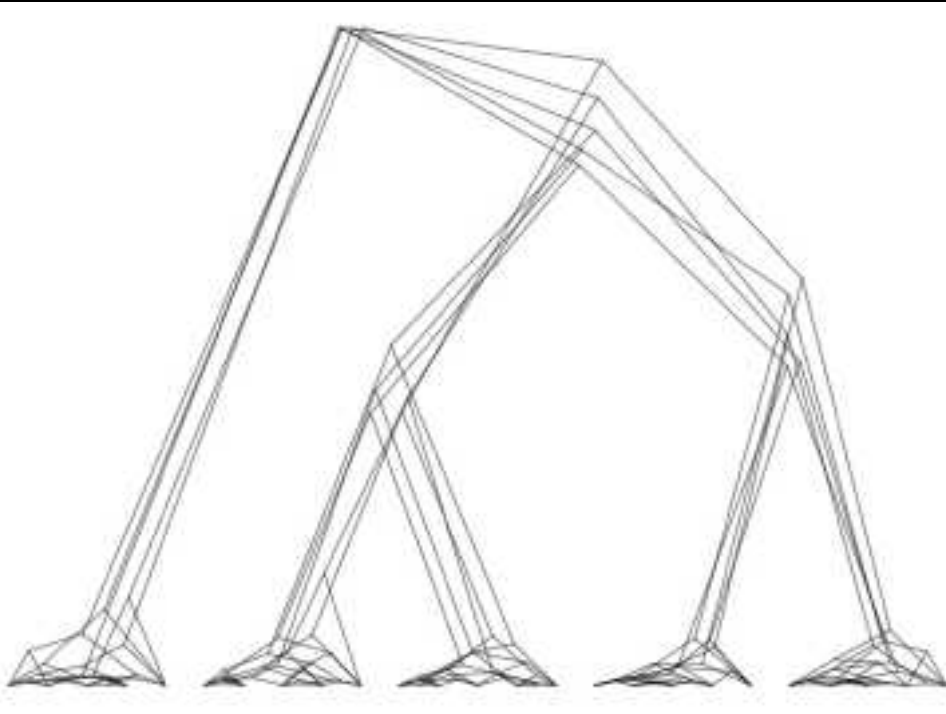
A hierarchy of species concepts: the denouement in the saga of the species problem

R. L. Mayden

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

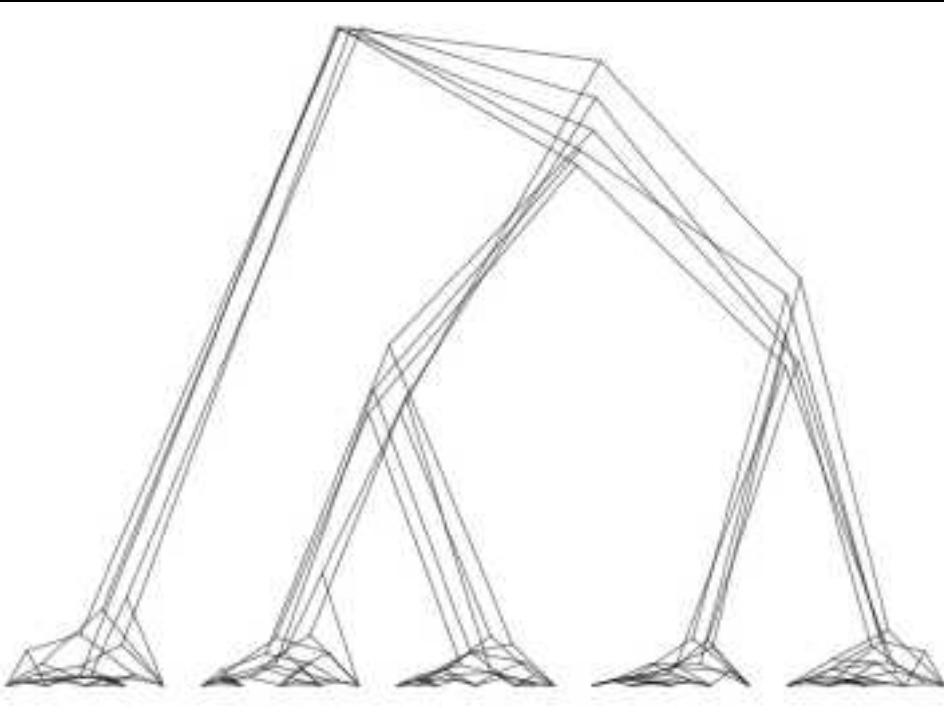
**eukaryote
vs.
bacteria and archaea**

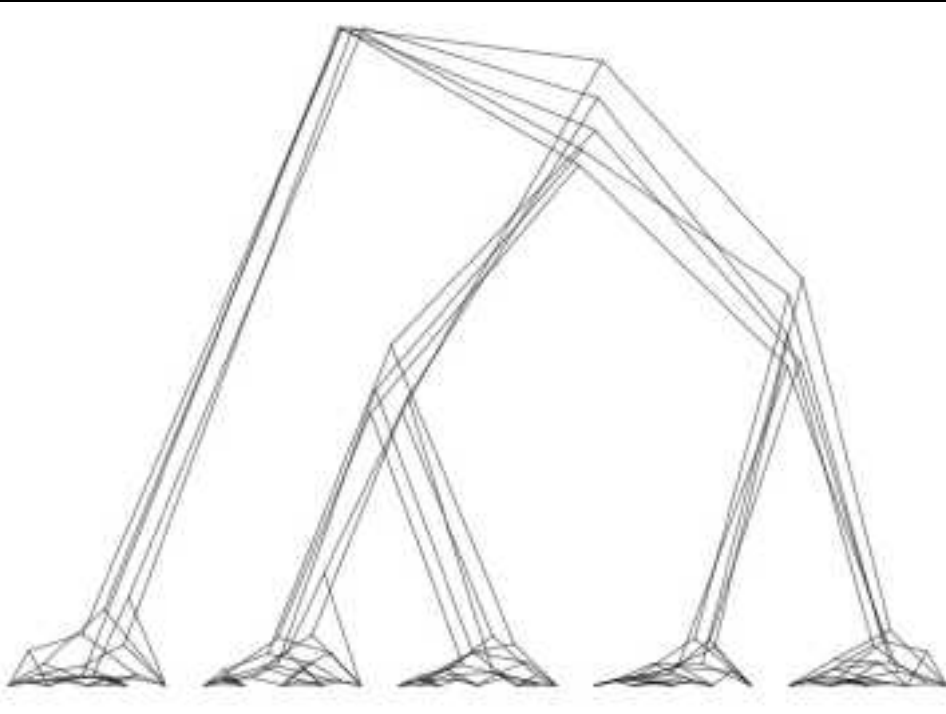
**sexual
vs.
asexual**



Barraclough (2019) The Evolutionary Biology of Species

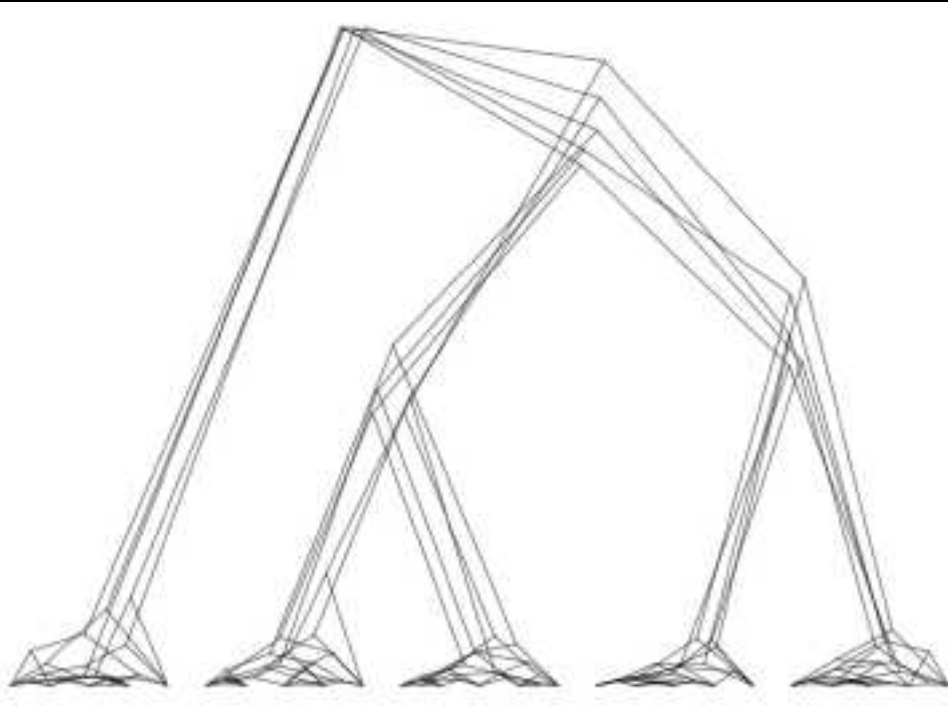
macro-evolutionary processes of phylogeny



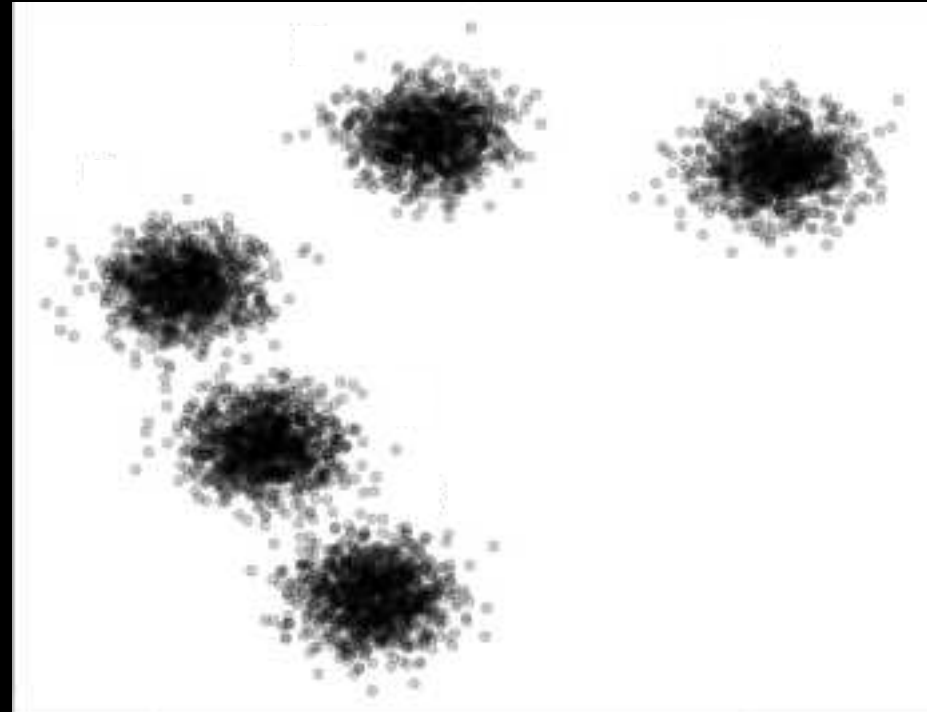


**macro-evolutionary
processes of phylogeny**

**micro-evolutionary
processes of population
genetics**



trait 2



trait 1

biological species

= actually or potentially inter-breeding natural populations, which are reproductively isolated

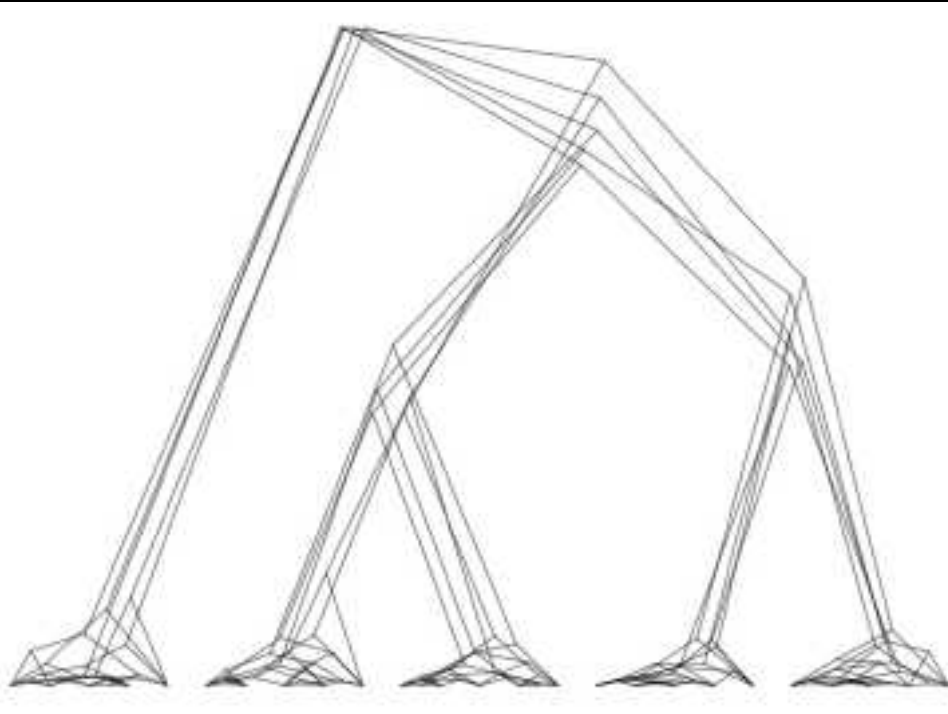
- Mayr 1942



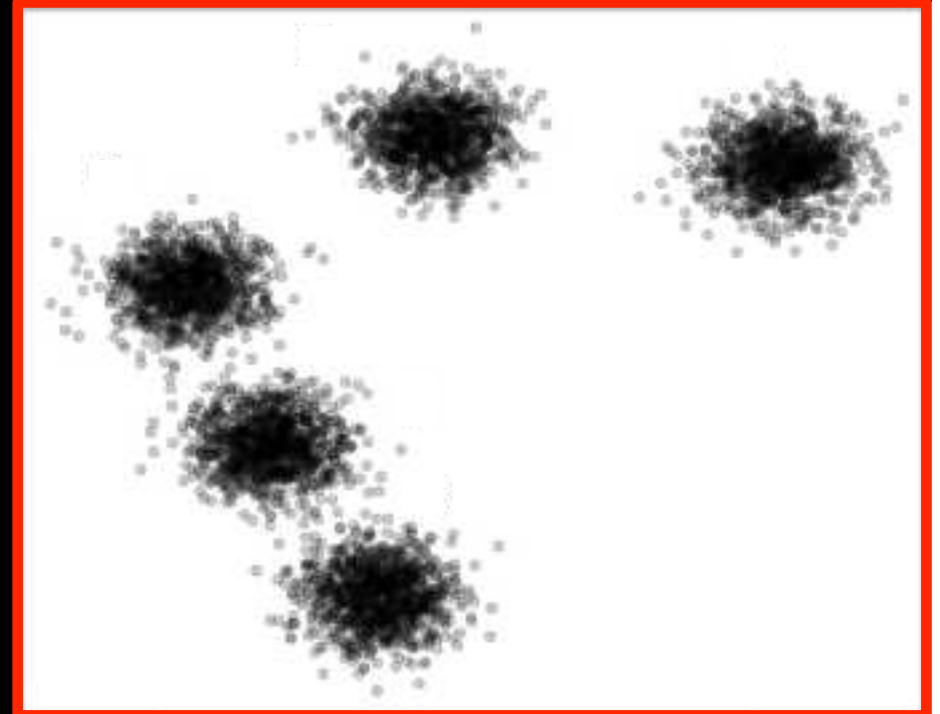
ciliate sex



Symbiodinium



trait 2



trait 1

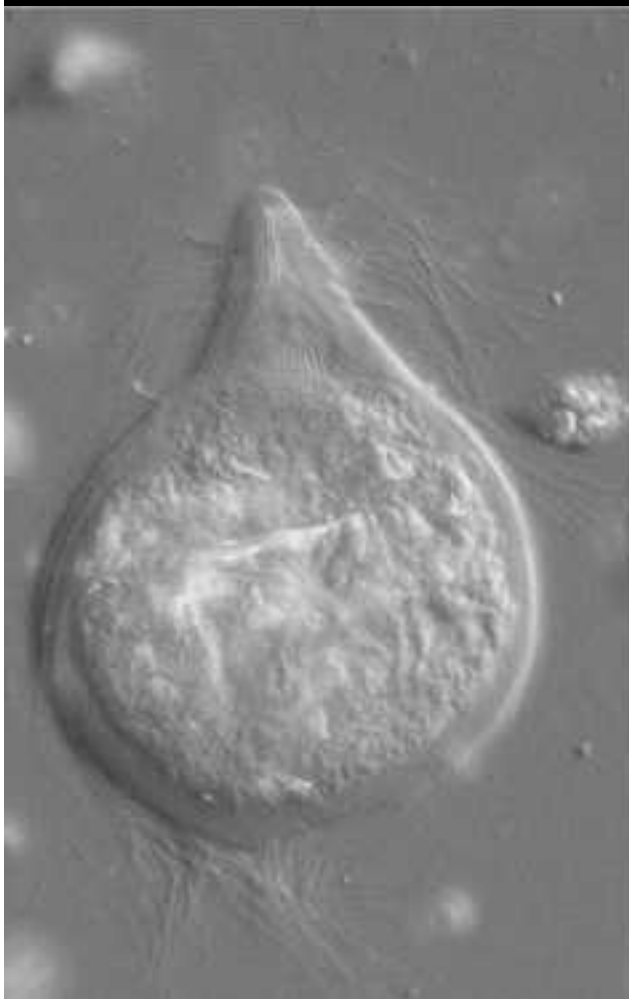
morphological species

**= the smallest groups that are
consistently and persistently distinct**

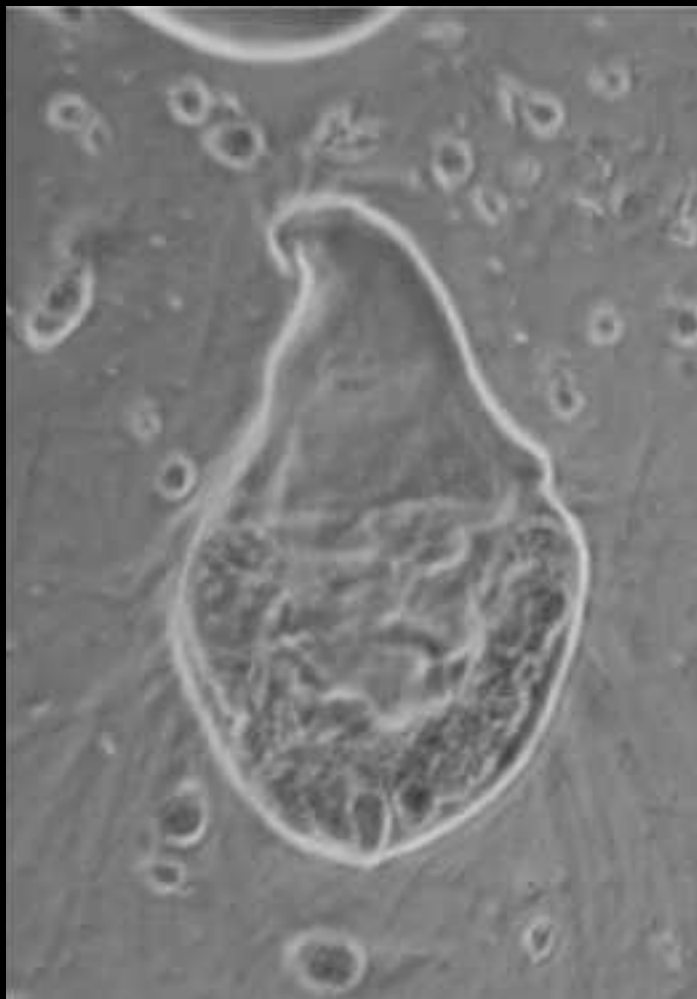
- Cronquist 1978



ciliates



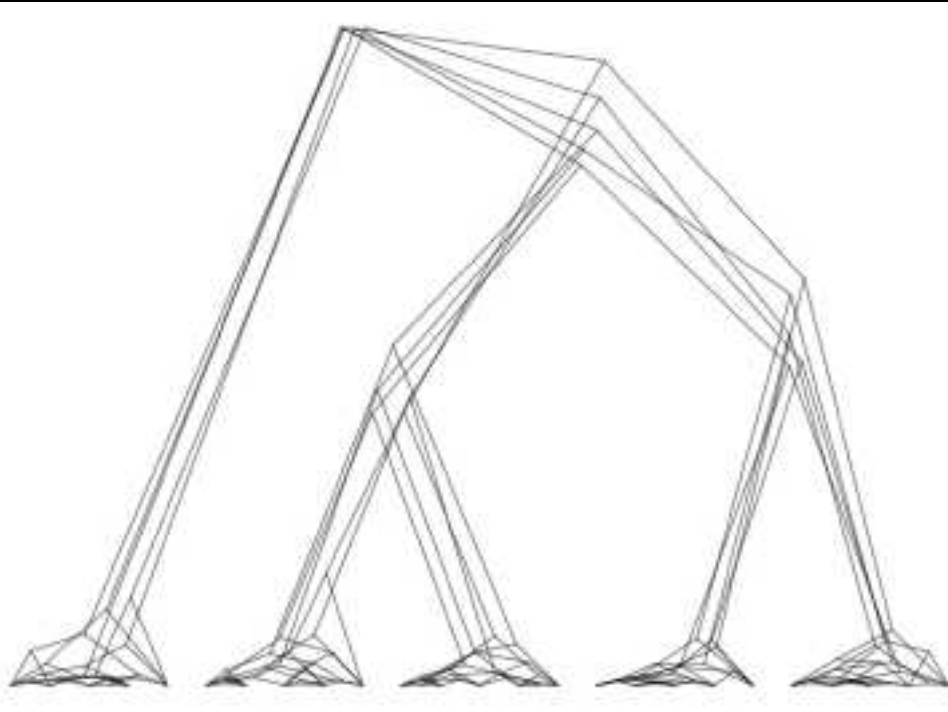
Trichonympha hueyi



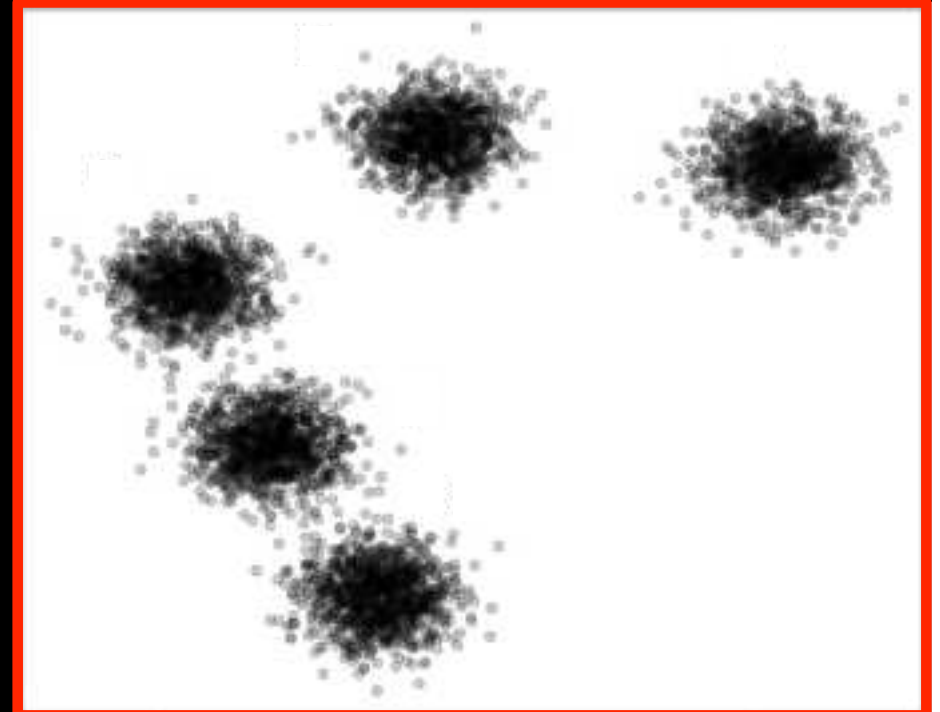
Trichonympha deweyi



Trichonympha louiei



trait 2



trait 1

phylogenetic species

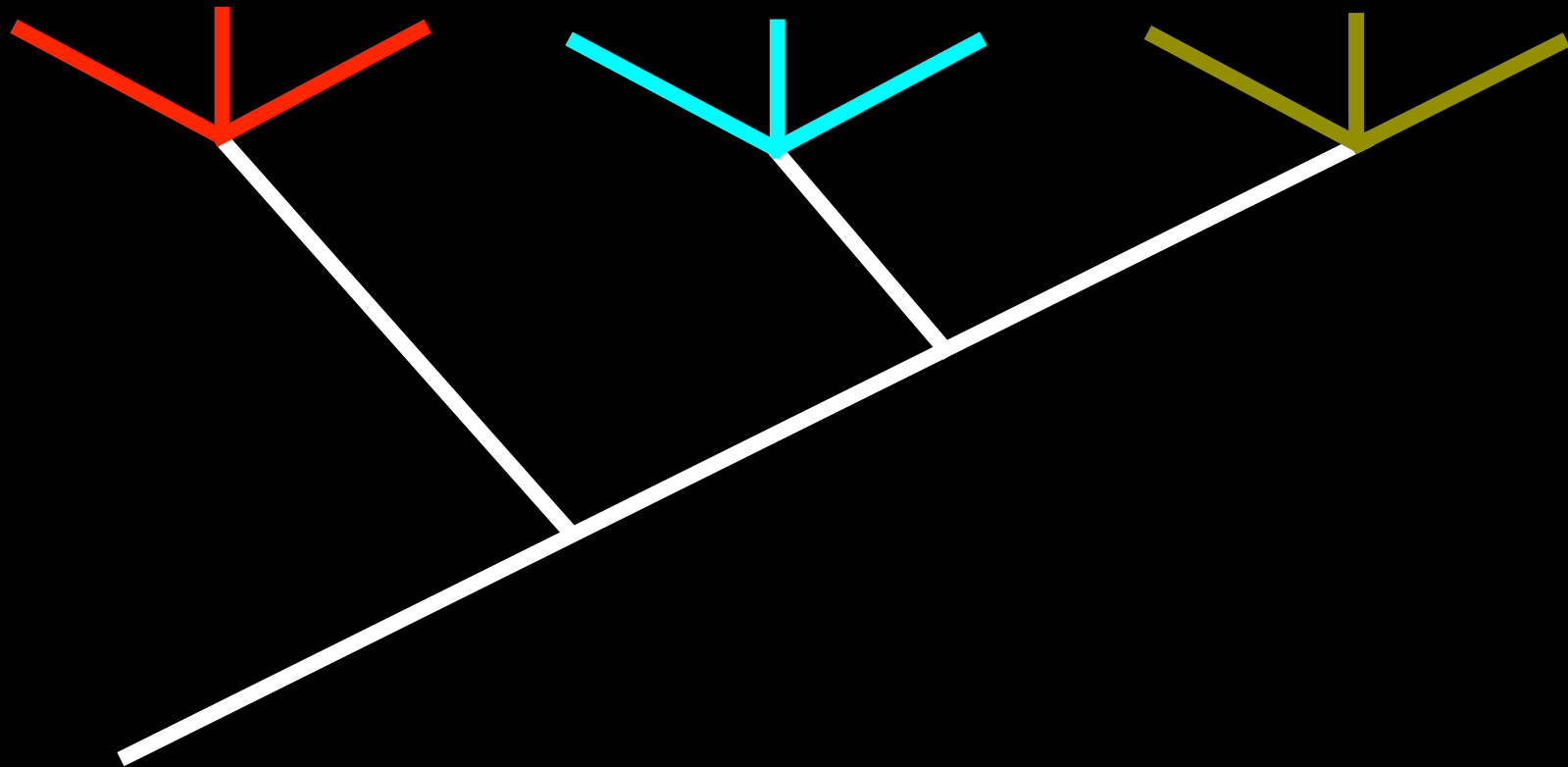
**= the smallest biological entities that
are diagnosable and/or monophyletic**

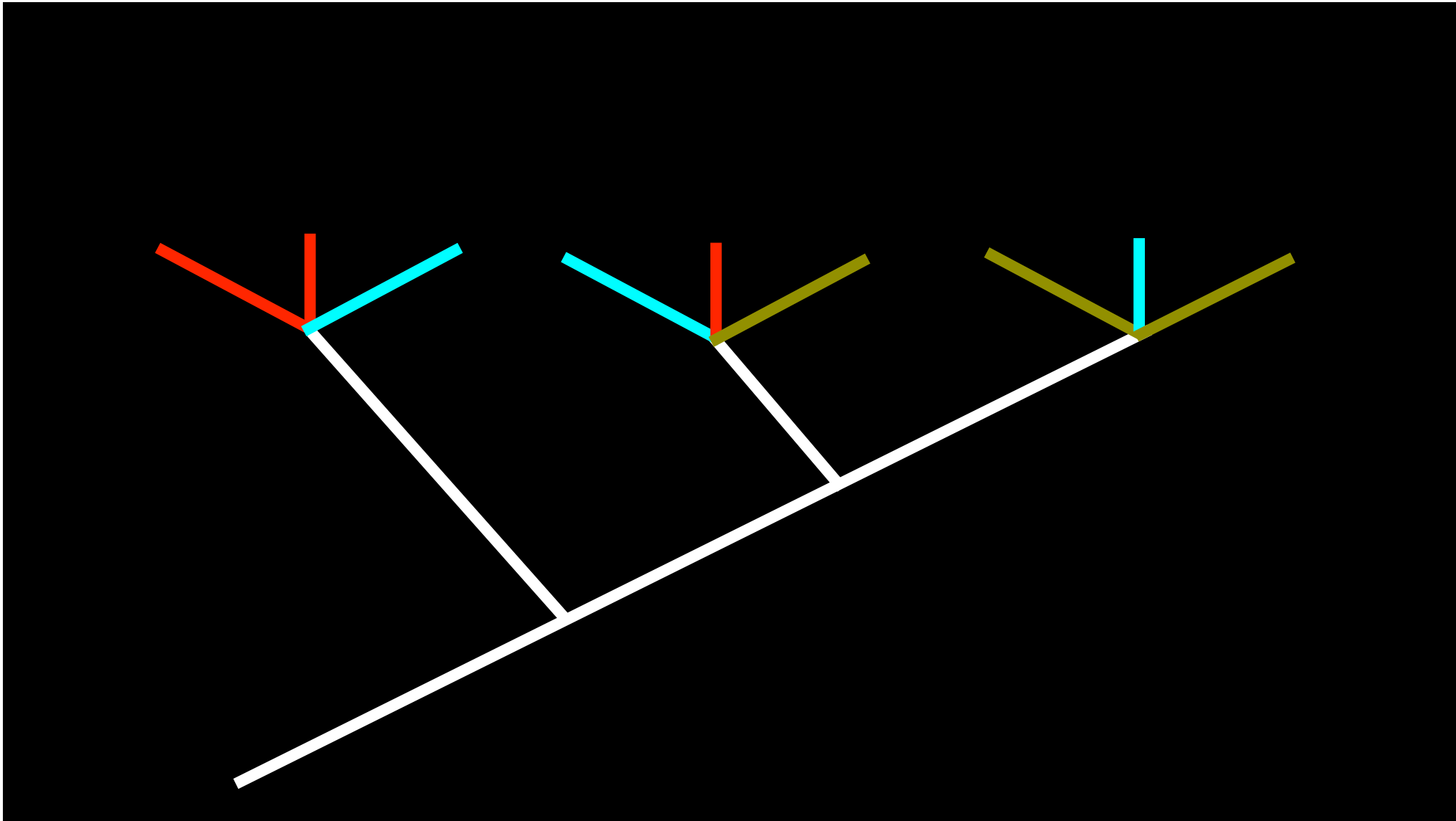
- Mayden 1997

species 1

species 2

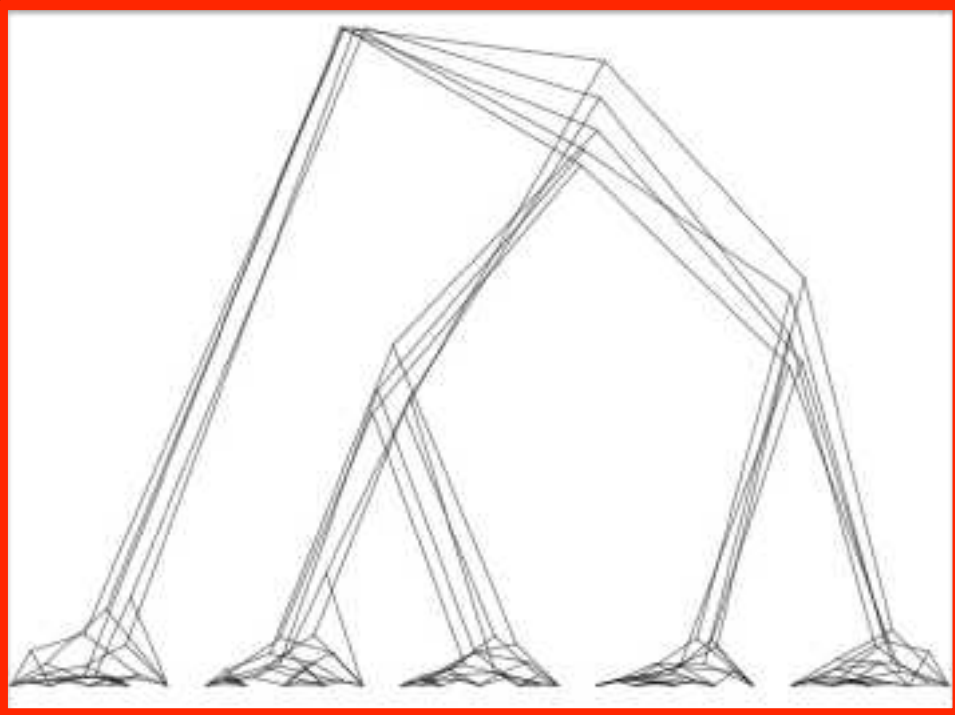
species 3



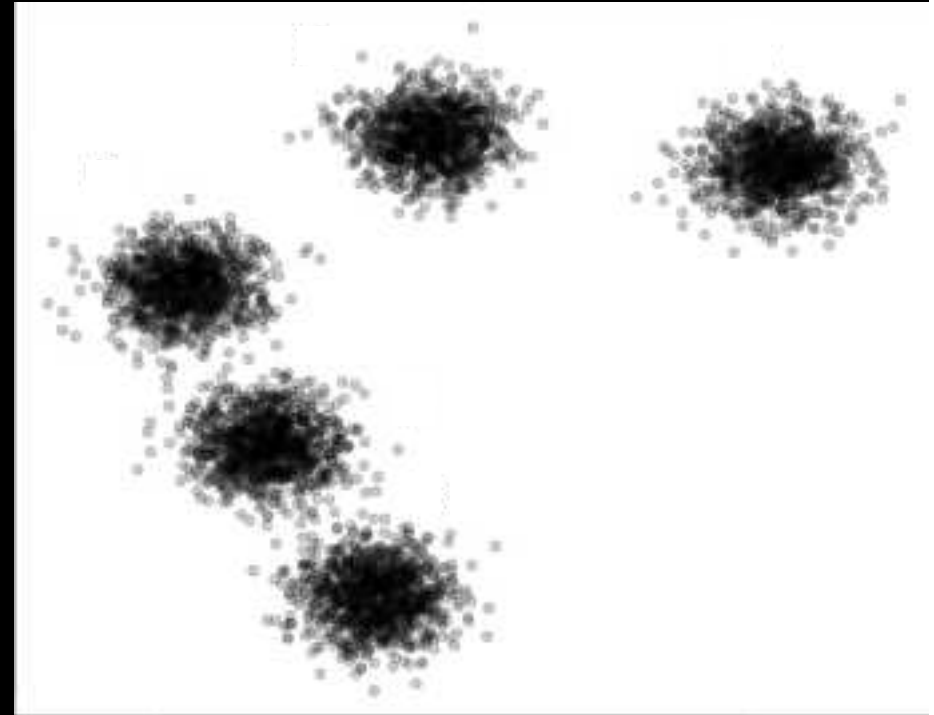




Paramecium



trait 2



trait 1

Syst. Biol. 56(6):879–886, 2007

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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

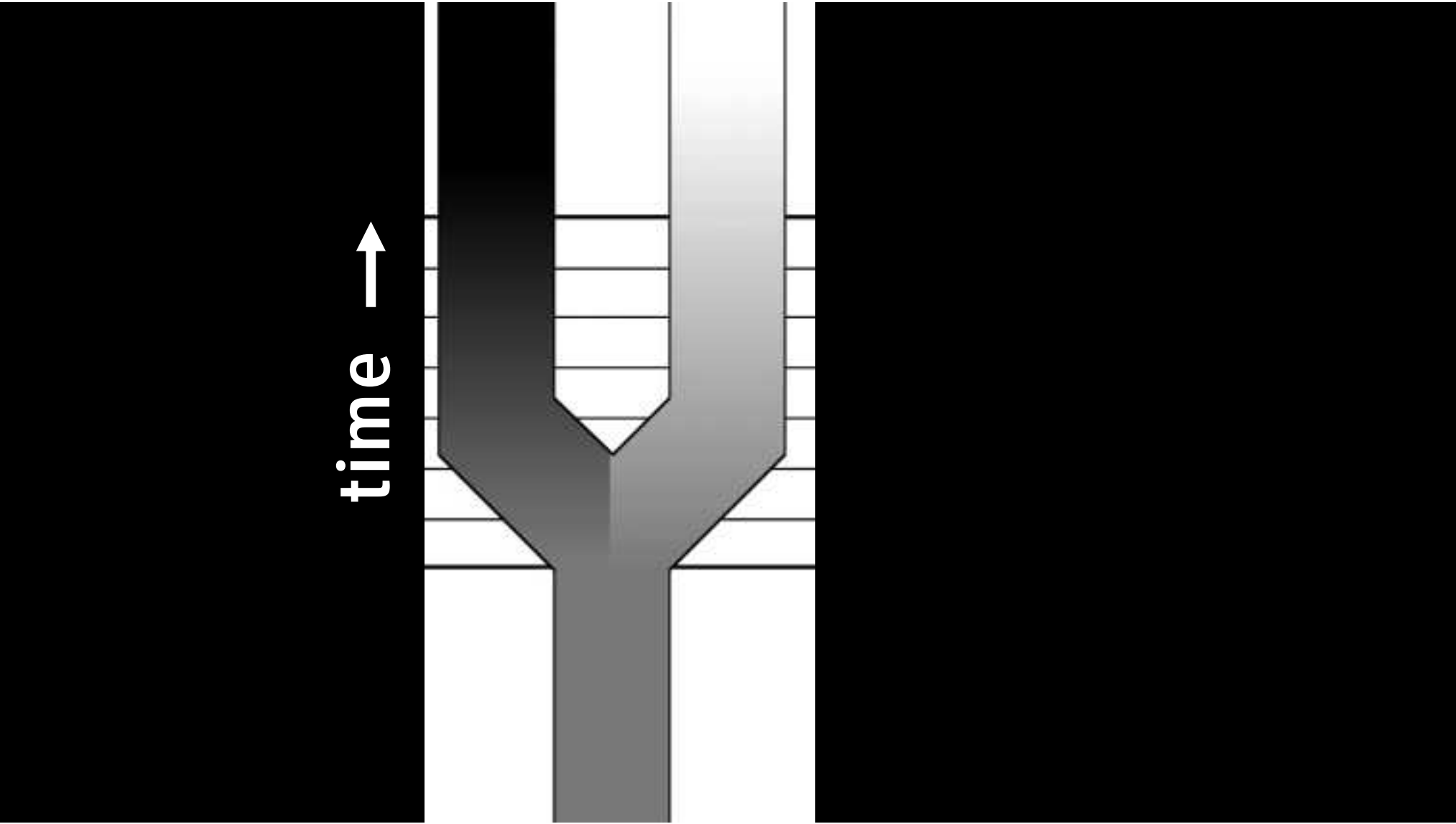
= separately evolving metapopulation
lineages

concept of a species

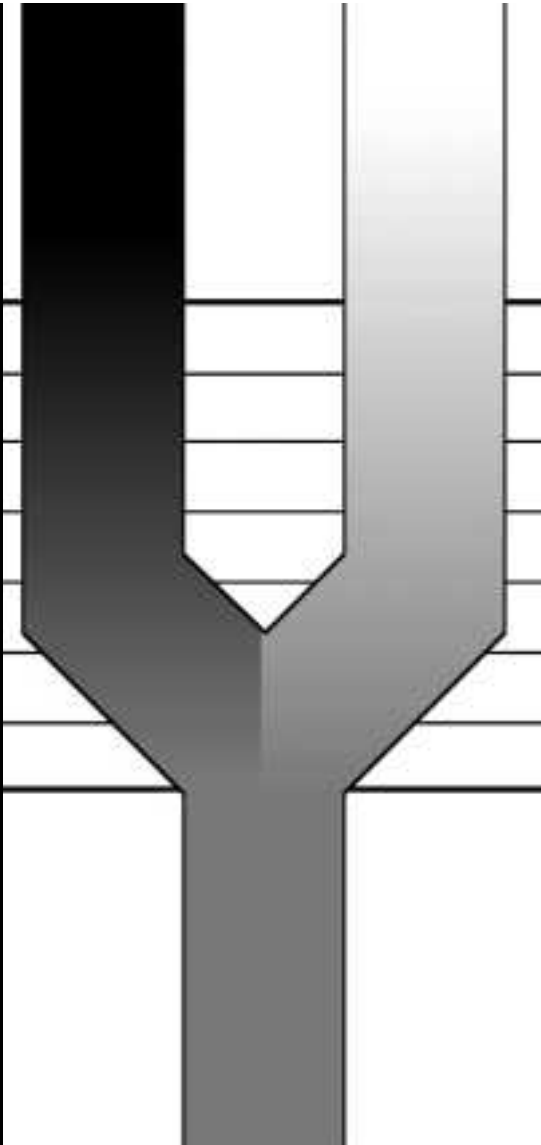
= separately evolving metapopulation lineages

operational criteria for delimitation

= Biological Species, Morphological Species, Phylogenetic Species, etc.



time ↑



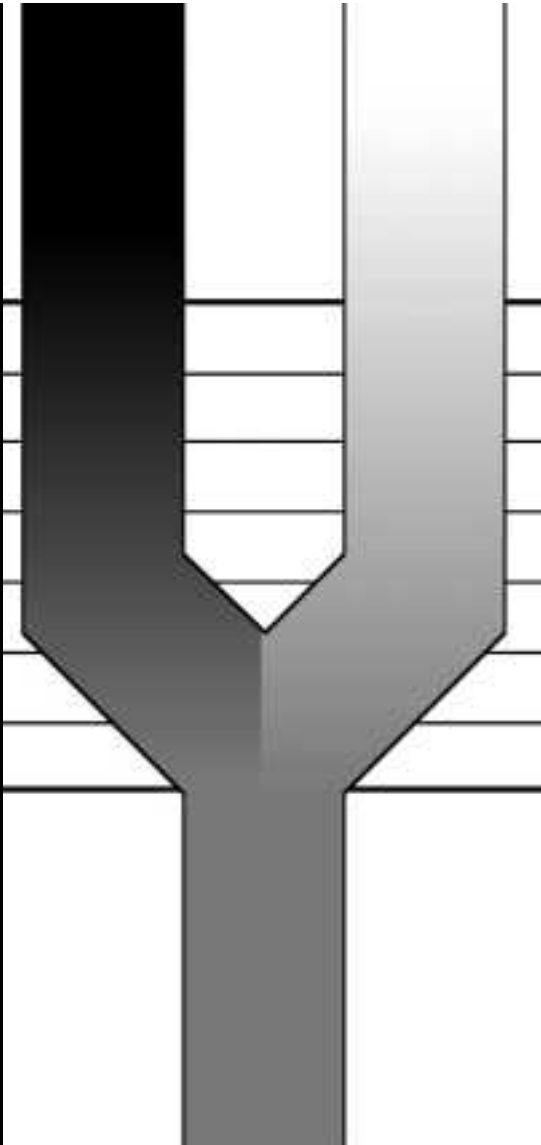
operational criteria

Phylogenetic

Morphological

Biological

time ↑



operational criteria

Morphological

Biological

Phylogenetic

clustering methods

just clustering
vs.
clustering and cleaning

**de novo clustering
vs.
closed-reference clustering**

**metabarcoding data
vs.
metagenomic/
metatranscriptomic**

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**

**VSEARCH/
Mothur**

DADA2

**local clustering
thresholds**

Swarm

**multi-rate
PTP**

**pairwise
comparisons**

**phylogenetic
comparisons**

**global clustering
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**VSEARCH/
Mothur**



DADA2

**local clustering
thresholds**

Swarm

**multi-rate
PTP**

2016 4:e2584

PeerJ

VSEARCH: a versatile open source tool for metagenomics

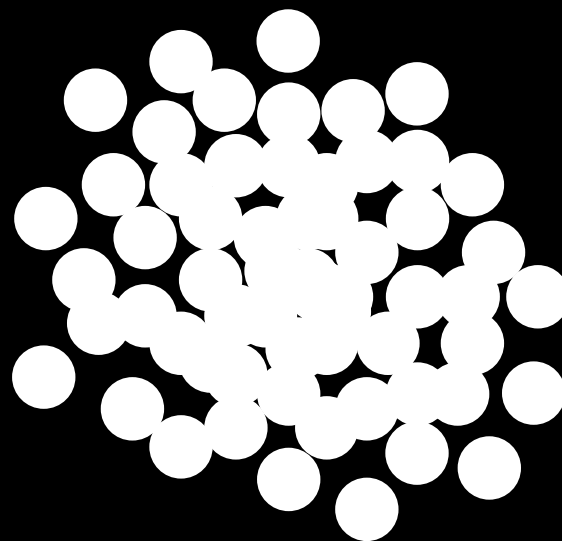
Torbjørn Rognes^{1,2}, Tomáš Flouri^{3,4}, Ben Nichols⁵, Christopher Quince^{5,6} and Frédéric Mahé^{7,8}

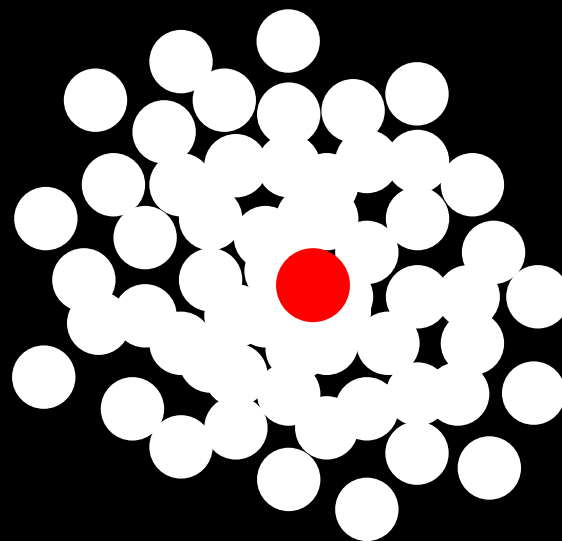
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2009, p. 7537–7541
0099-2240/09/\$12.00 doi:10.1128/AEM.01541-09
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

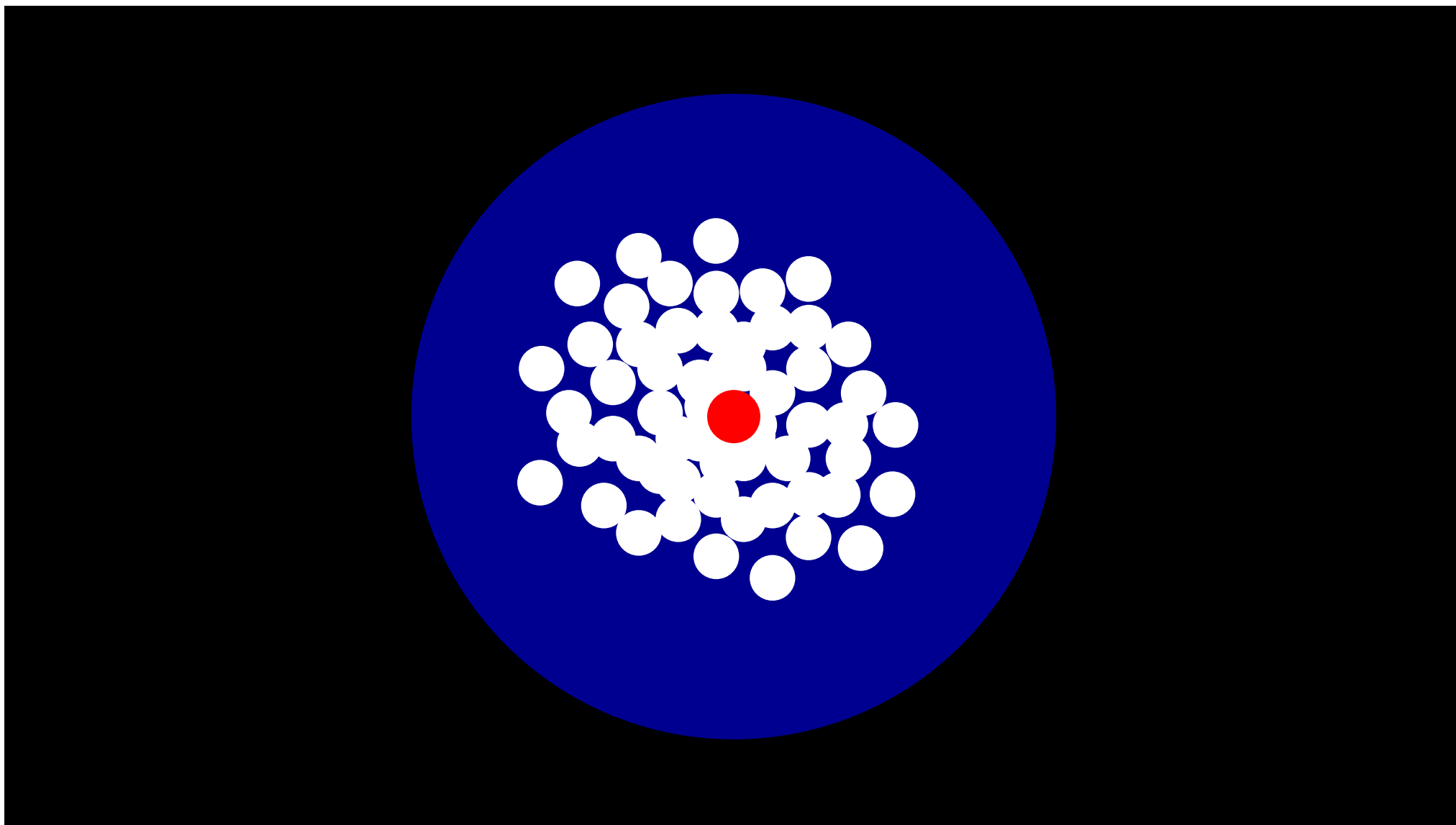
Vol. 75, No. 23

Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities[▽]

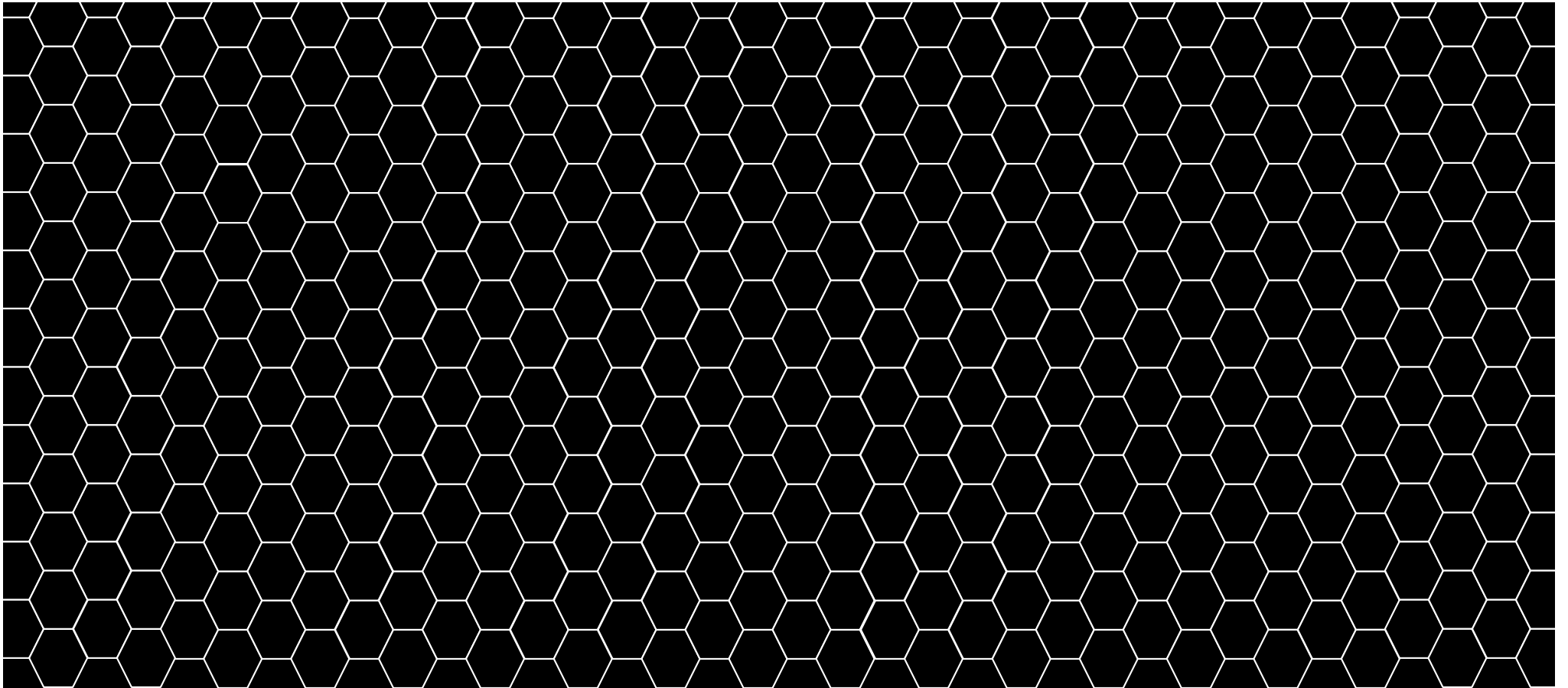
Patrick D. Schloss,^{1,2*} Sarah L. Westcott,^{1,2} Thomas Ryabin,¹ Justine R. Hall,³ Martin Hartmann,⁴ Emily B. Hollister,⁵ Ryan A. Lesniewski,⁶ Brian B. Oakley,⁷ Donovan H. Parks,⁸ Courtney J. Robinson,² Jason W. Sahl,⁹ Blaz Stres,¹⁰ Gerhard G. Thallinger,¹¹ David J. Van Horn,² and Carolyn F. Weber¹²

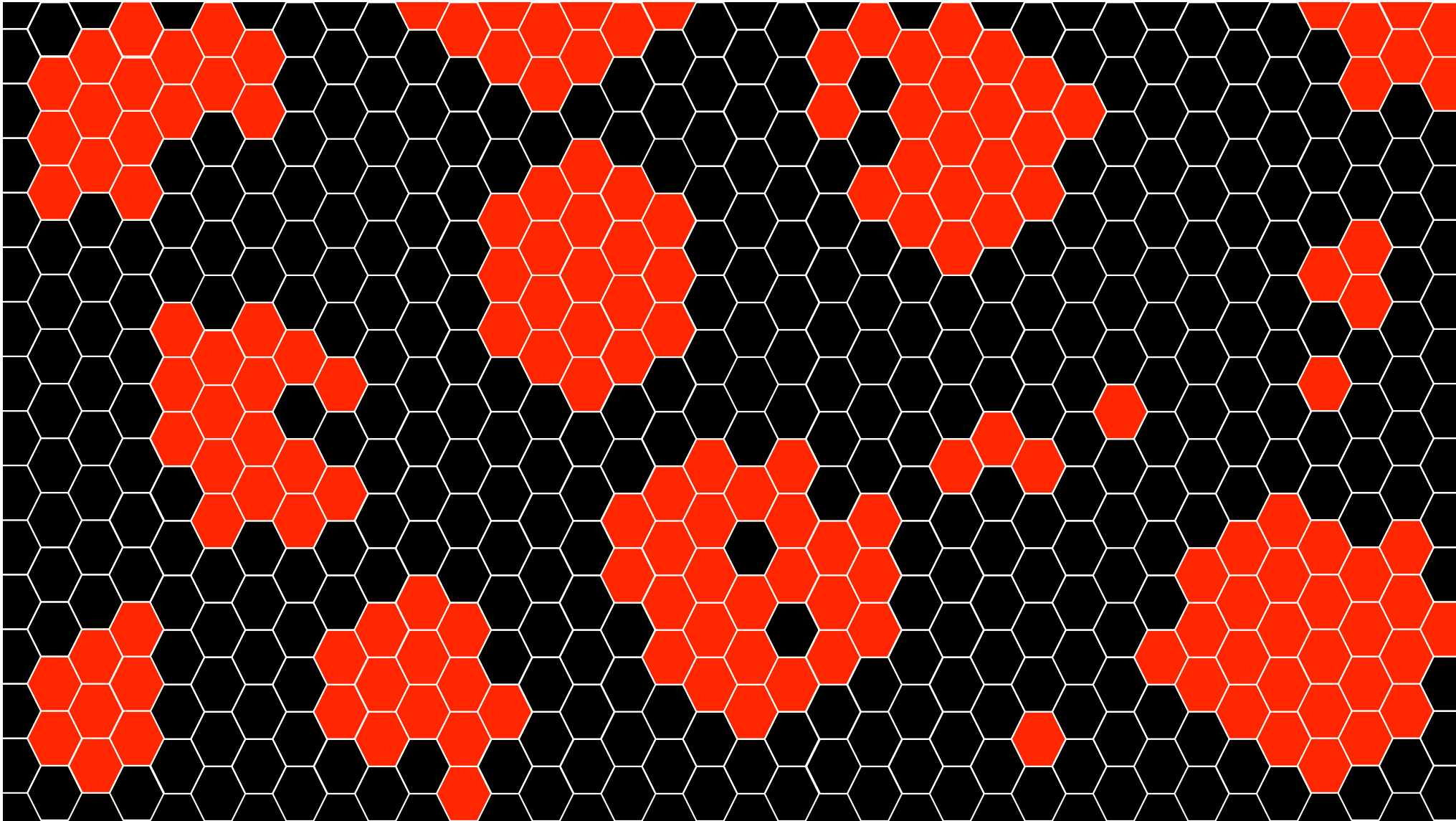


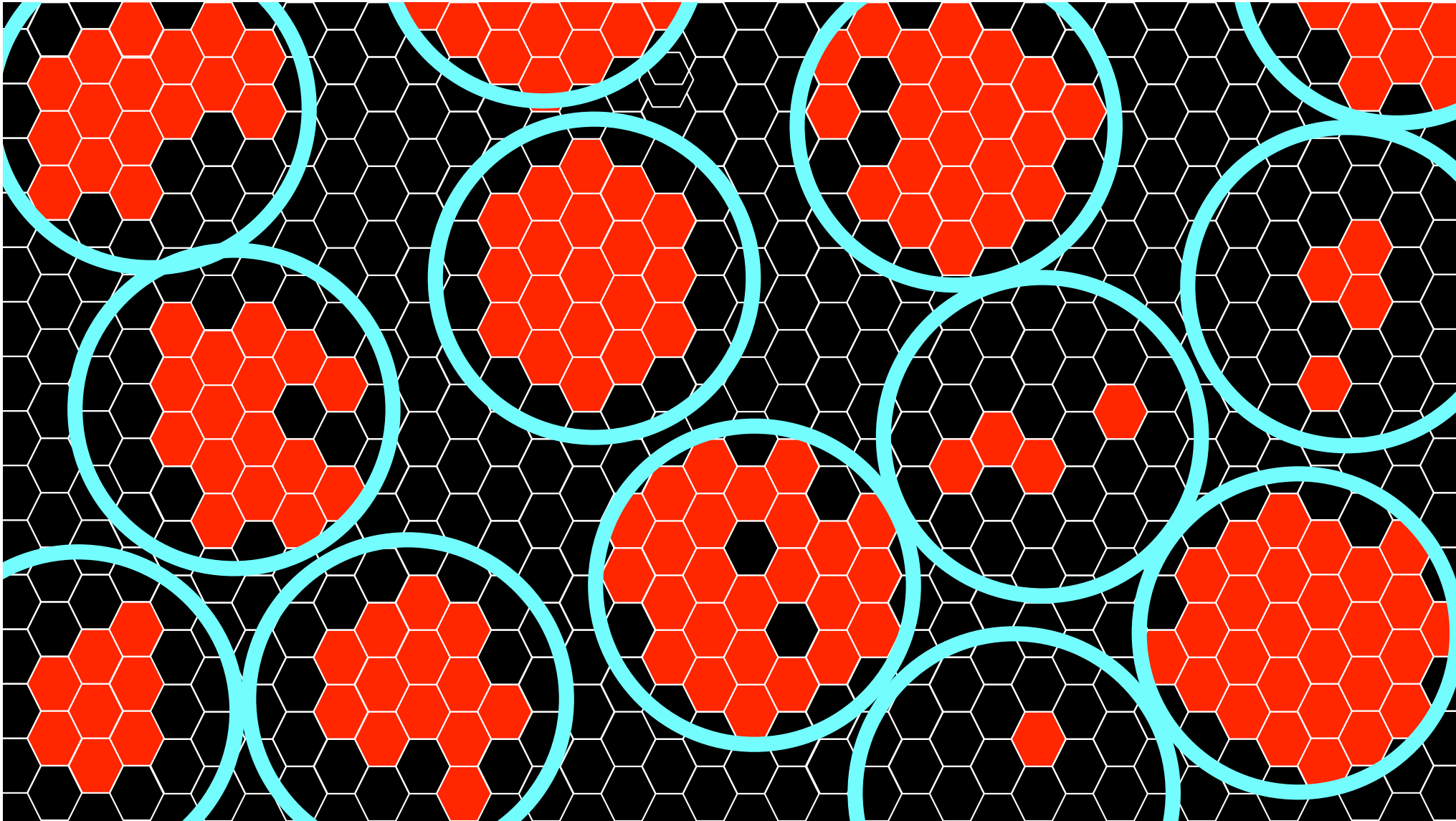


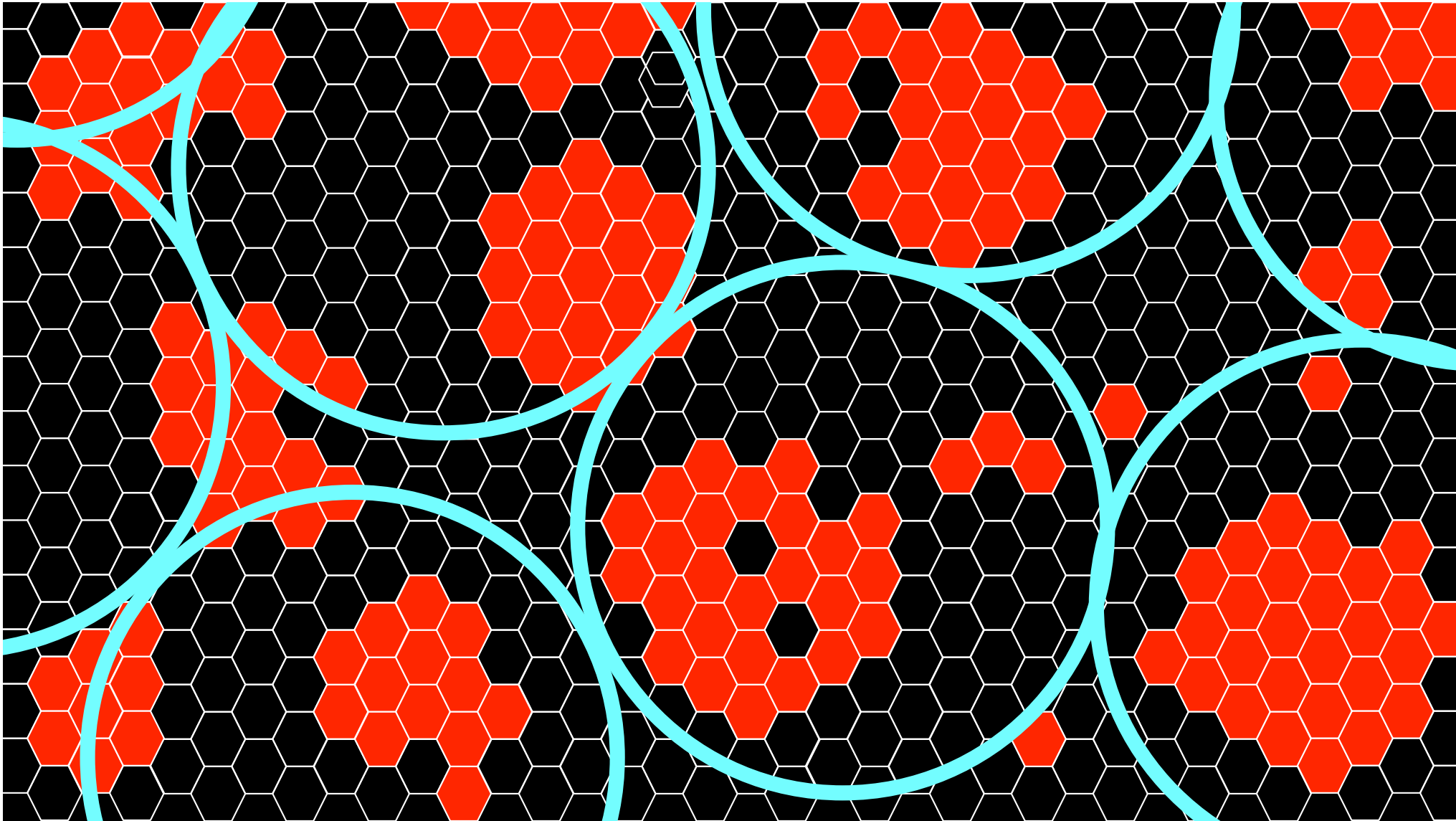


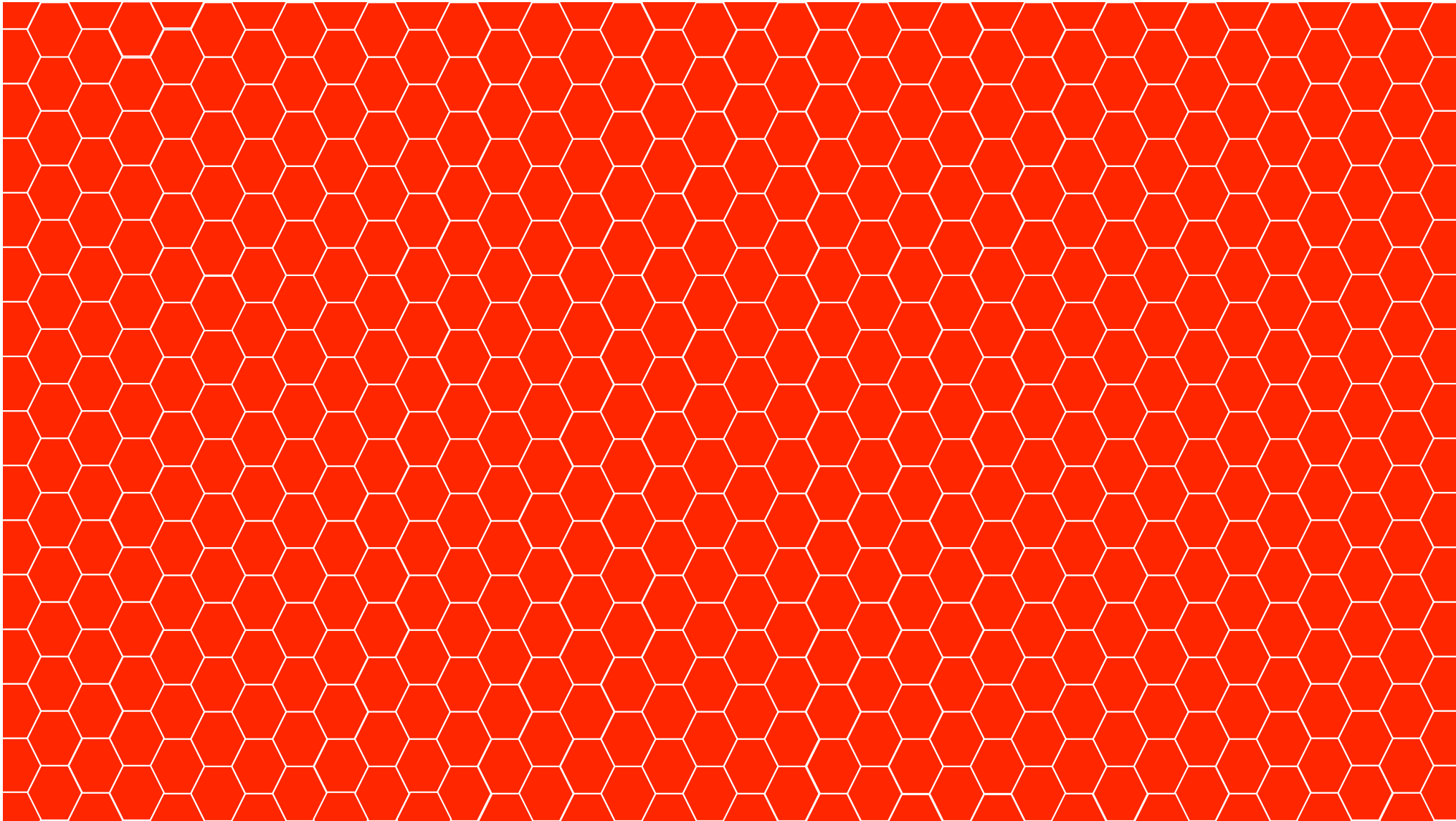
**each hexagon is a unique sequence,
neighboring hexagons differ by one base pair**

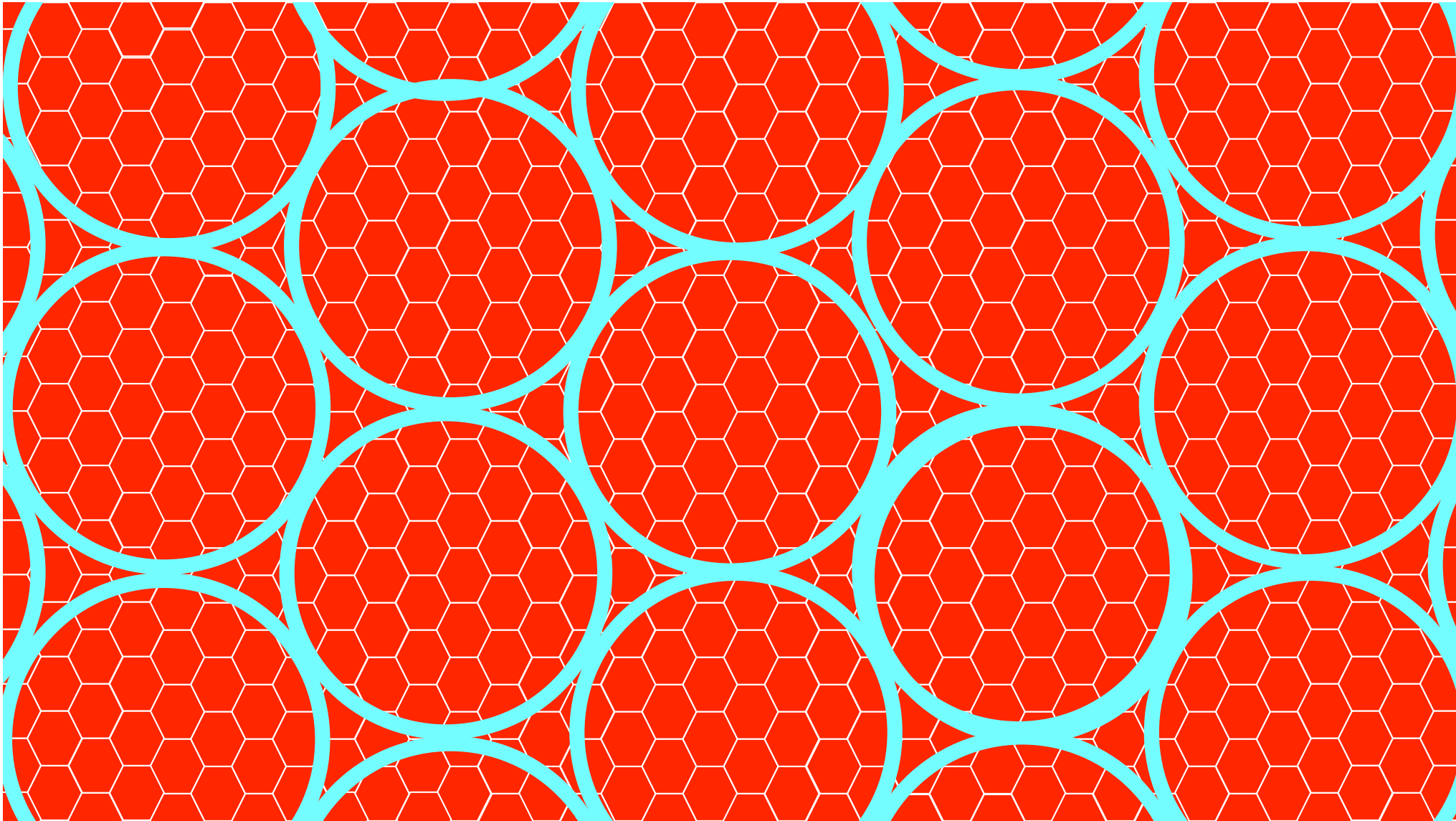


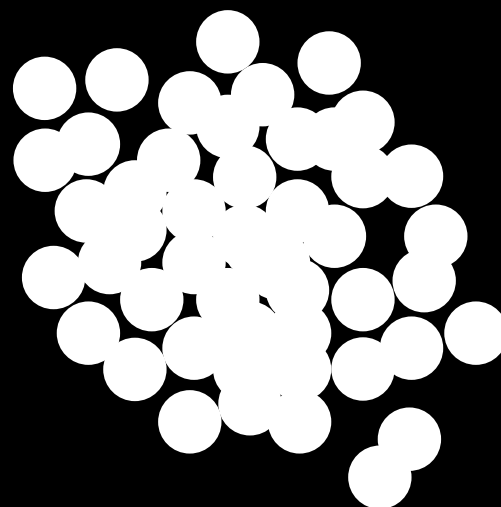
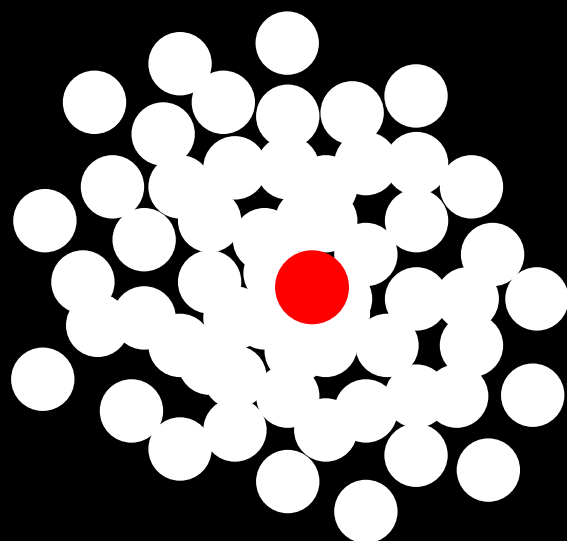


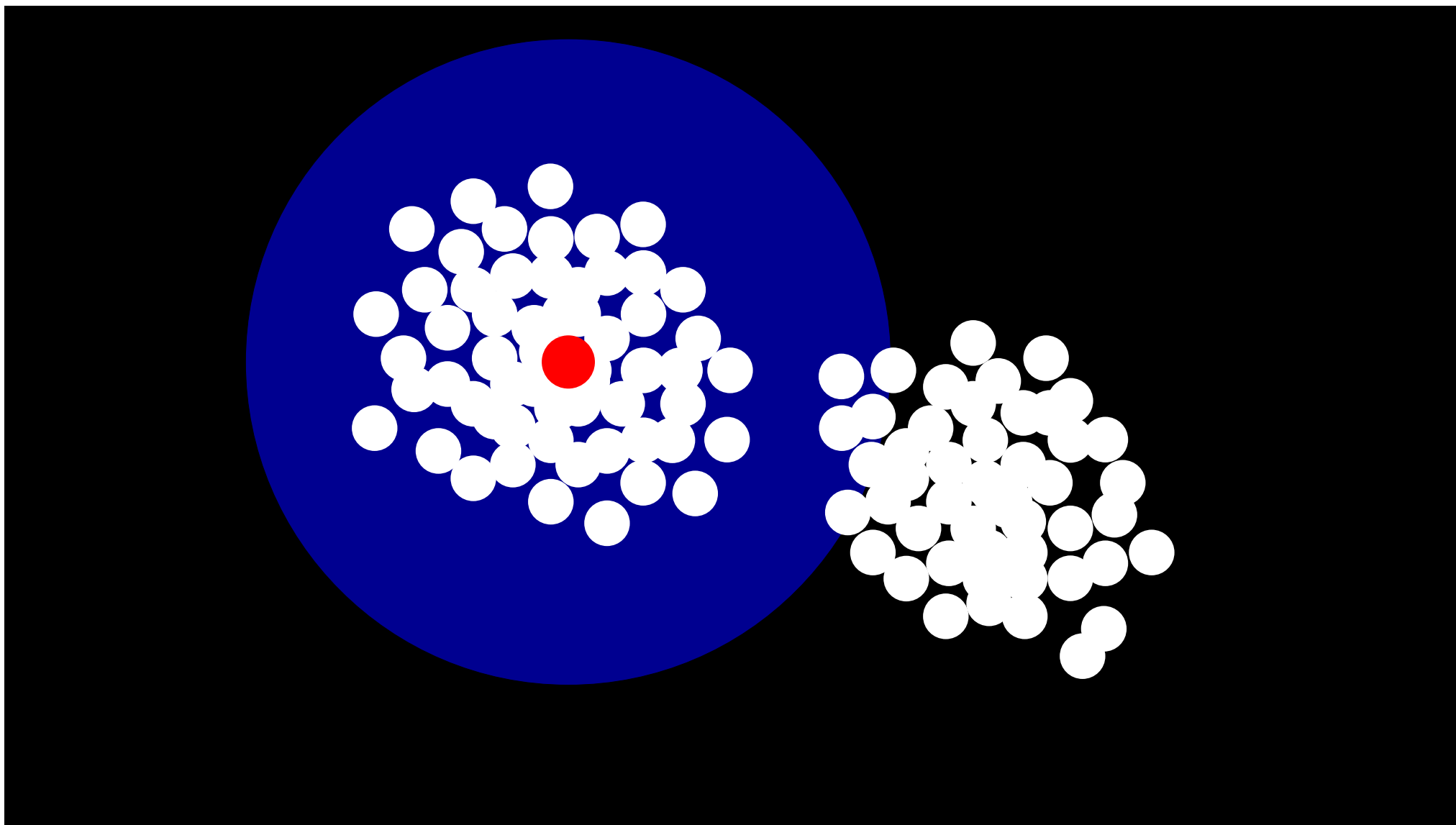


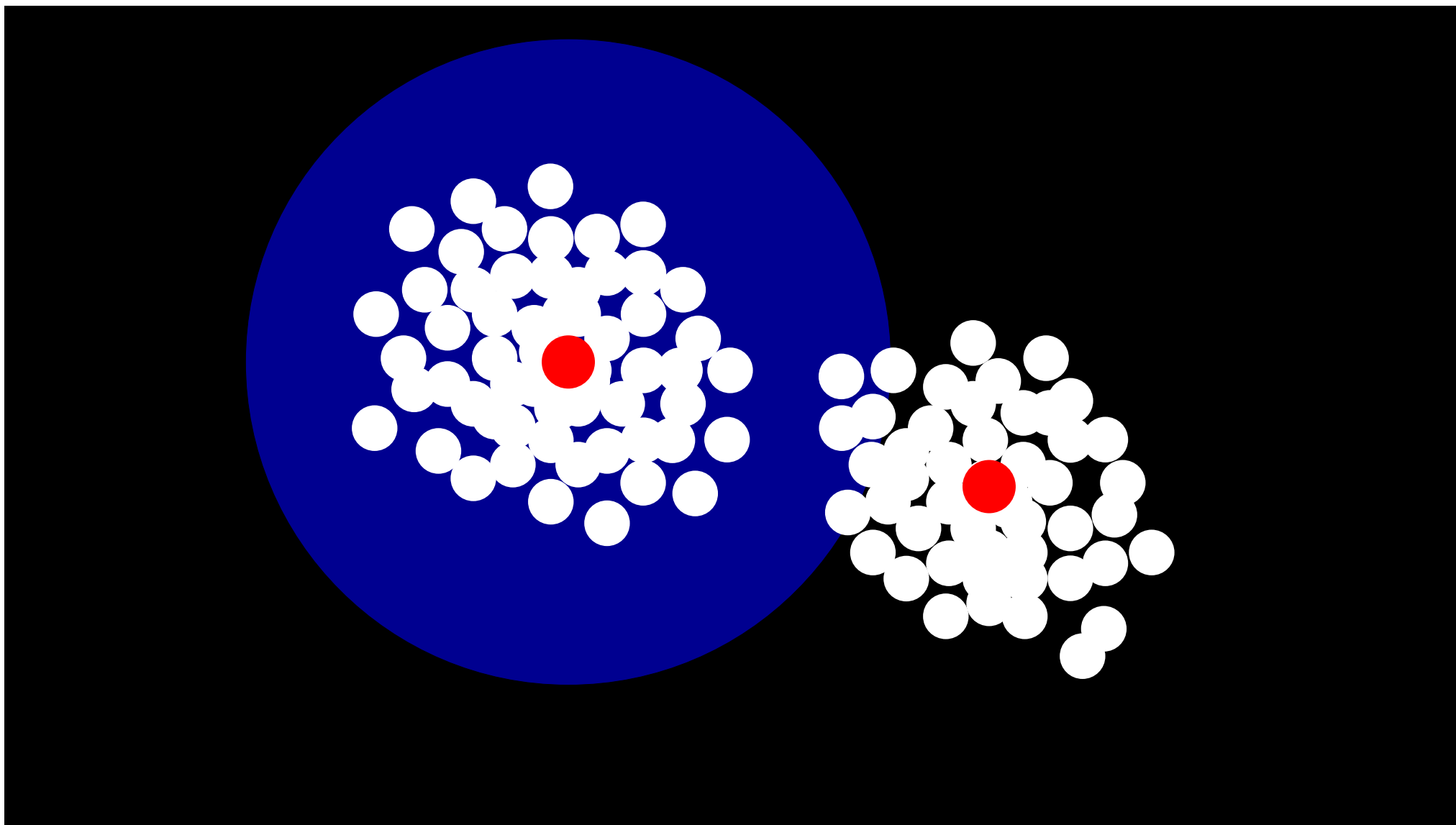


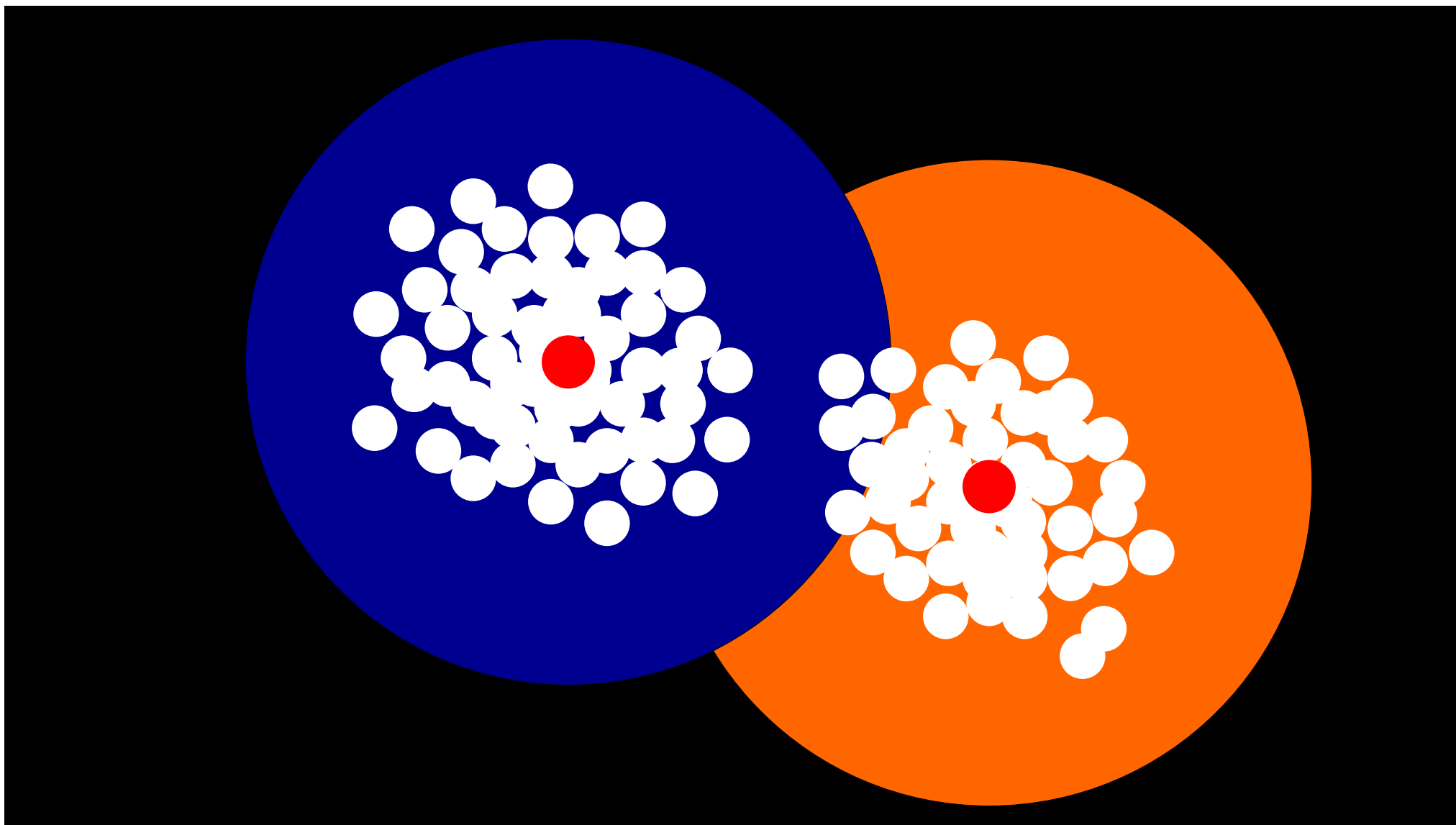


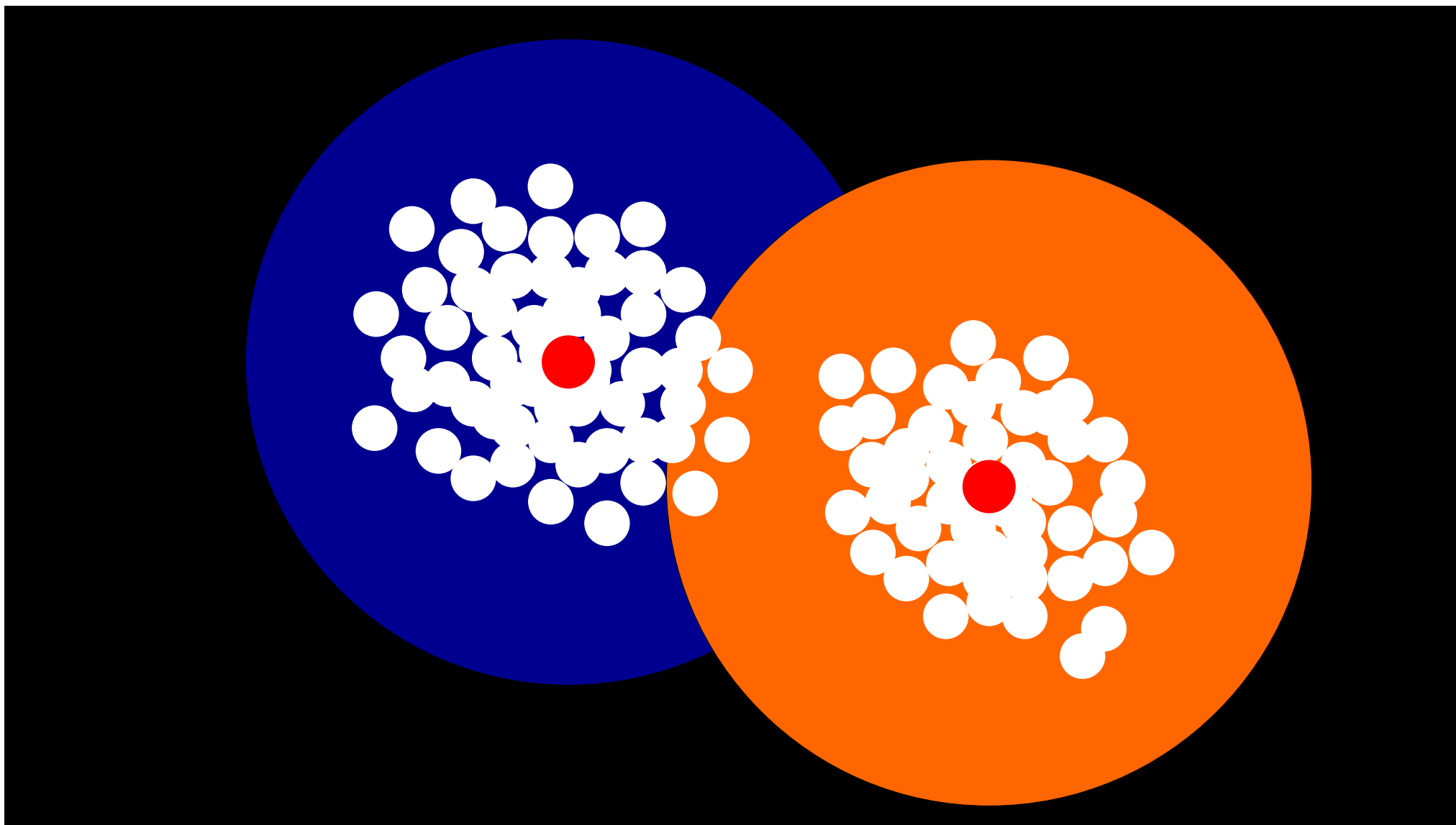












97%



Ian Marshall


@ianpgm.bsky.social

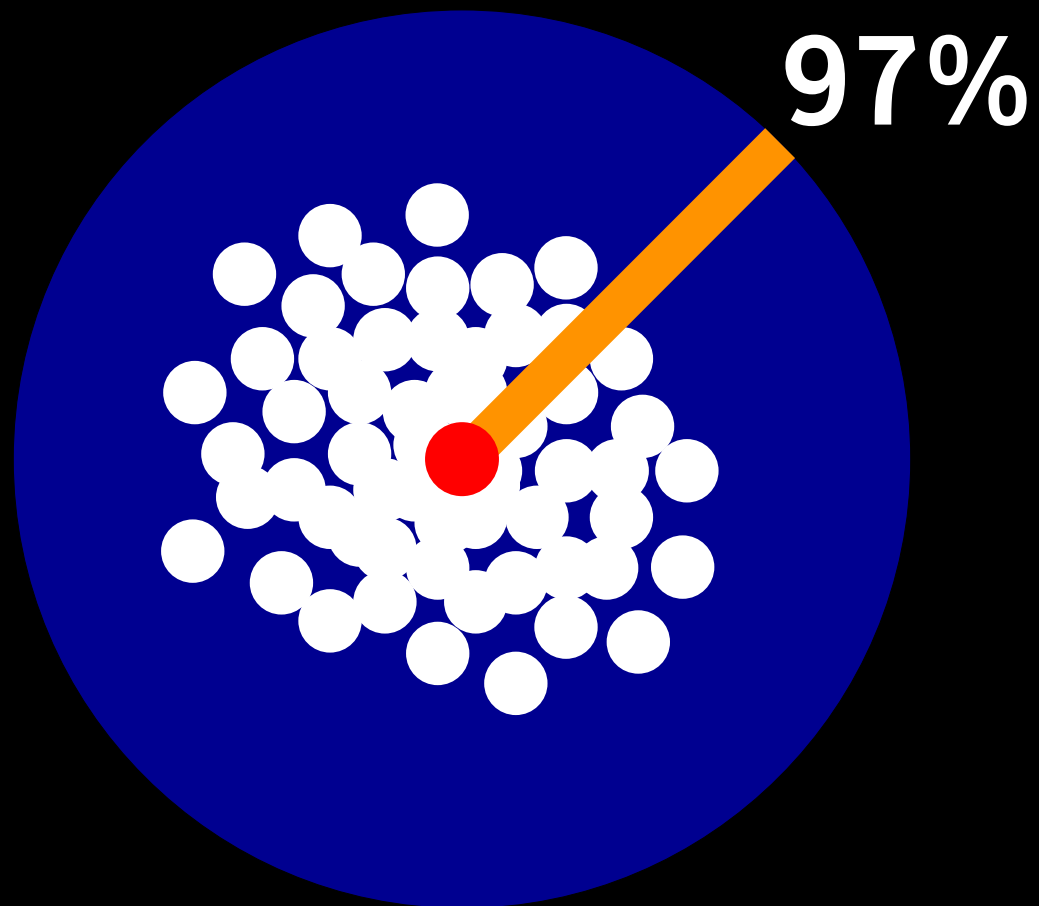
I know people teaching with old slides that never updated the 97% species threshold to 98.7%... looks like they've waited long enough that now those slides are almost accurate again :-)

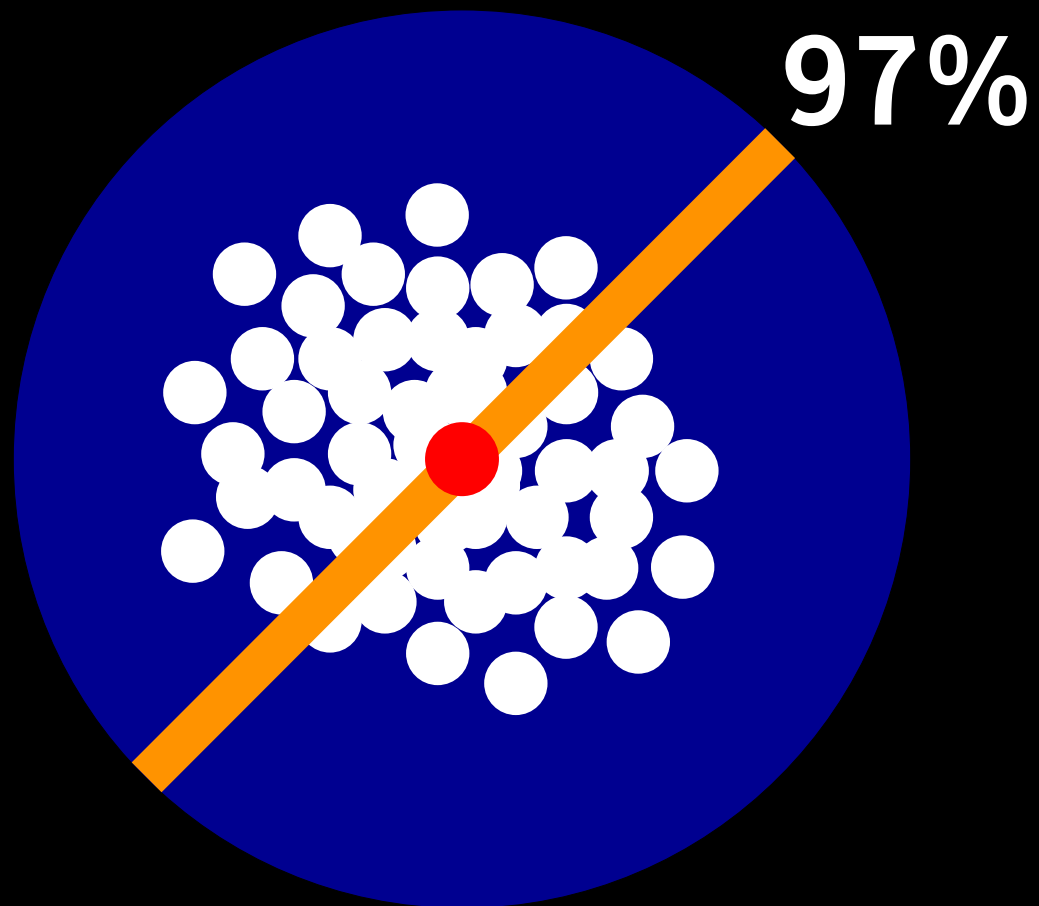


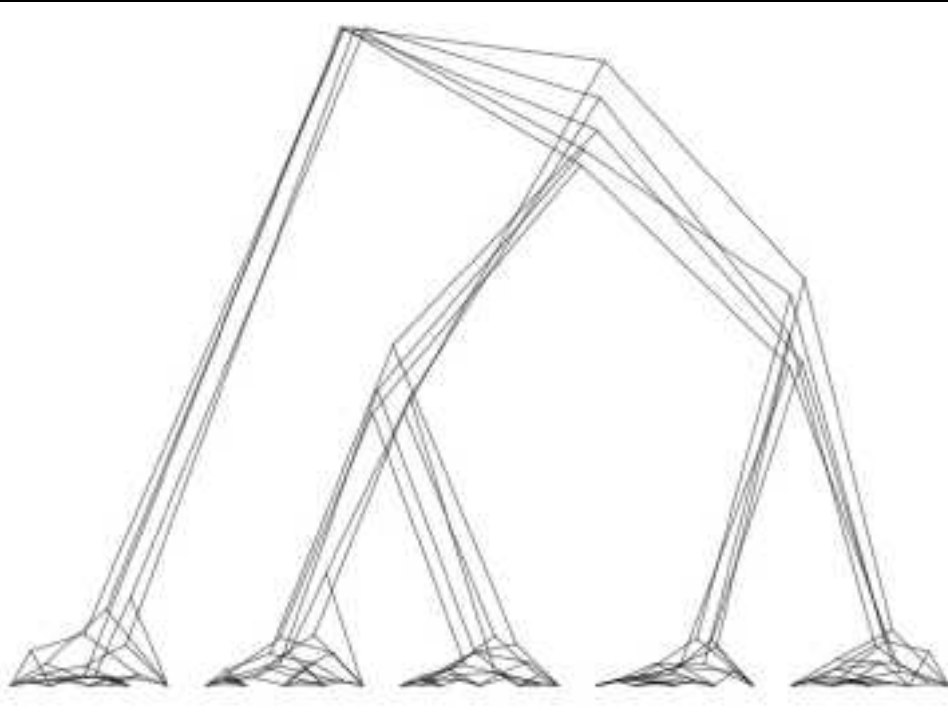
Cameron Thrash @jcamthrash.bsky.s... · 5h

Setting new boundaries of 16S rRNA gene identity for prokaryotic taxonomy
www.microbiologyresearch.org/content/jour...
#jcampubs

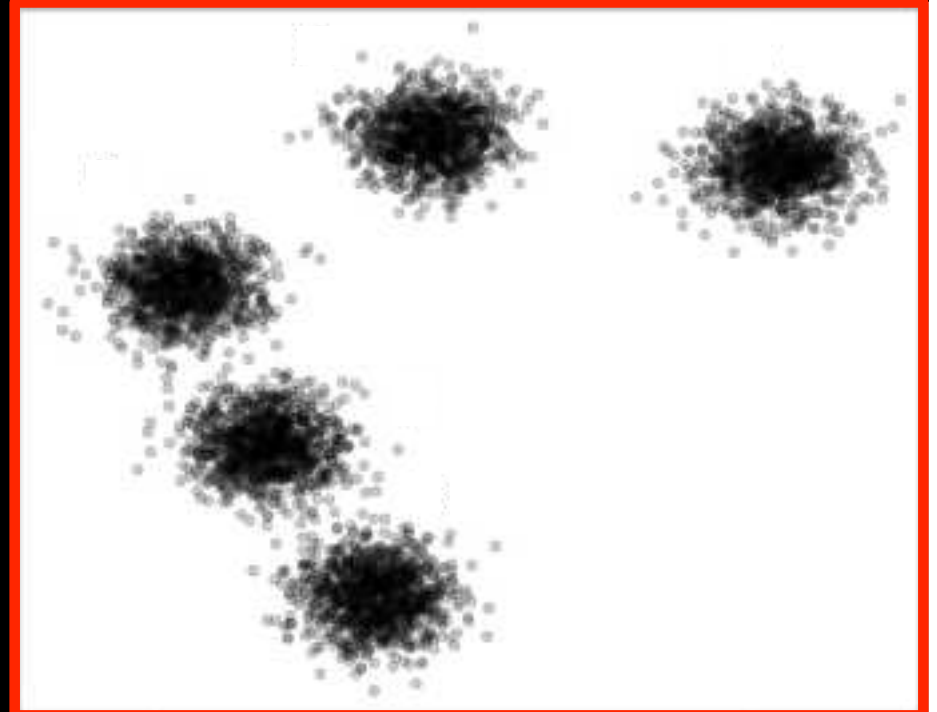
April 9, 2025 at 7:22 PM  Everybody can reply







trait 2



trait 1

**pairwise
comparisons**

**phylogenetic
comparisons**

**global clustering
thresholds**

**VSEARCH/
Mothur**

DADA2



**local clustering
thresholds**

Swarm

**multi-rate
PTP**

2016 13:581-583

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

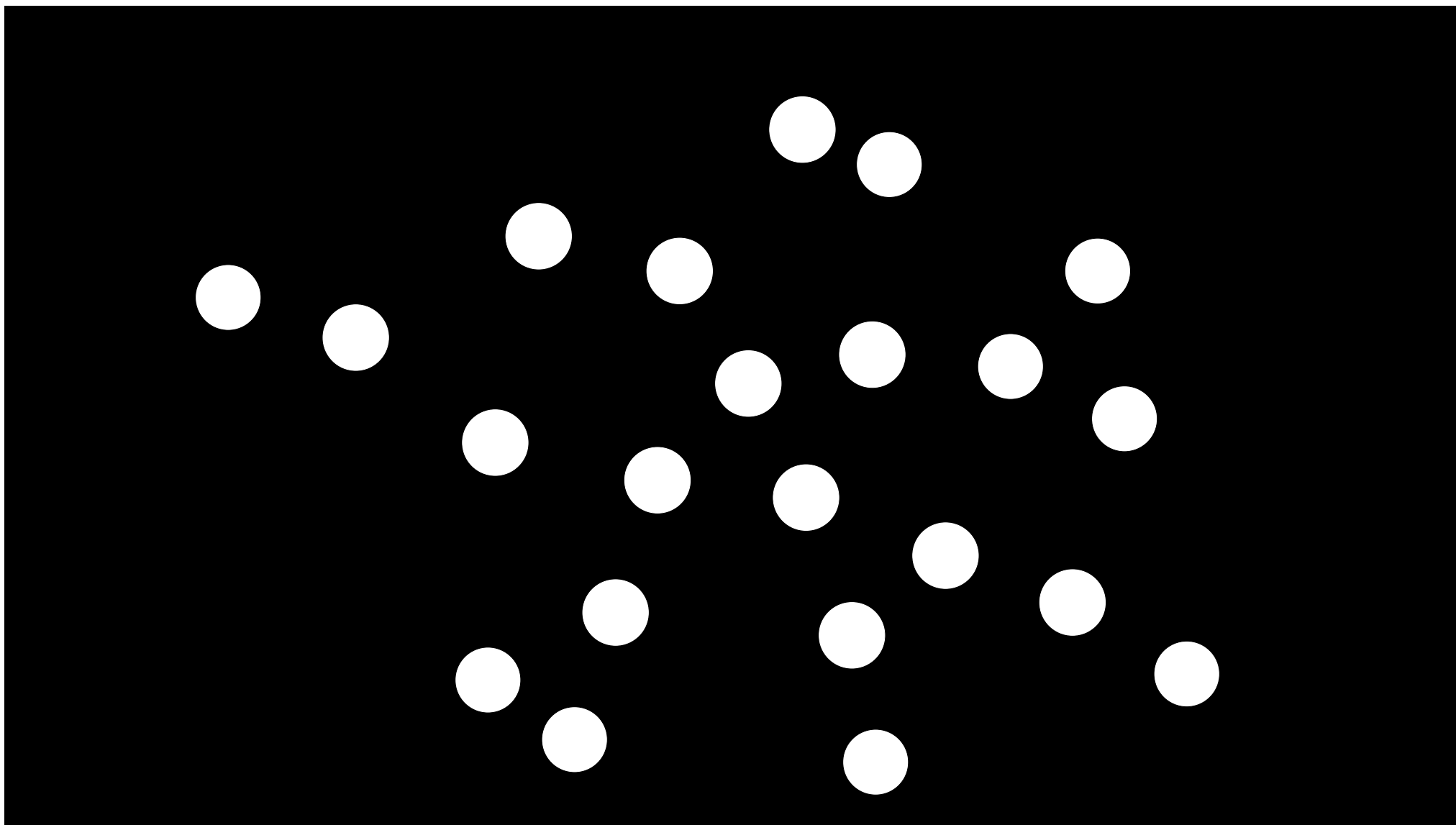
Nature Methods

Benjamin J Callahan¹, Paul J McMurdie²,
Michael J Rosen³, Andrew W Han², Amy Jo A Johnson² &
Susan P Holmes¹

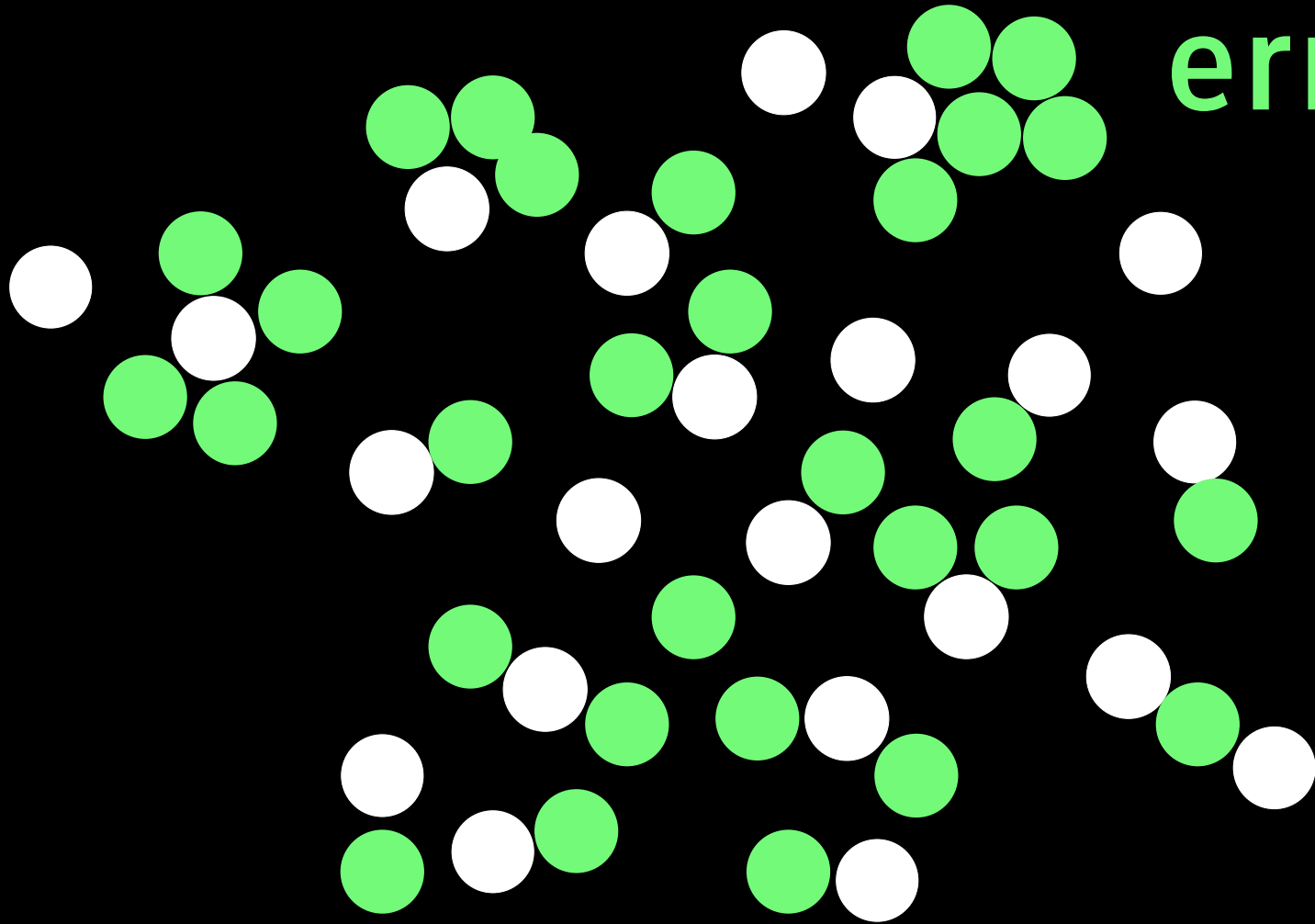
**amplicon sequence variants
(ASVs)**

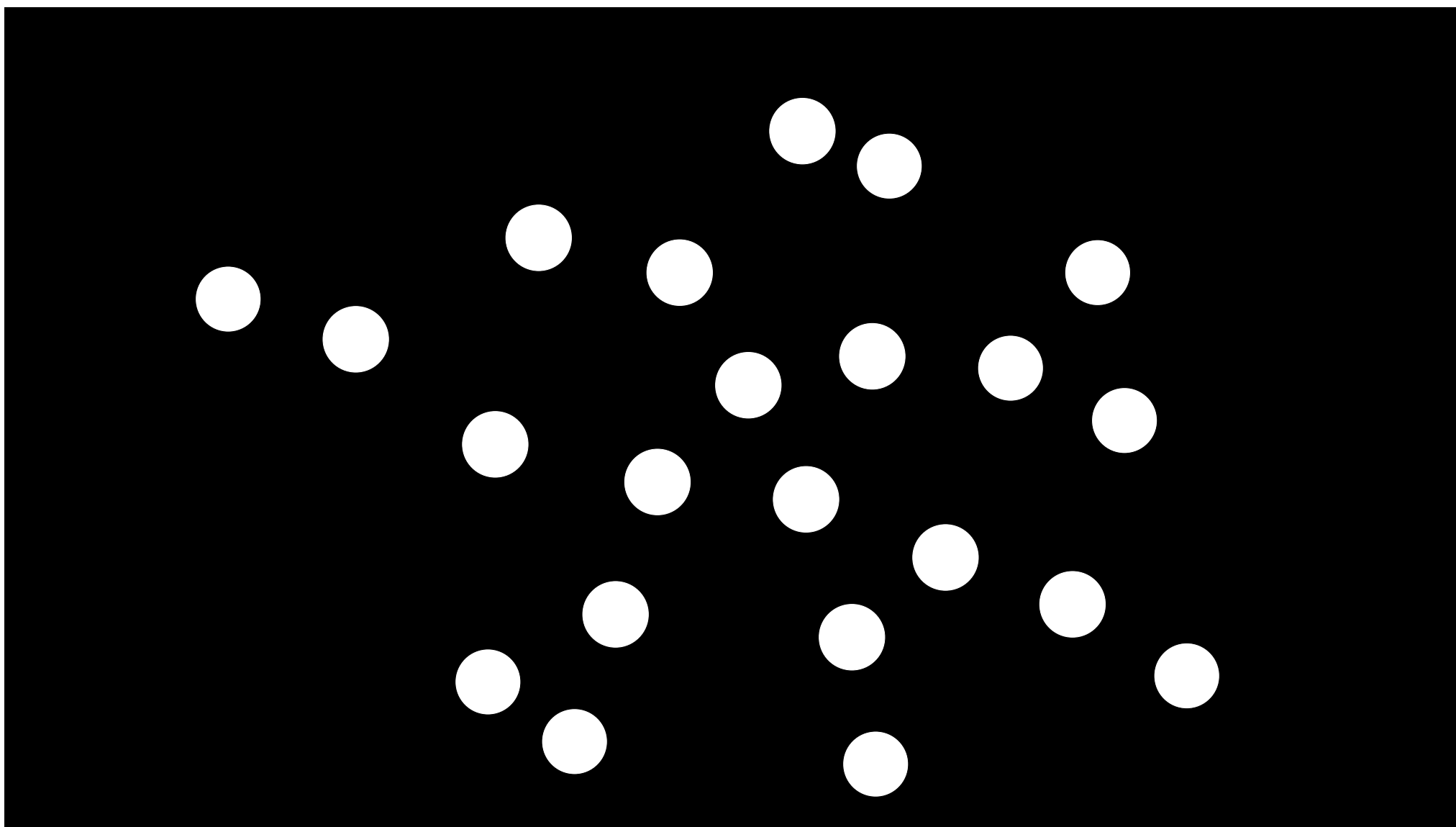
**amplicon sequence variants
(ASVs)**

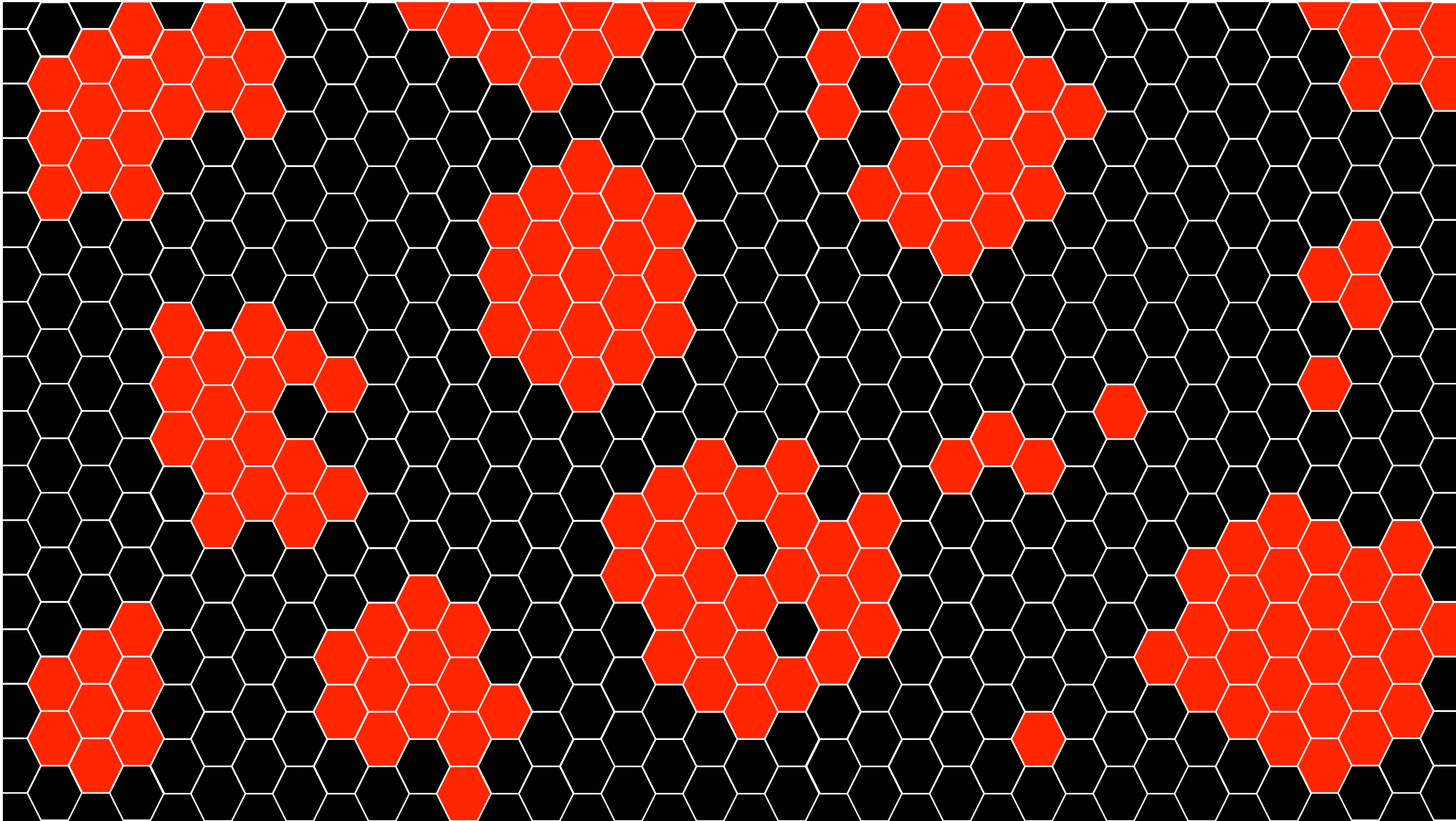
clustering by another name

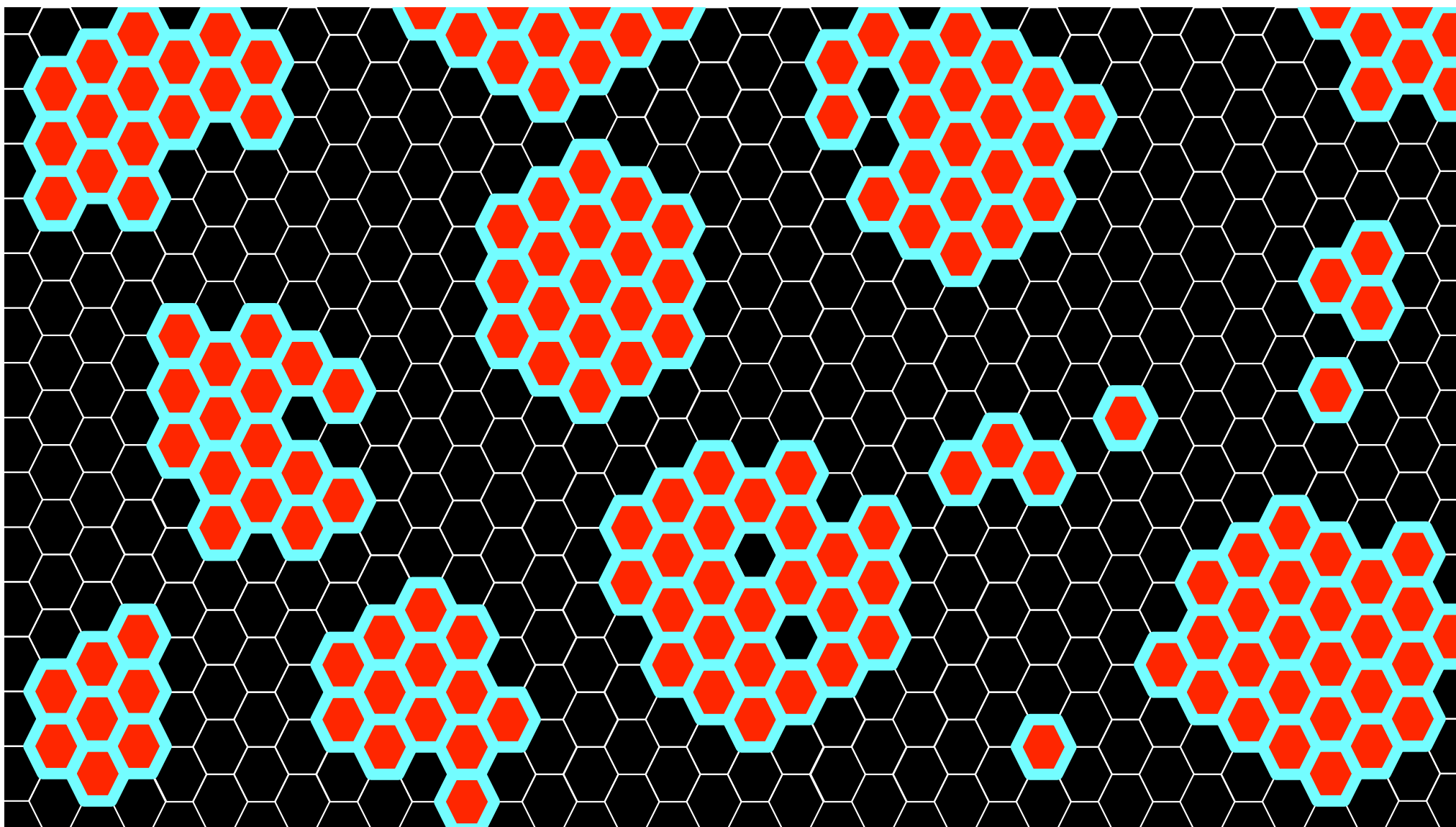


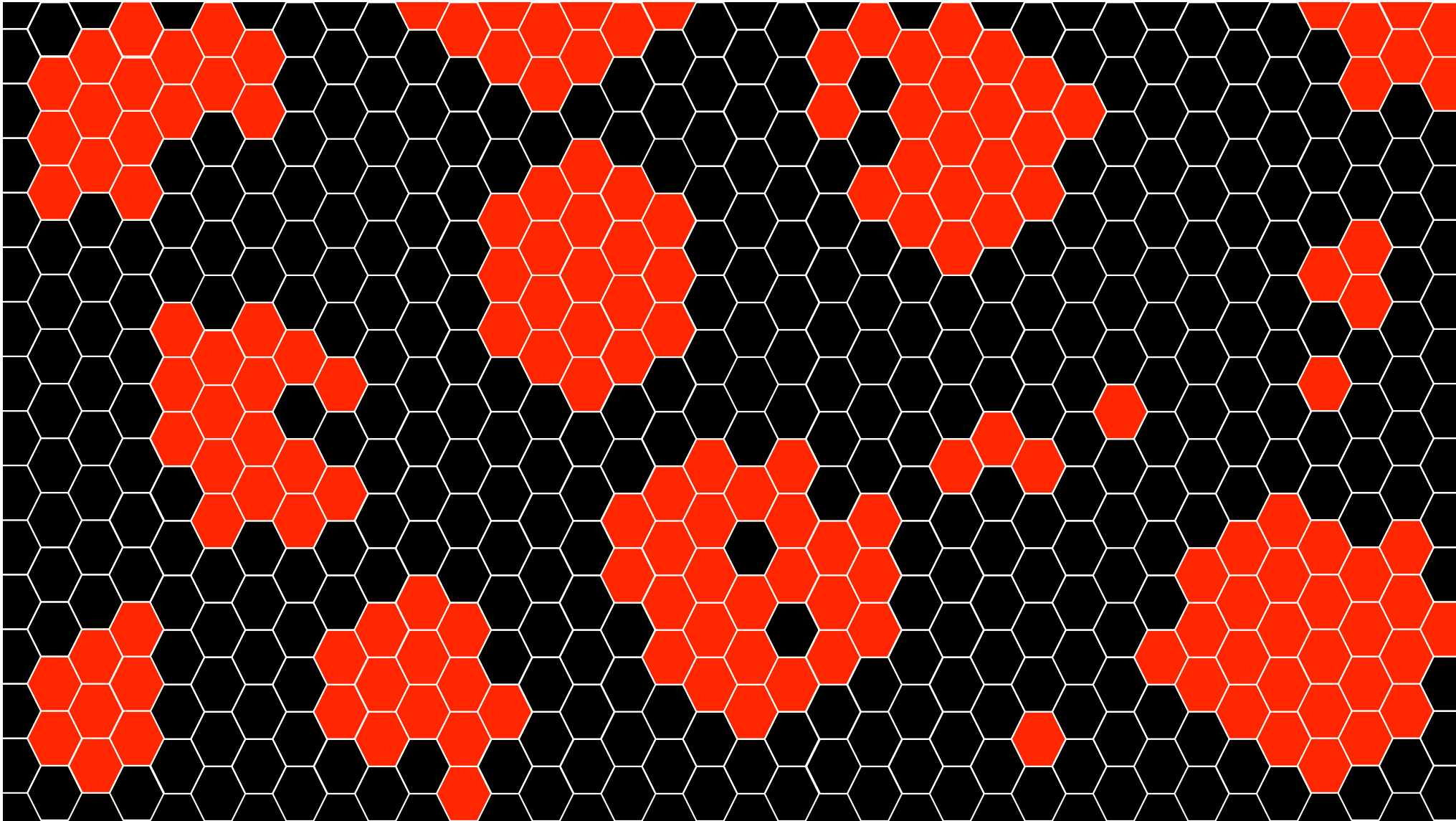
errors

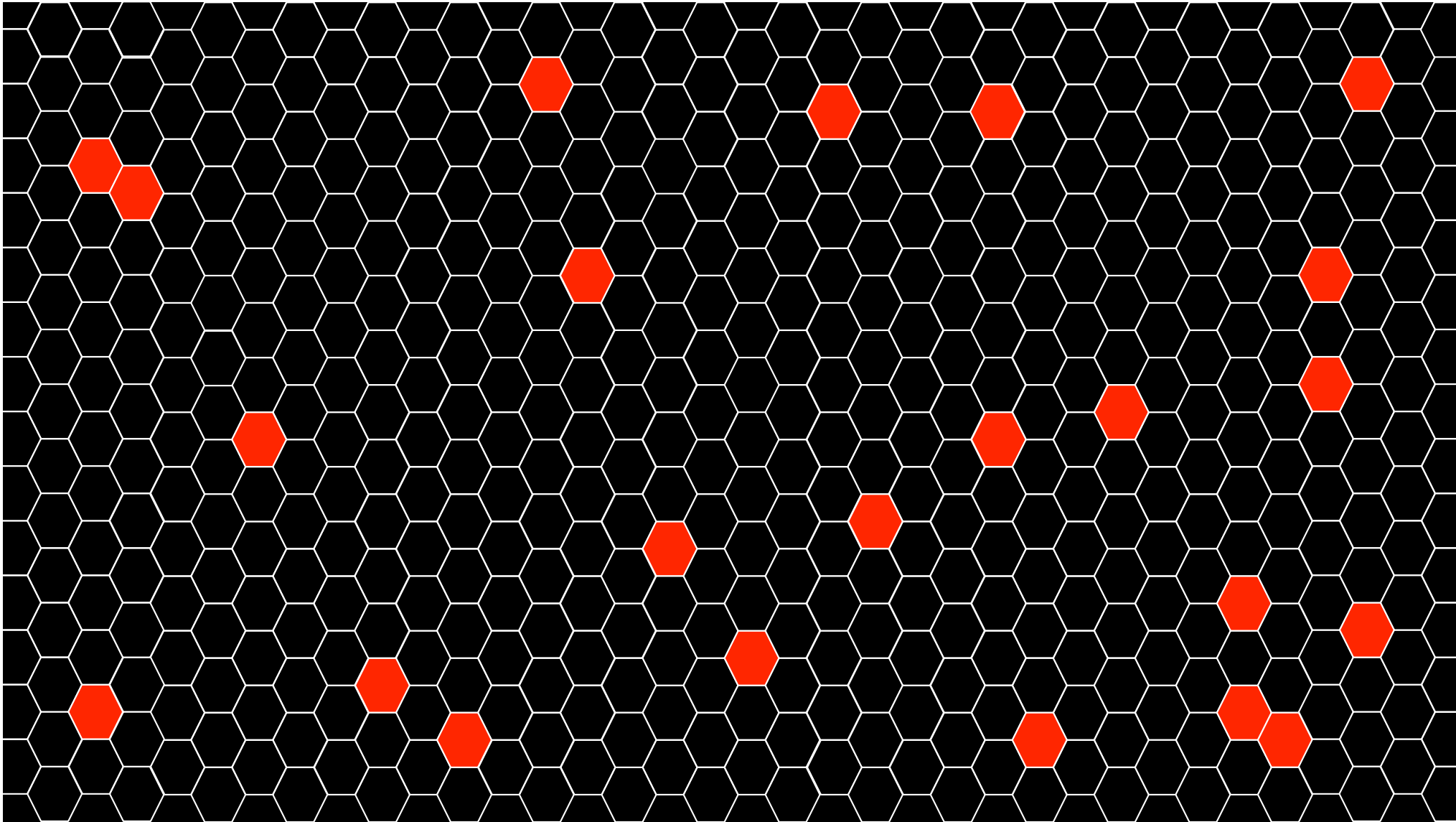


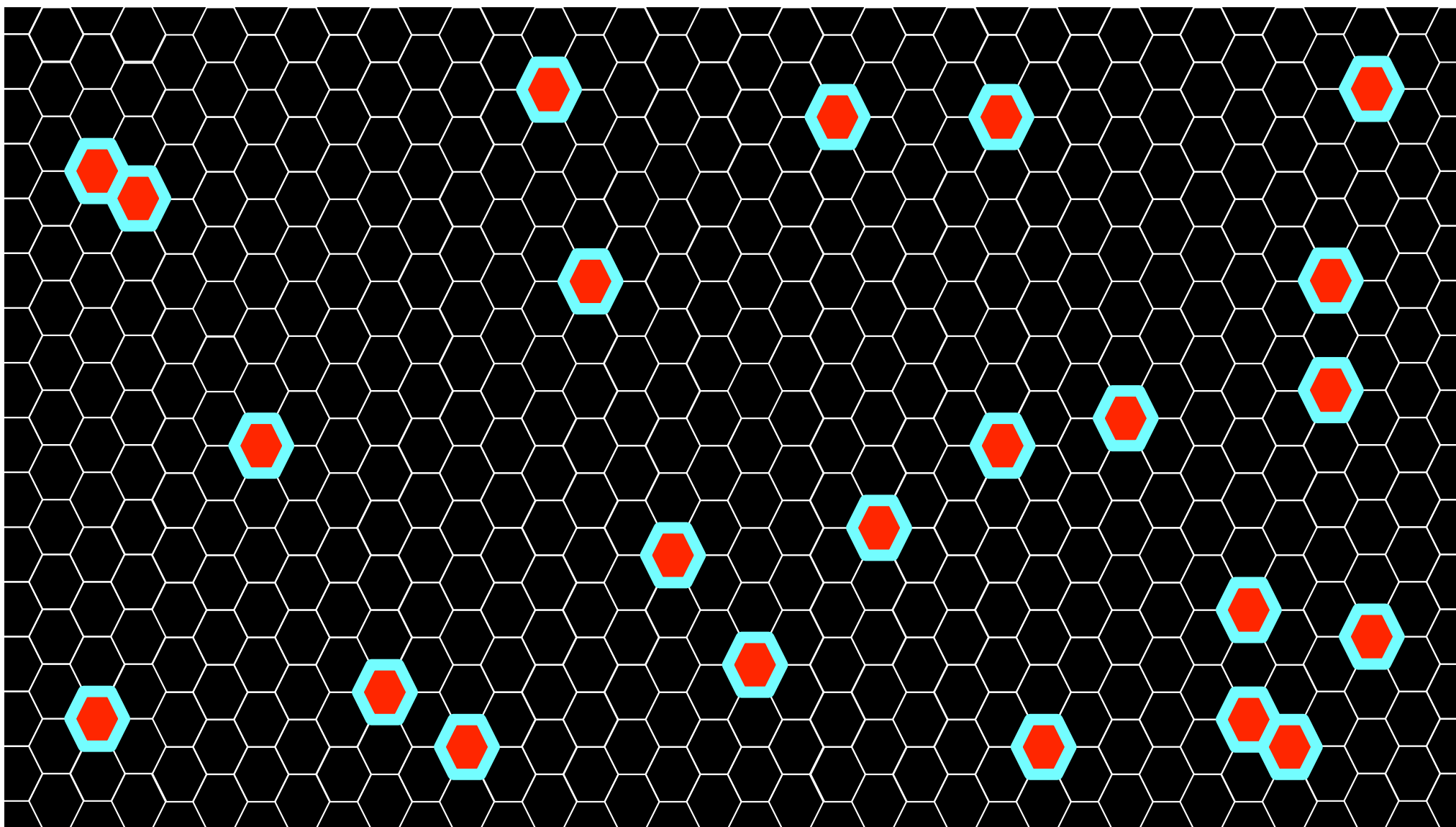












100%

population genetics doesn't matter

population genetics doesn't matter



Sewall Wright



J.B.S. Haldane



Ronald Fisher

sampling,
storage



nucleotide
extraction



amplification,
sequencing

cleaning,
dereplication



clustering



more
cleaning

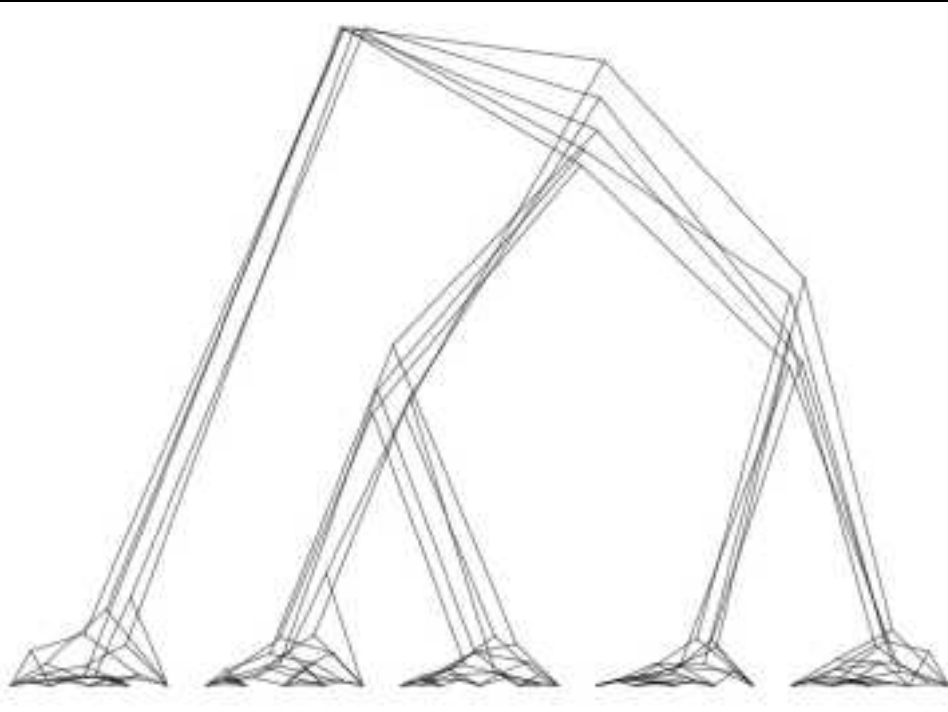
taxonomic
assignments



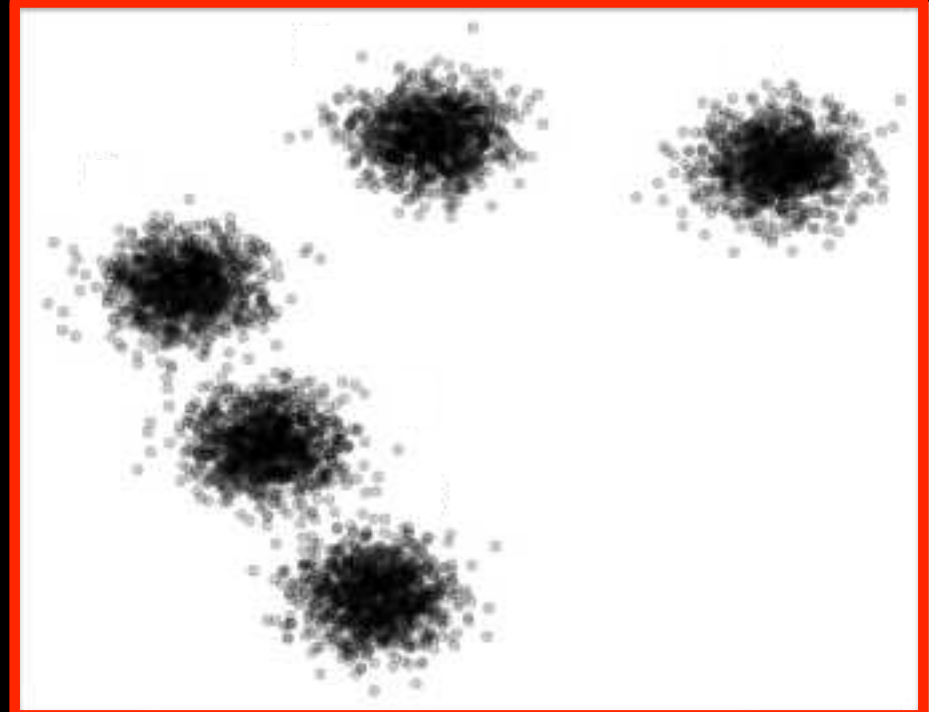
phylogenetic
placements



α, β diversities,
co-occurrences



trait 2



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DADA2



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Bioinformatics, 38(1), 2022, 267–269

doi: 10.1093/bioinformatics/btab493

Advance Access Publication Date: 9 July 2021

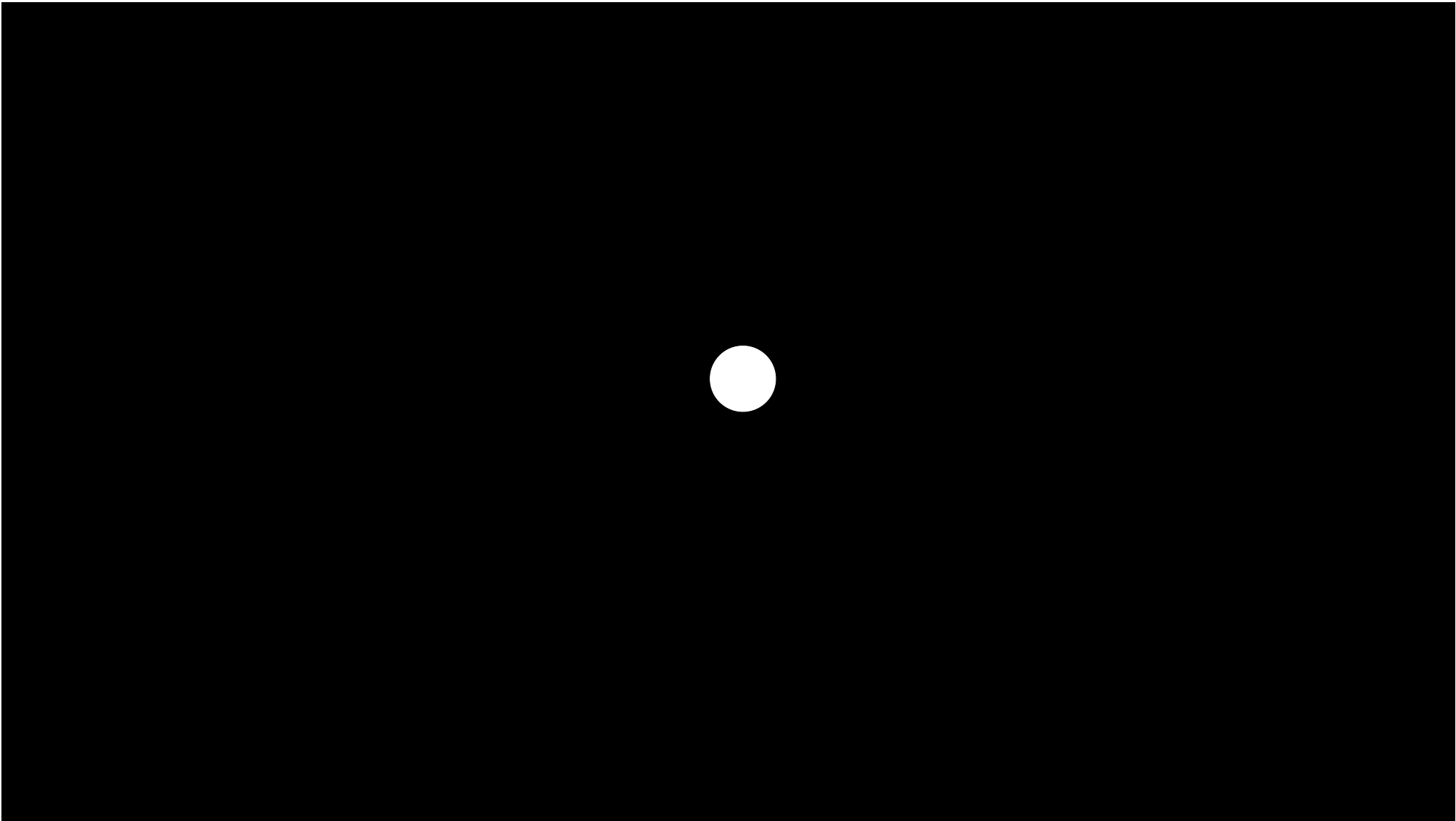
Applications Note

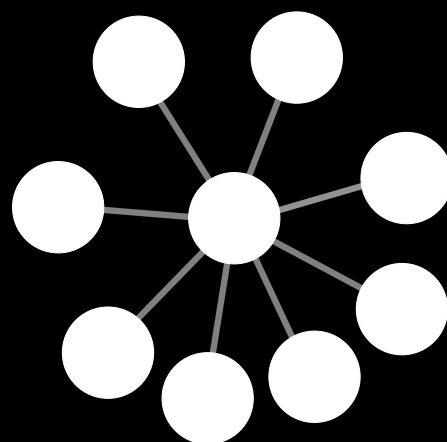
OXFORD

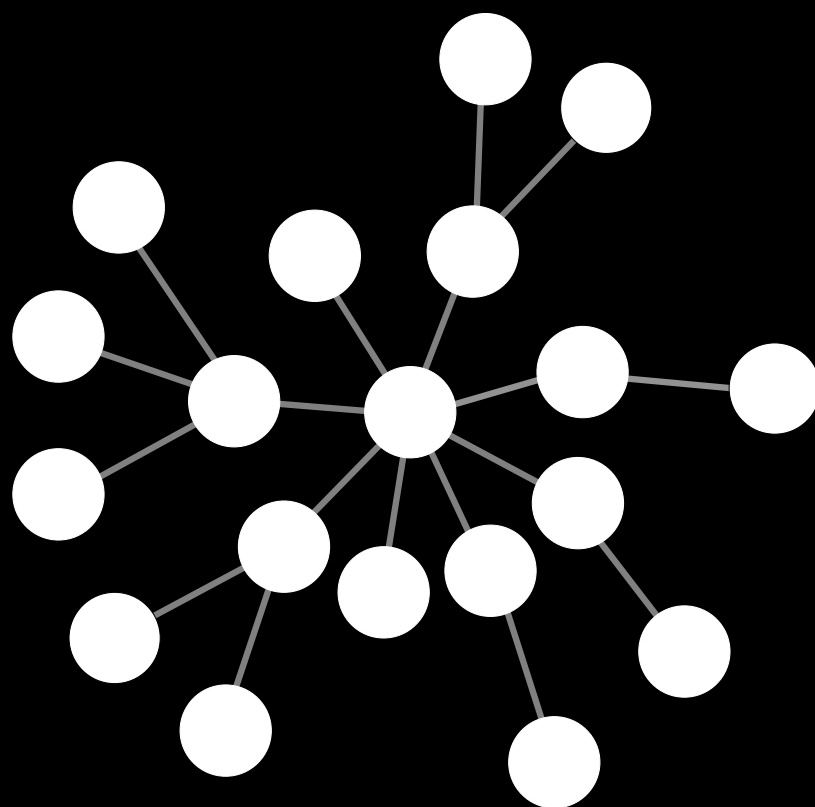
Sequence analysis

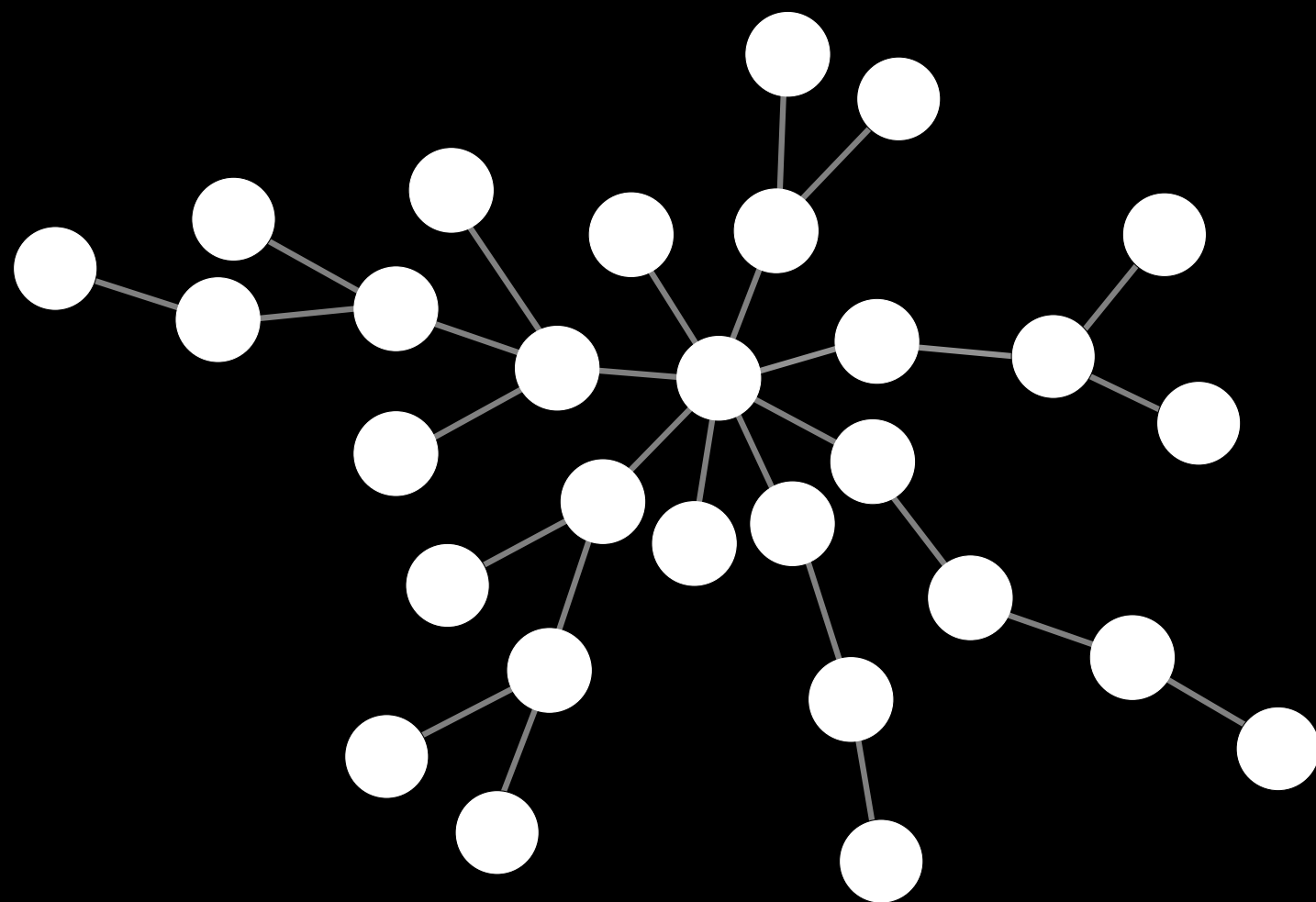
Swarm v3: towards tera-scale amplicon clustering

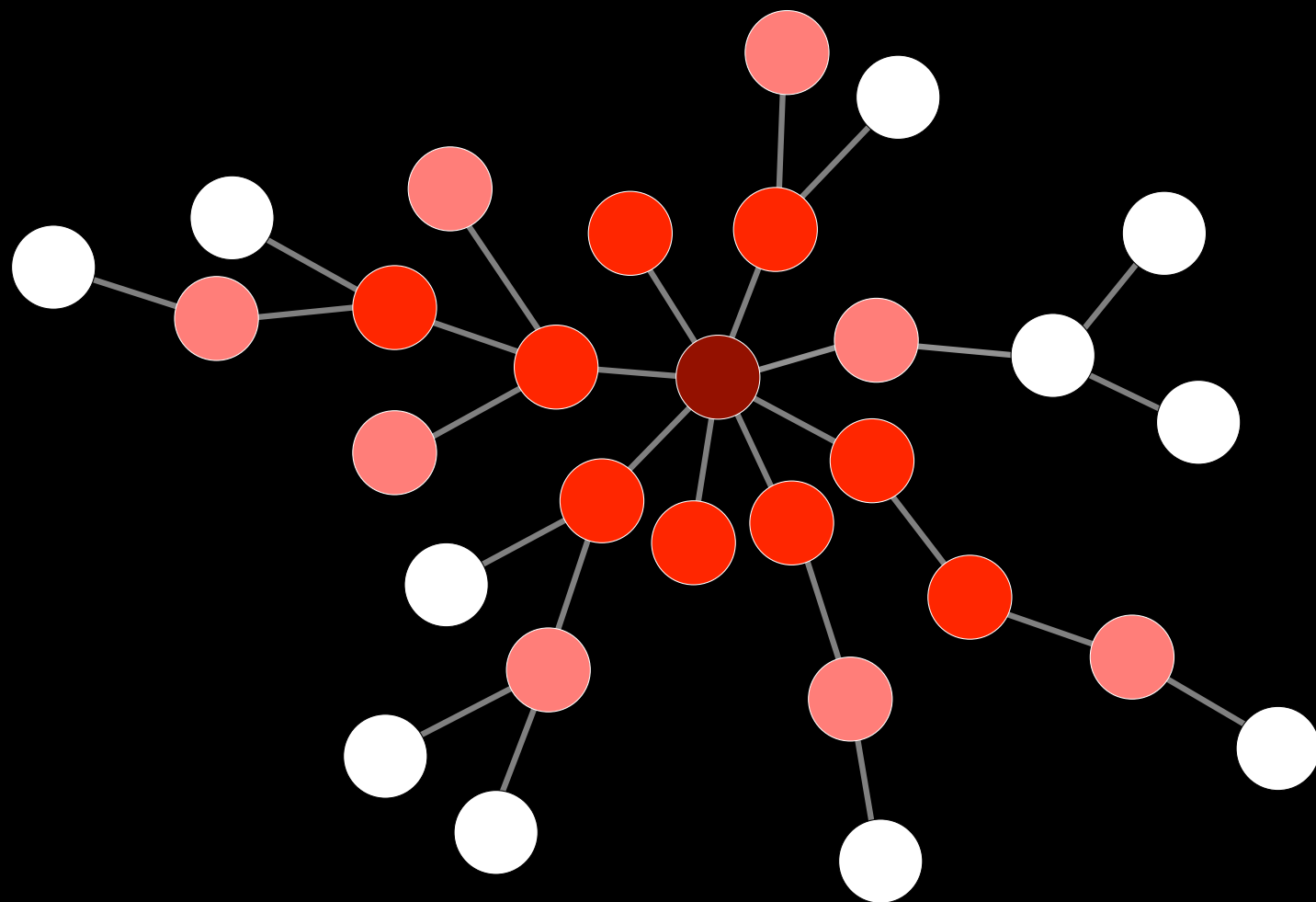
**Frédéric Mahé^{1,2,*}, Lucas Czech^{3,4}, Alexandros Stamatakis^{3,5},
Christopher Quince^{6,7,8}, Colomán de Vargas^{9,10}, Micah Dunthorn^{11,12} and
Torbjørn Rognes^{13,14}**

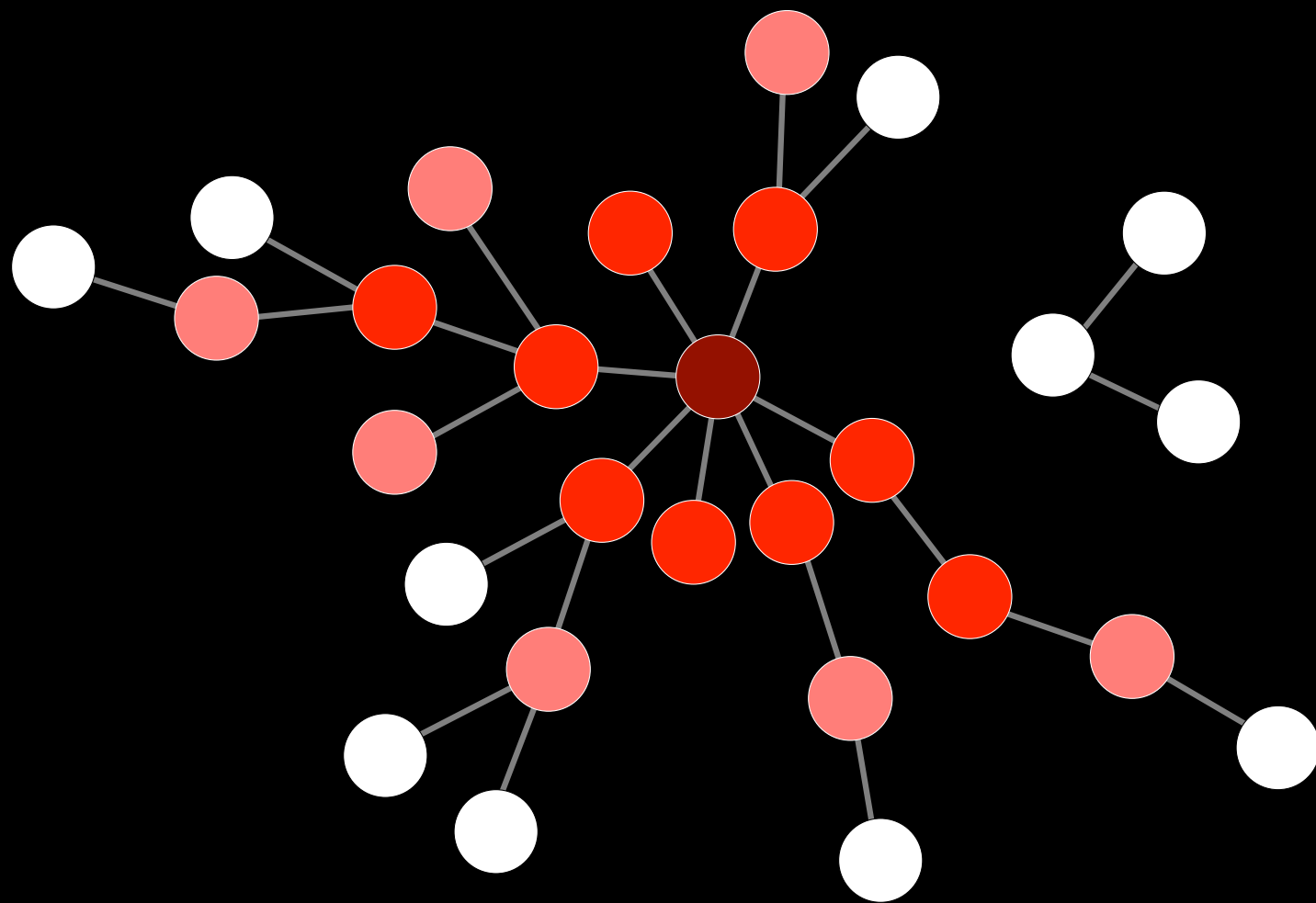


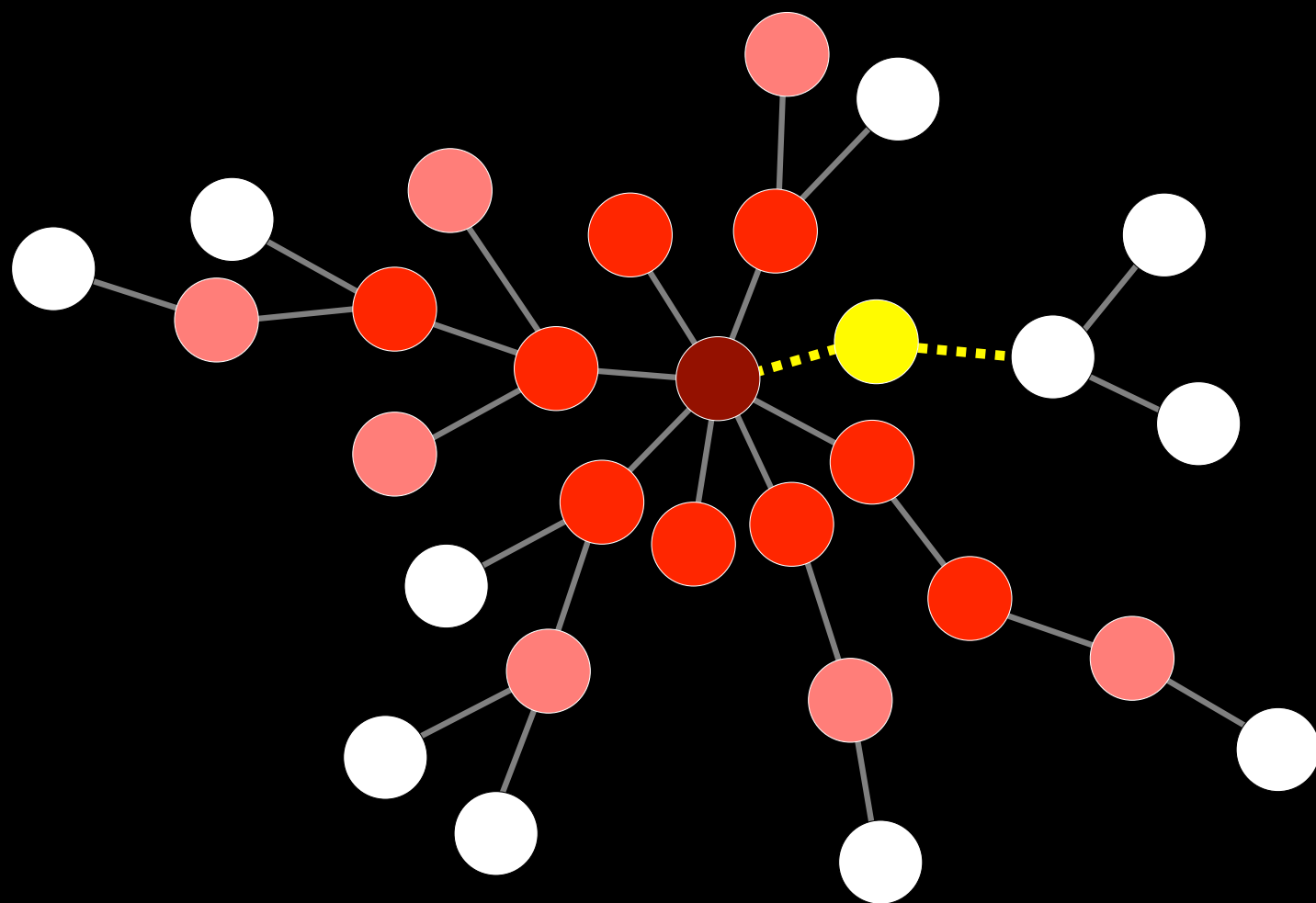


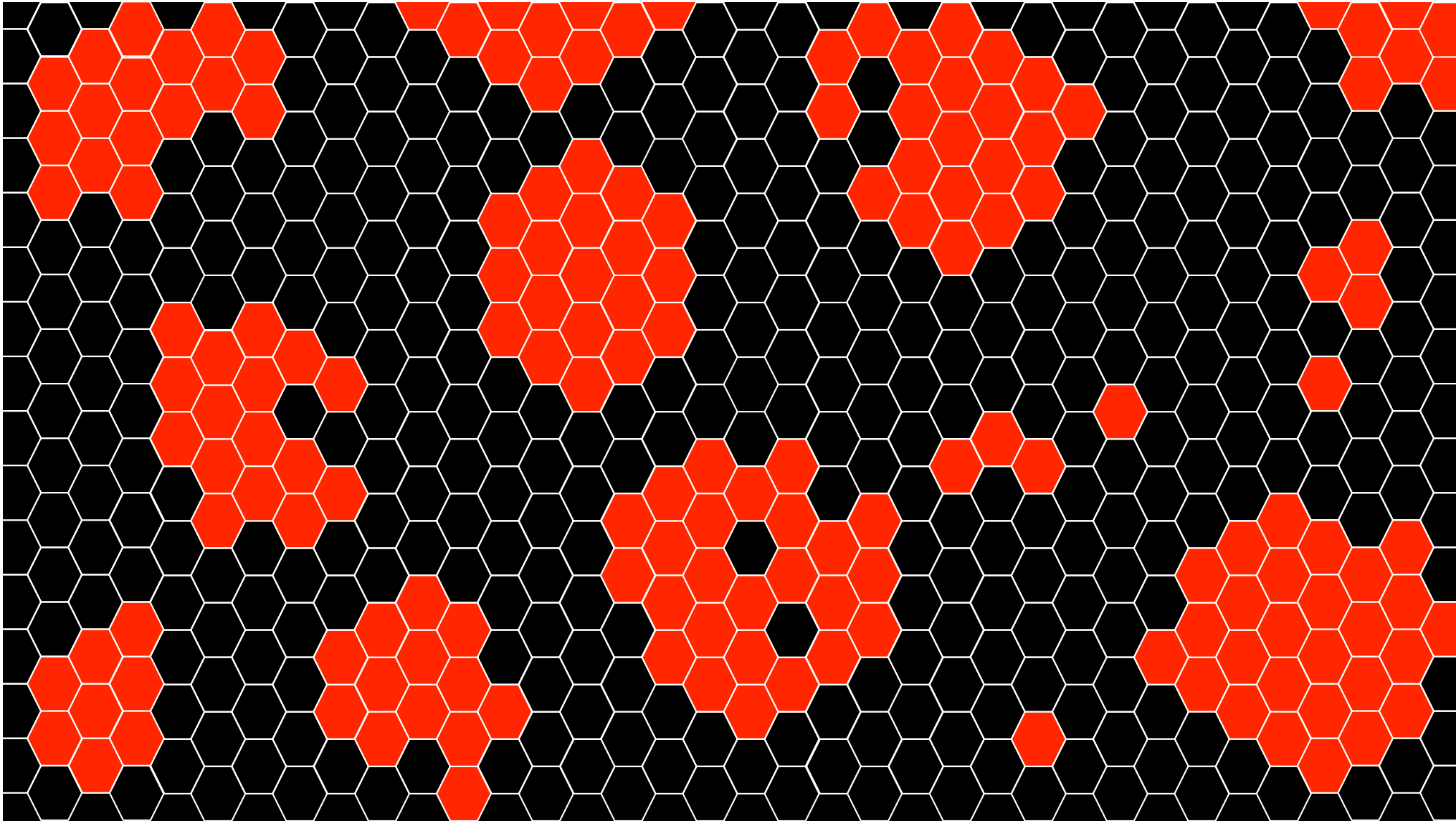


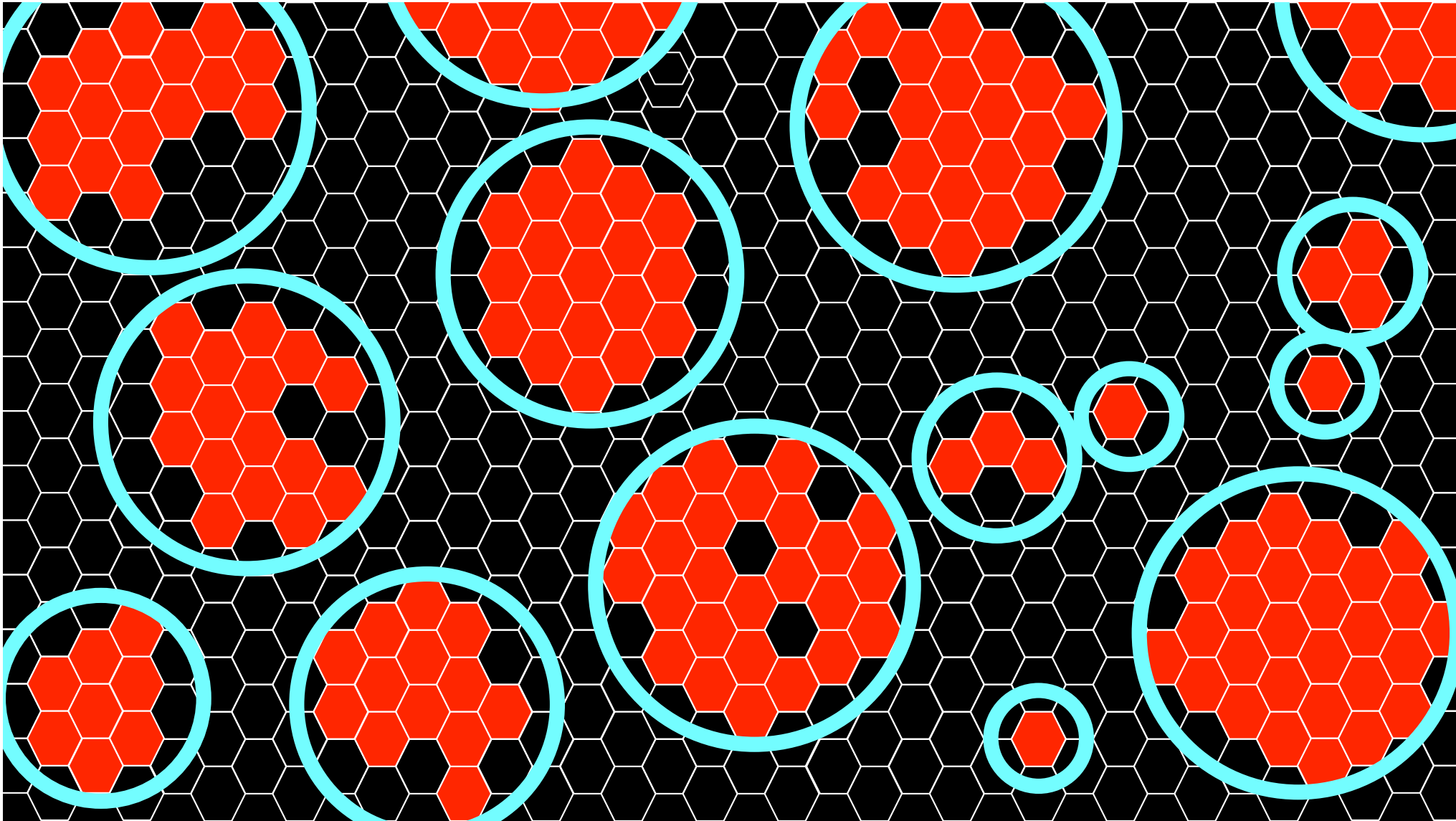


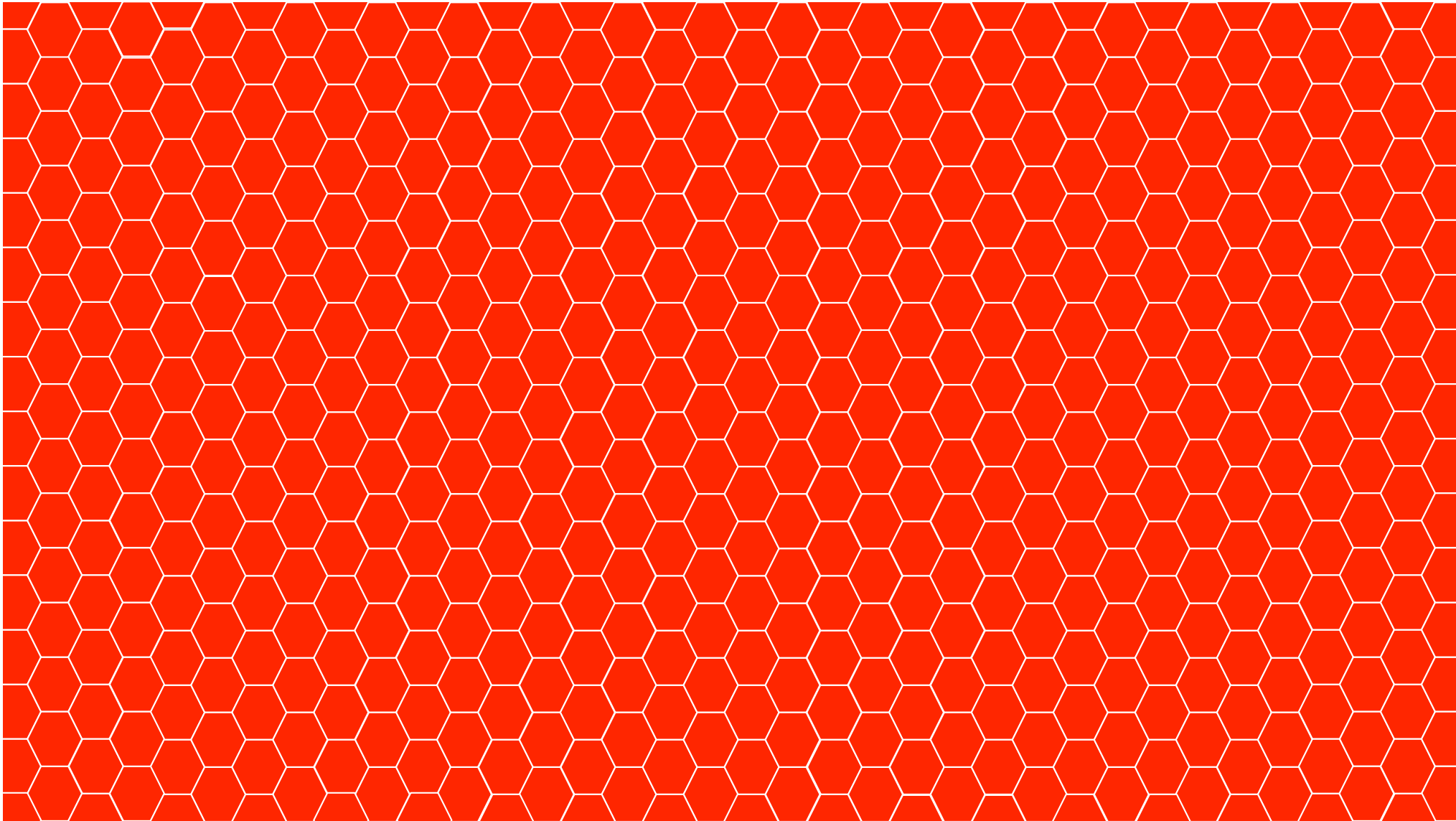


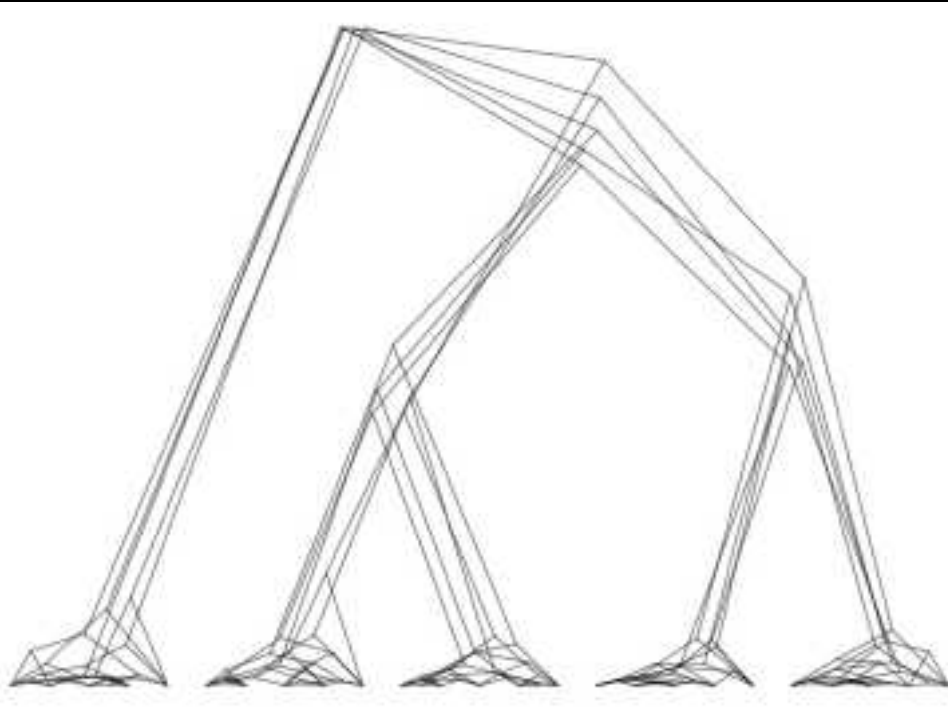




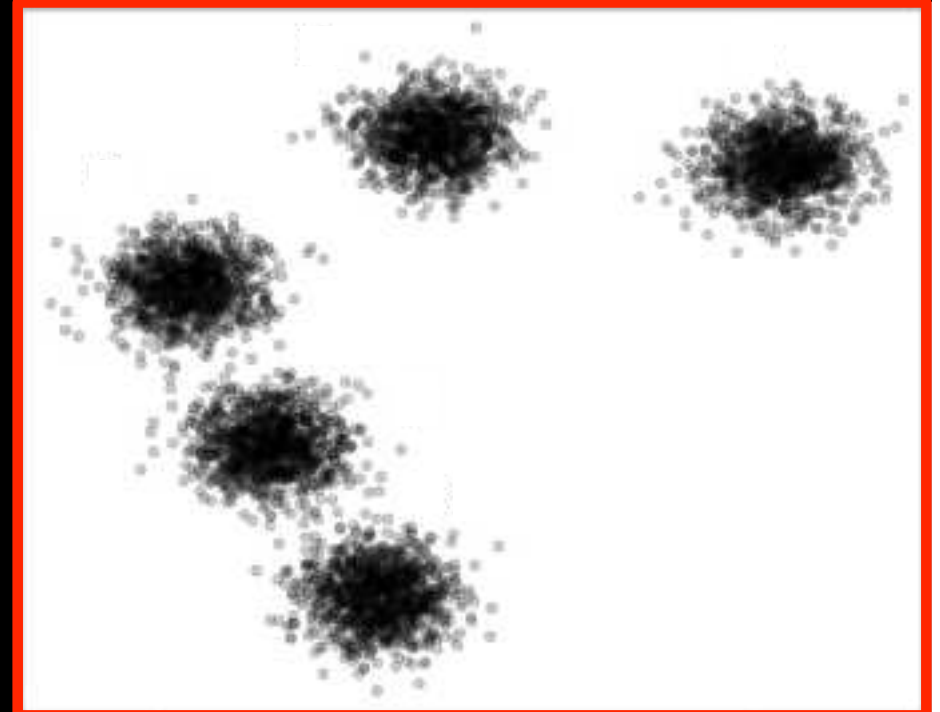








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PTP**

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

doi: 10.1093/bioinformatics/btx025

Advance Access Publication Date: 20 January 2017

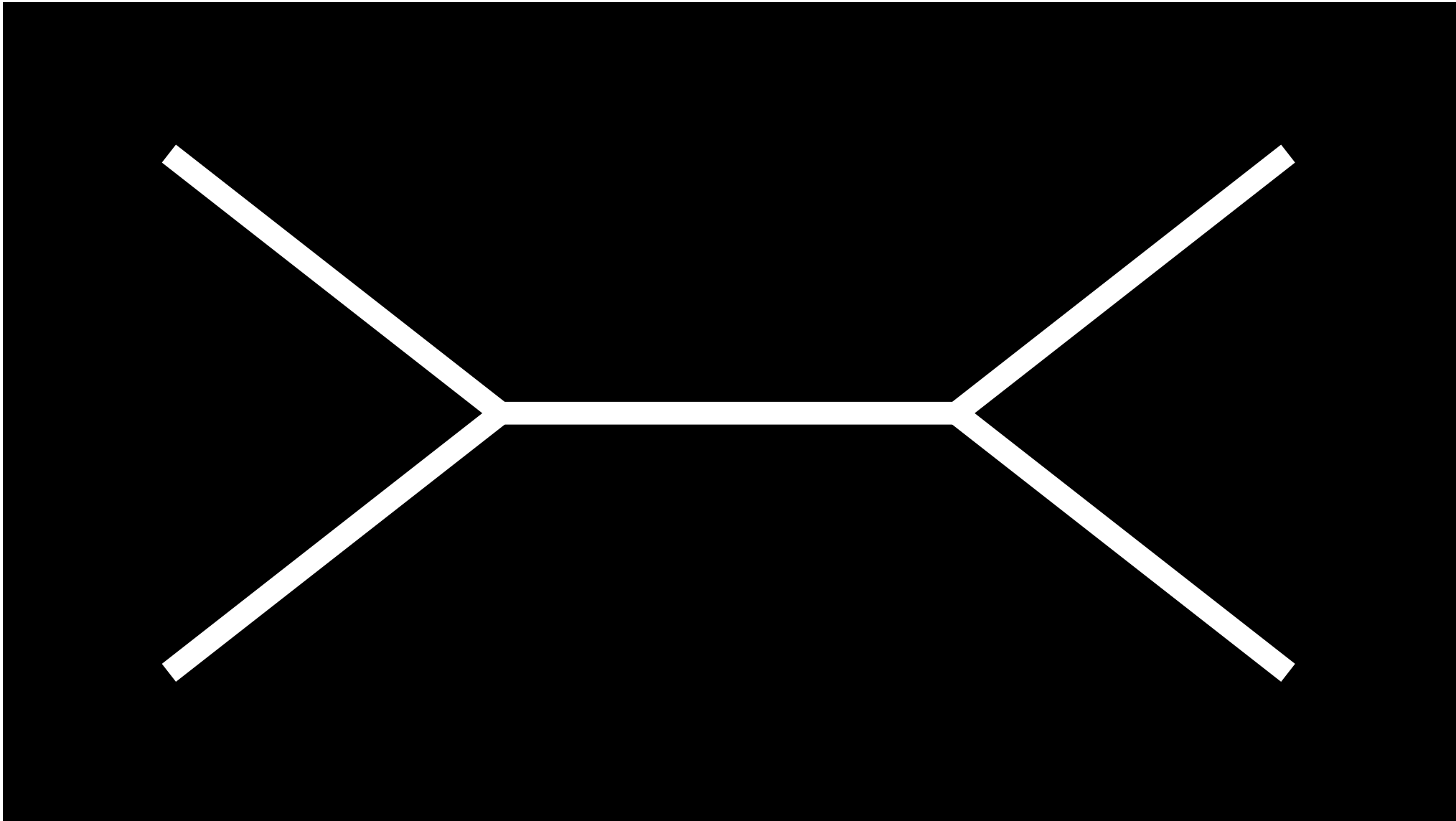
Original Paper

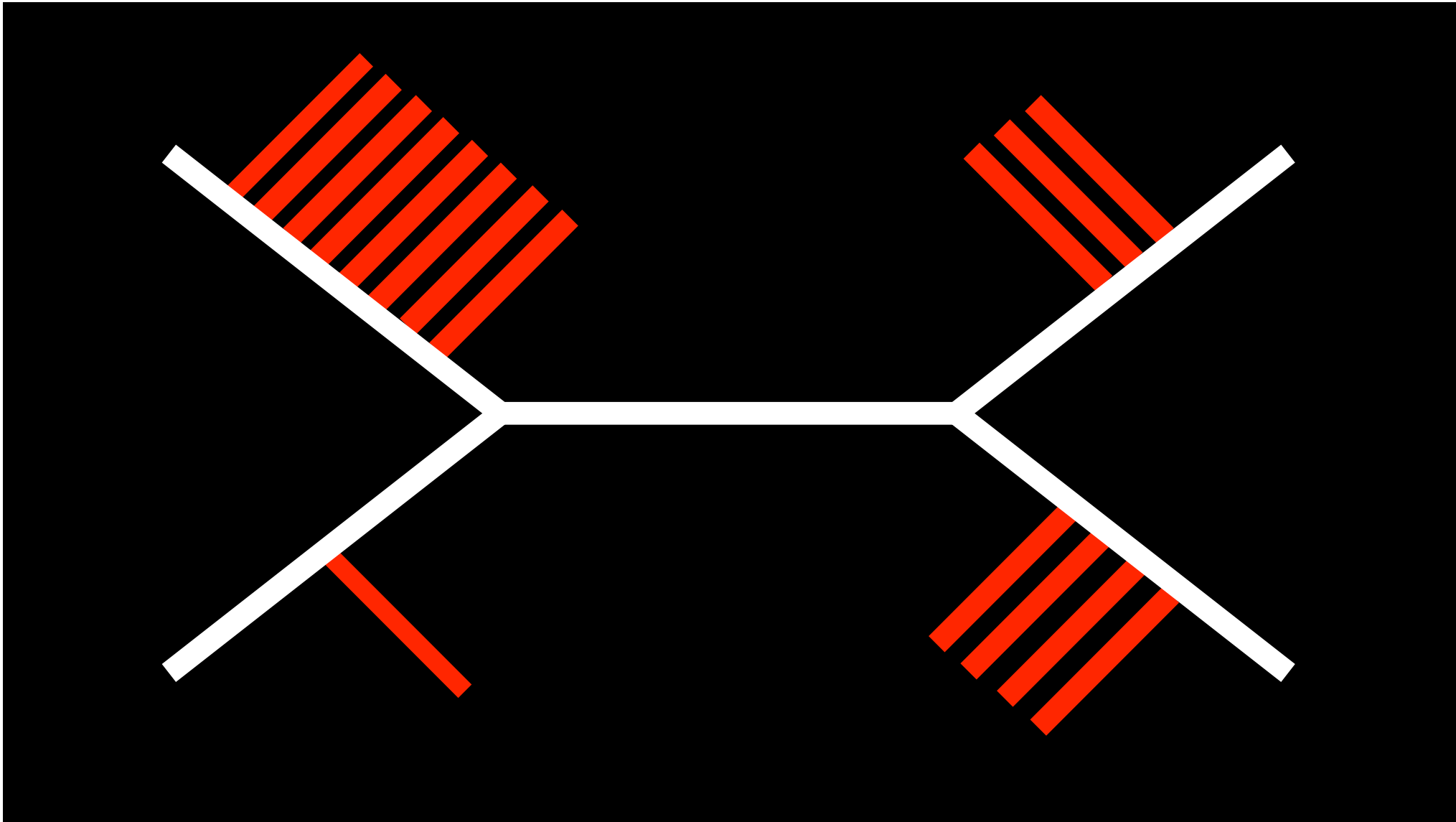


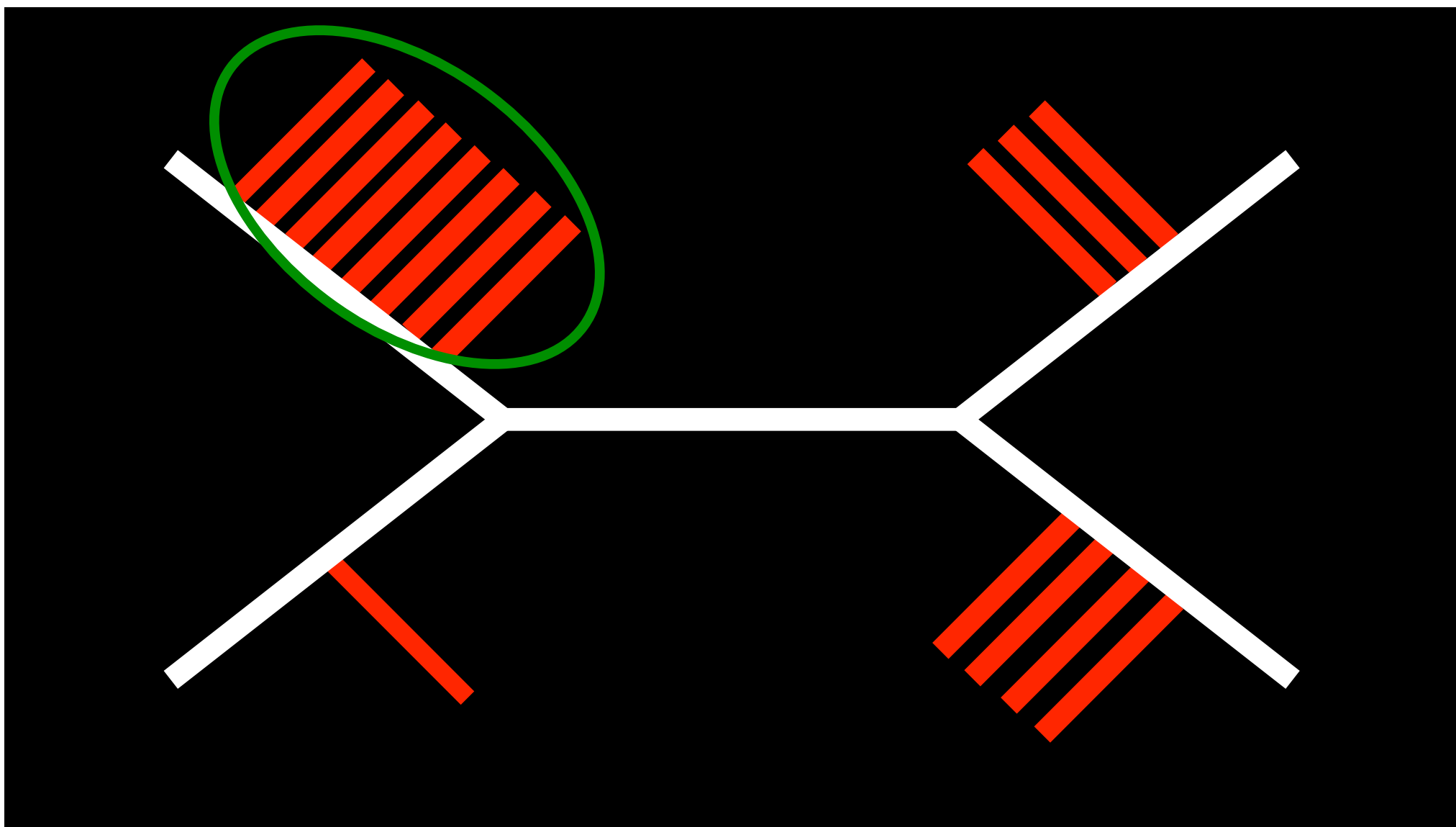
Phylogenetics

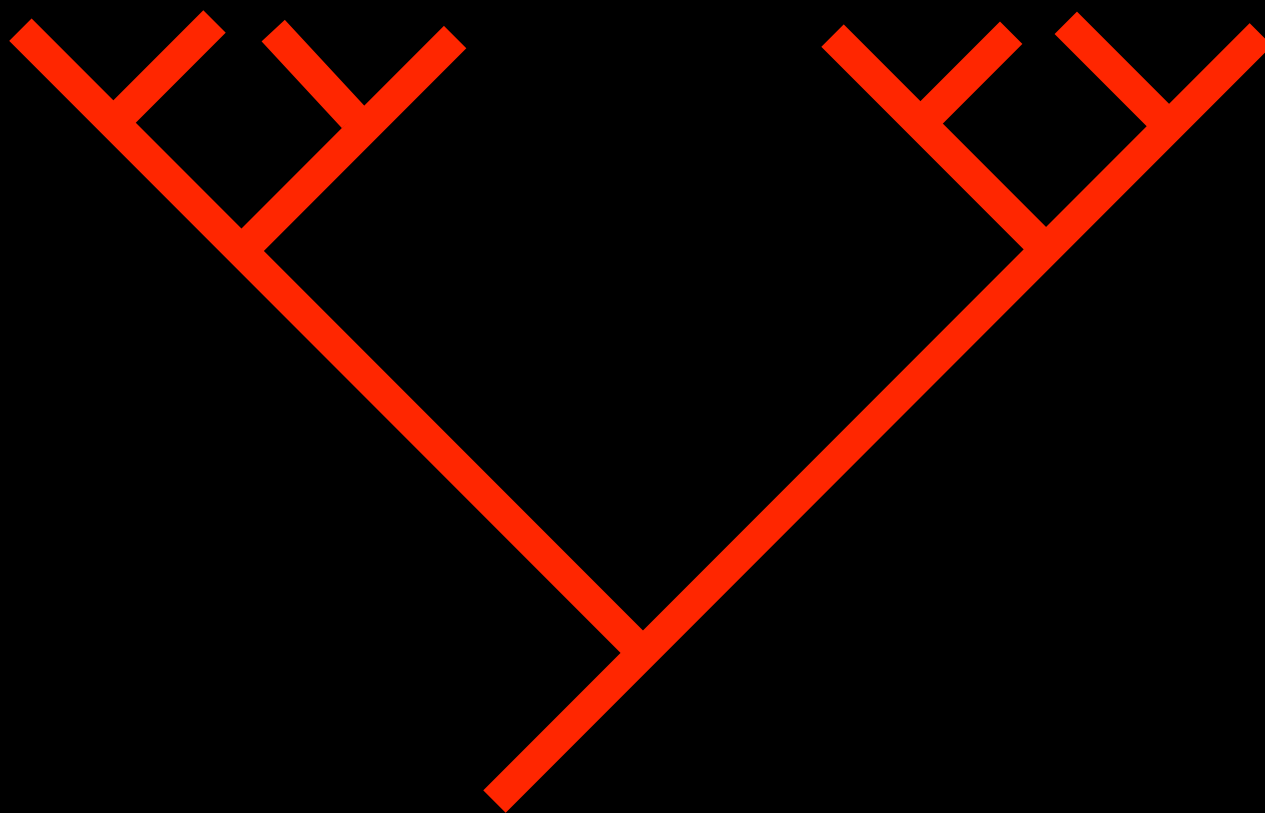
Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo

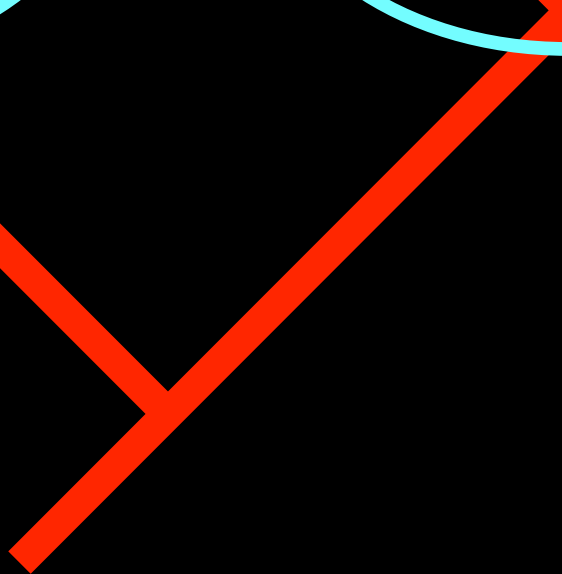
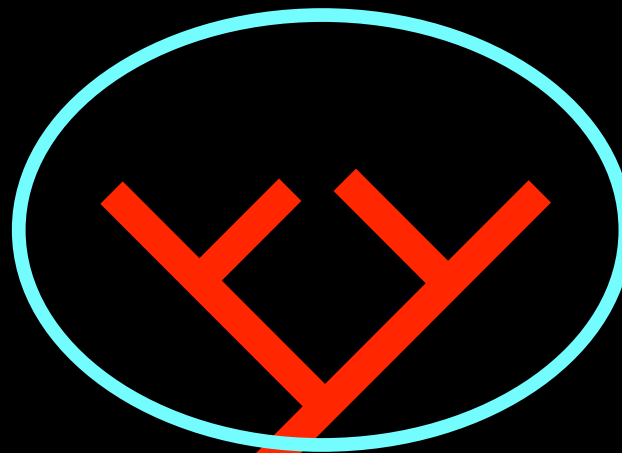
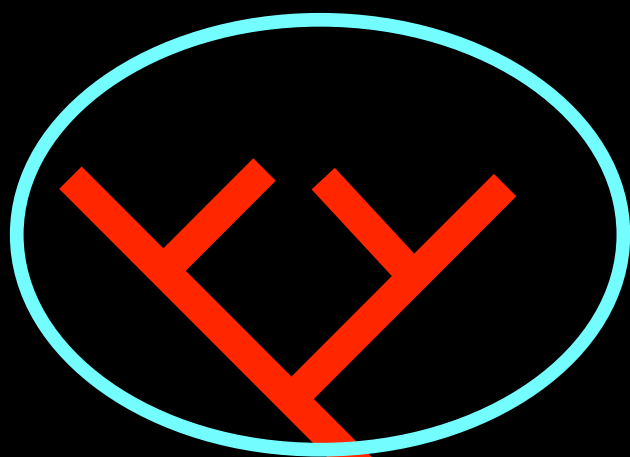
P. Kapli^{1,*}, S. Lutteropp^{1,2}, J. Zhang¹, K. Kobert¹, P. Pavlidis³,
A. Stamatakis^{1,2,*} and T. Flouri^{1,2,*}

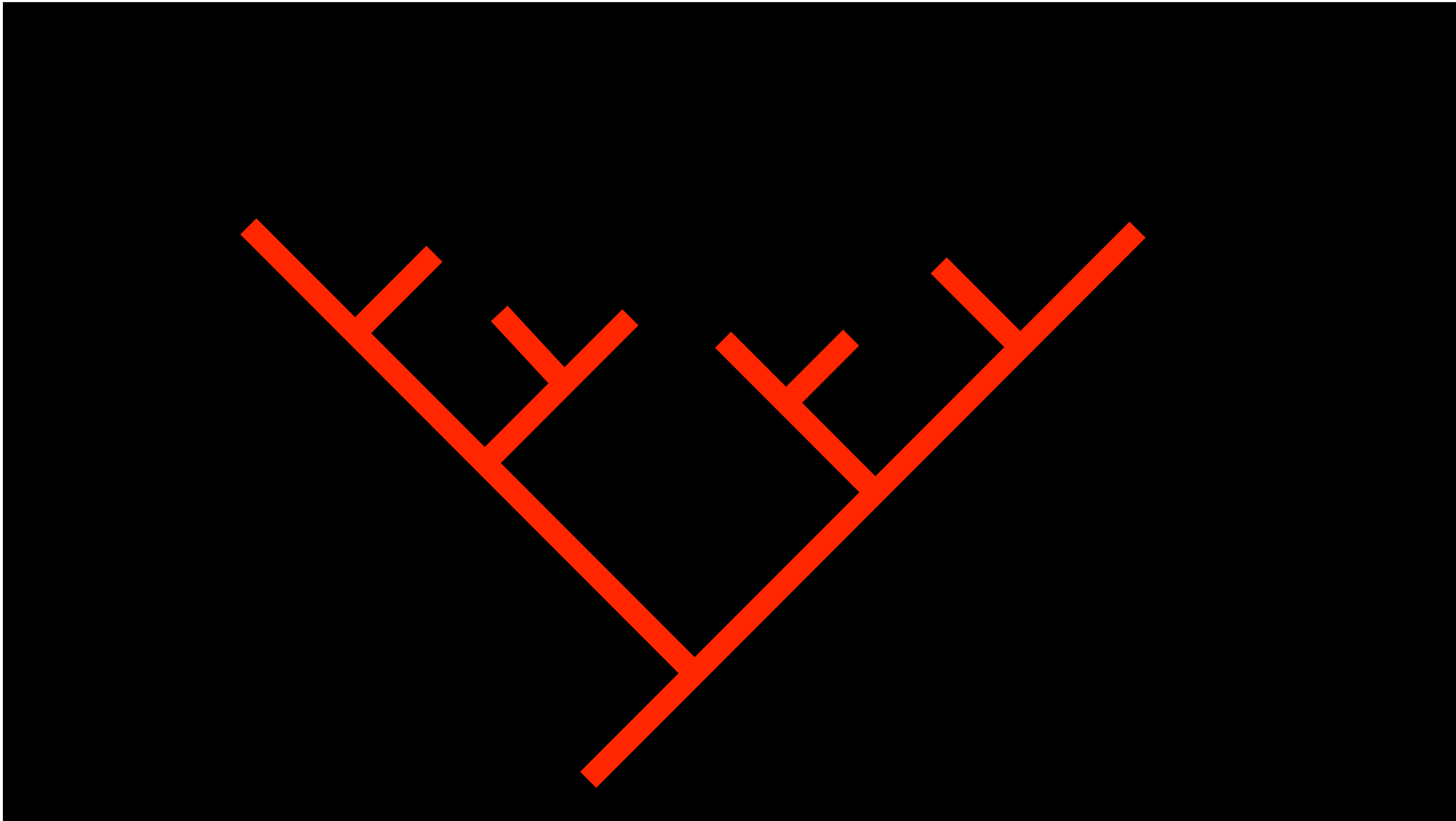


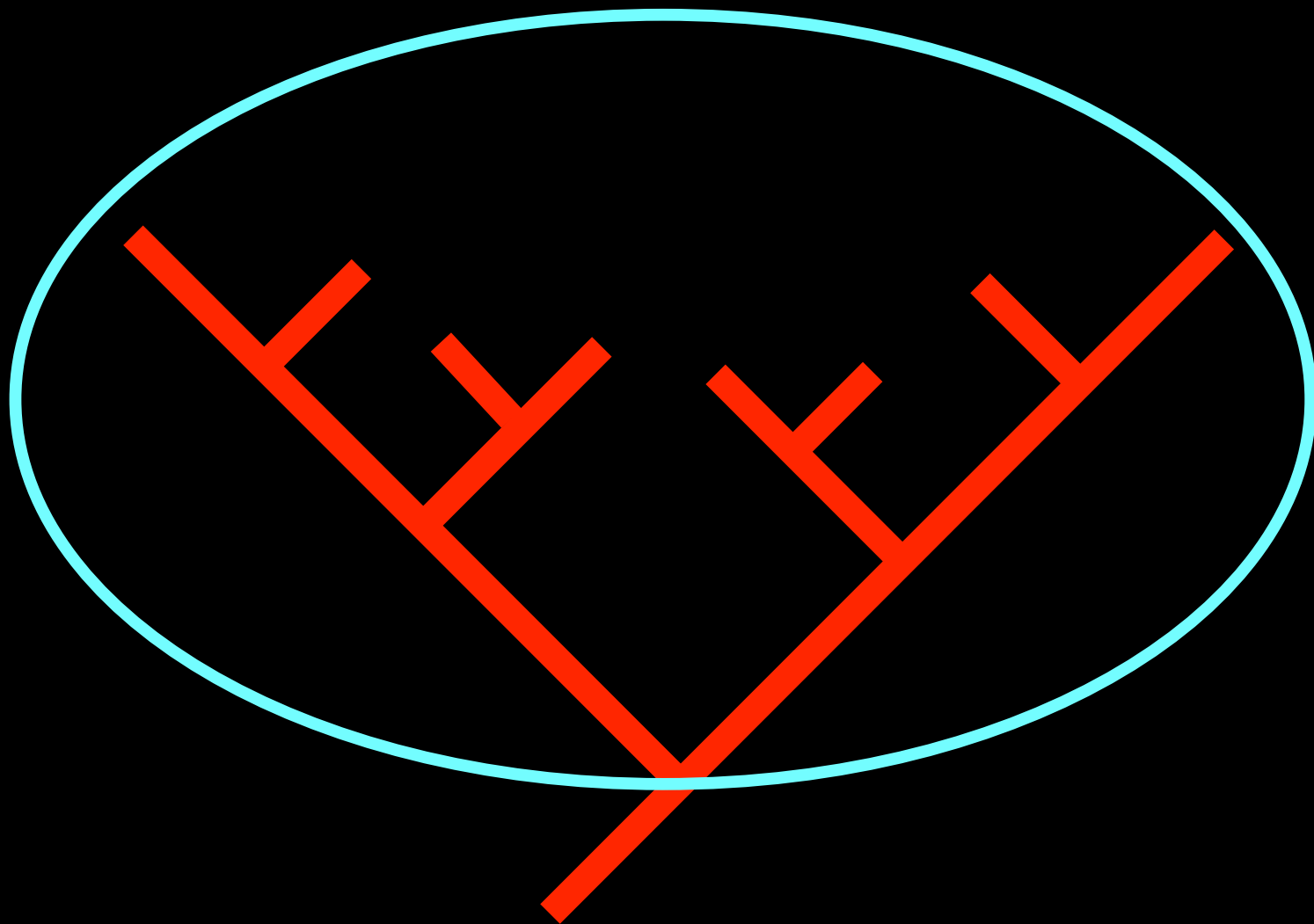


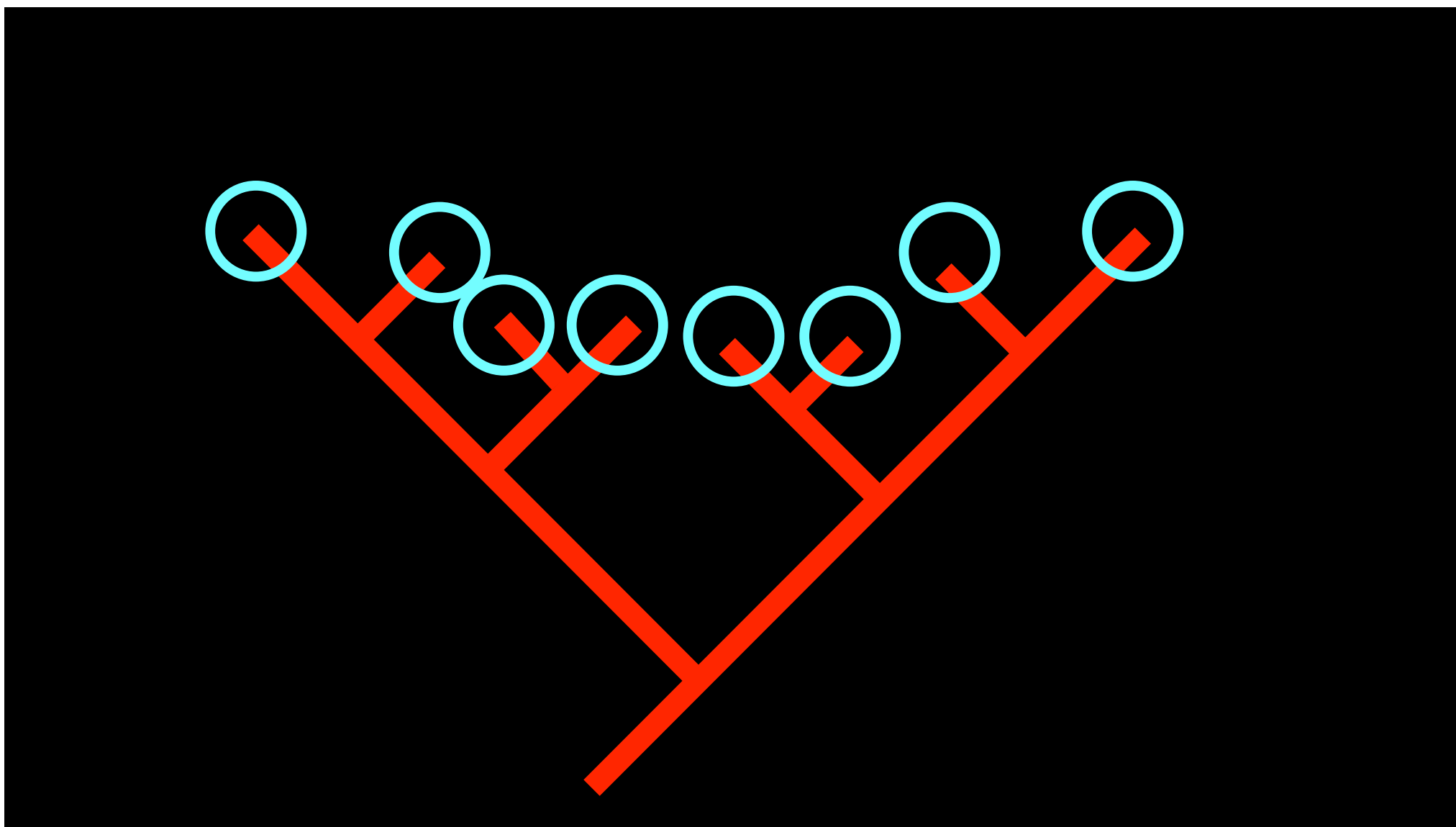


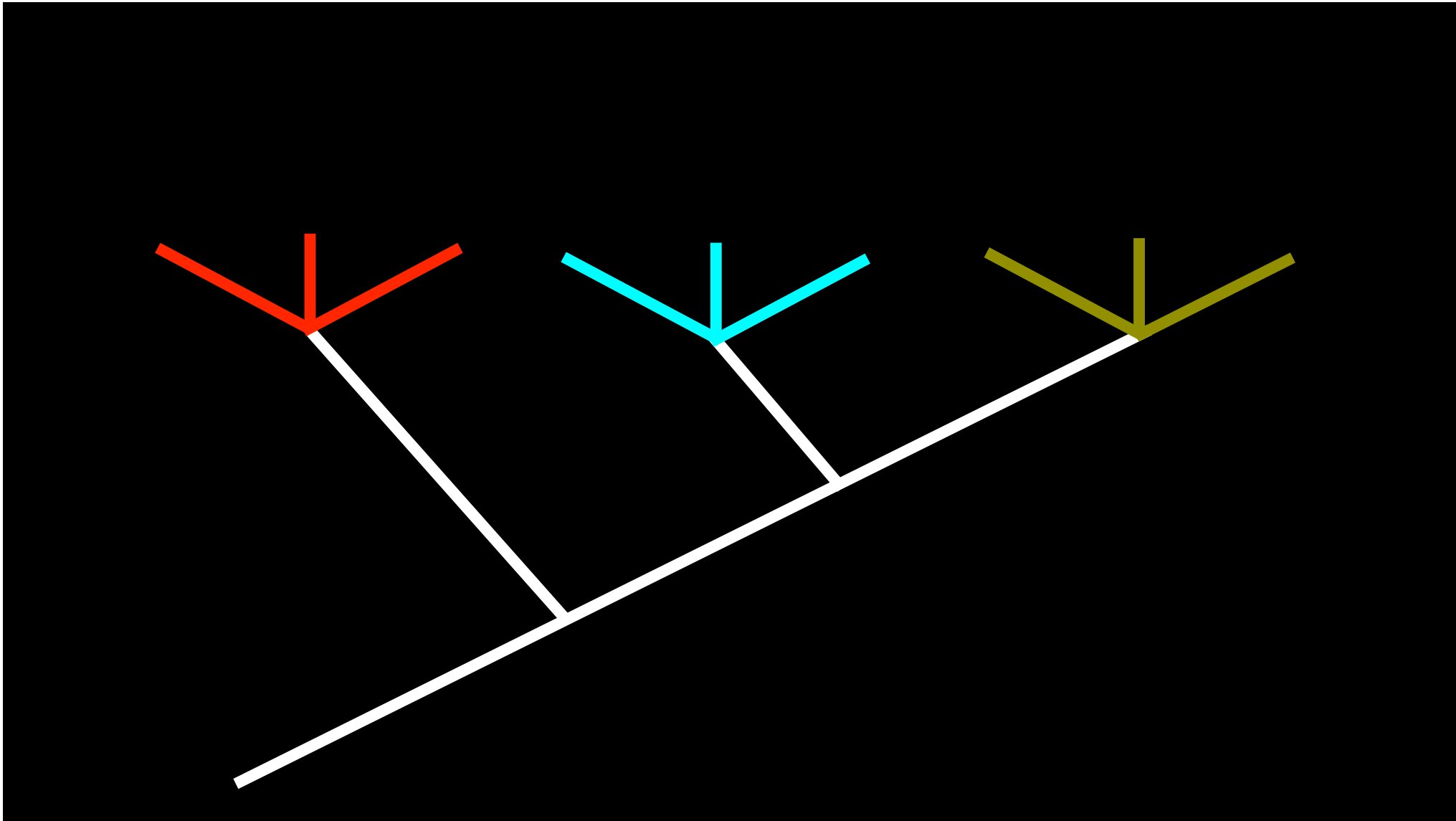


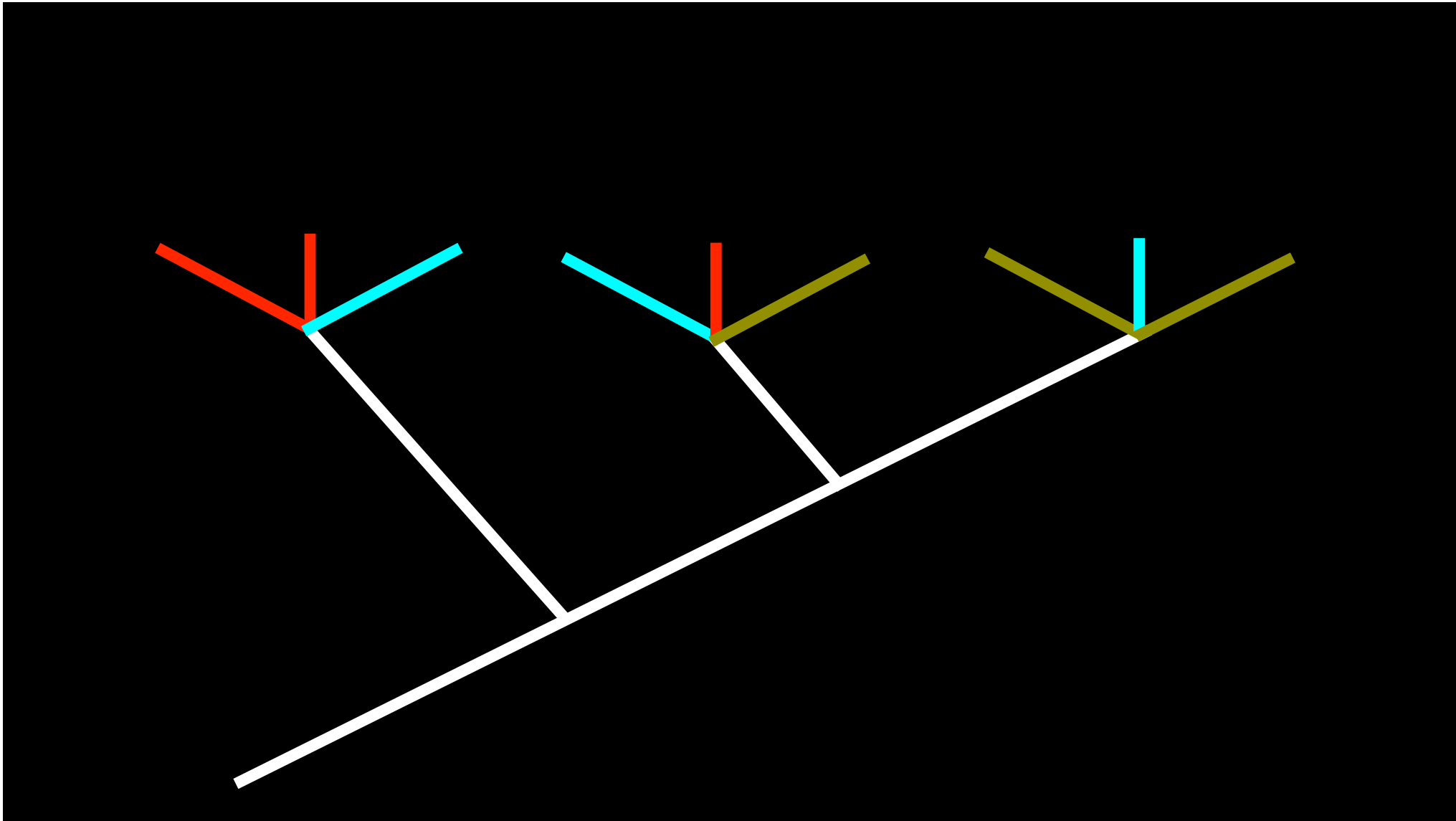


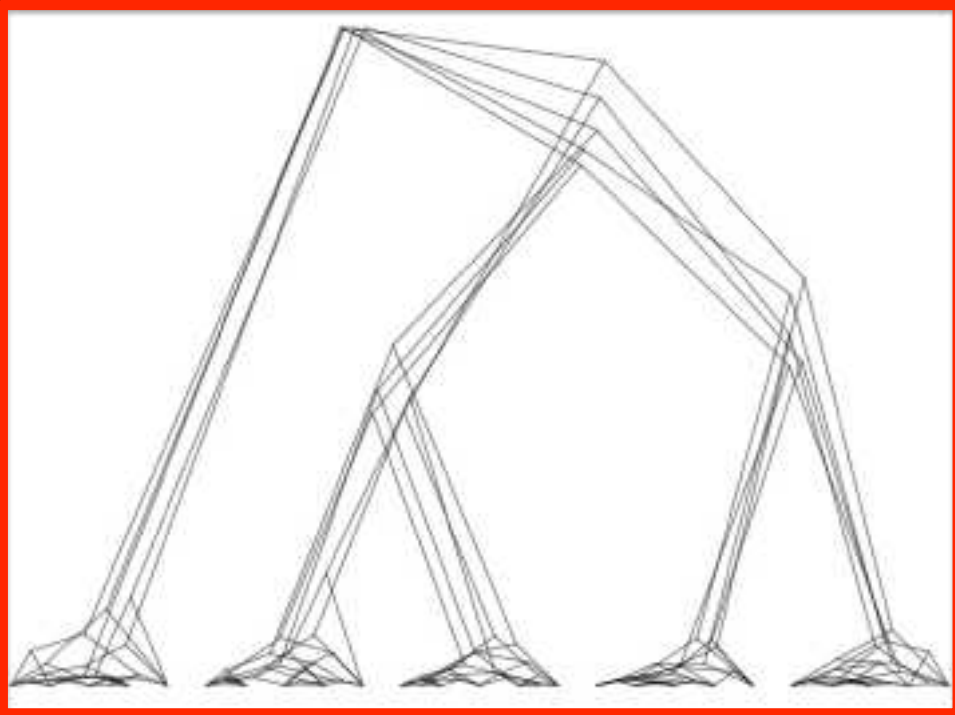




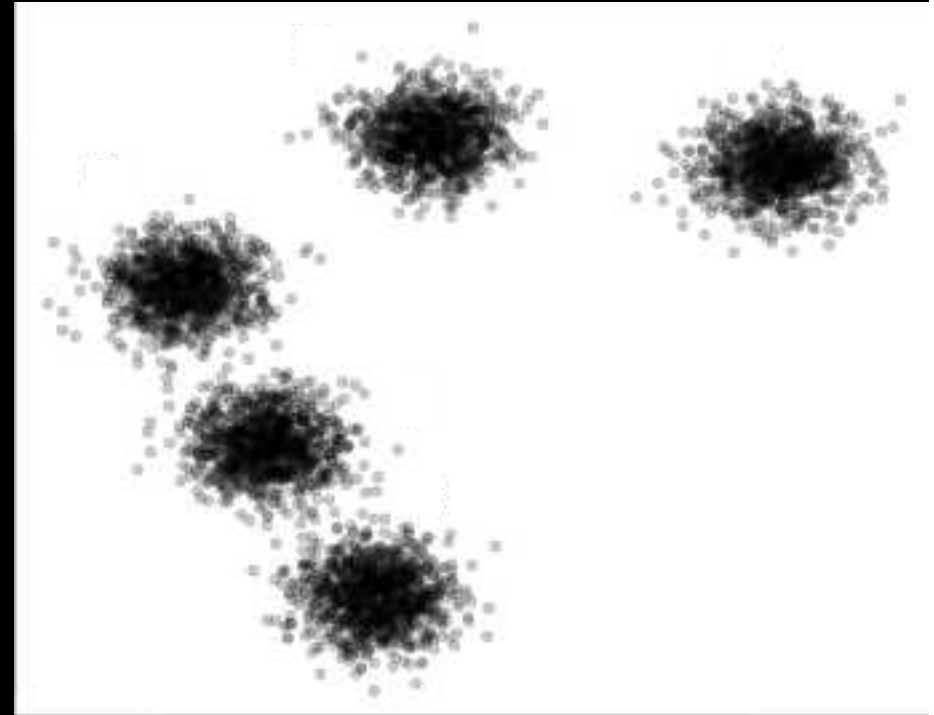








trait 2



trait 1

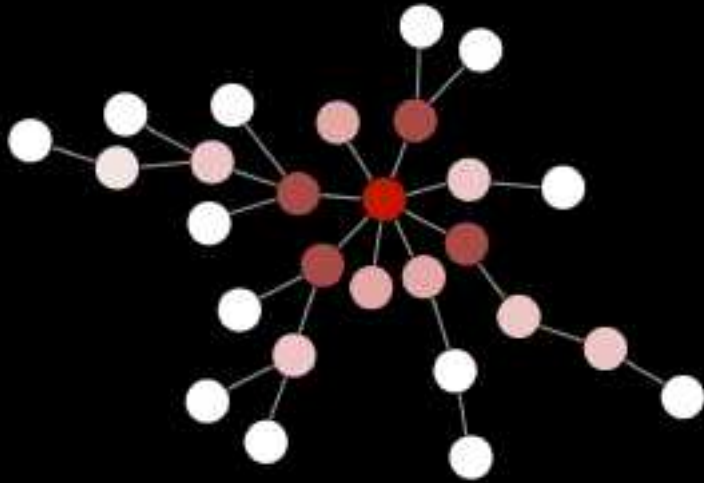
where to now with OTUs

operational taxonomic units

operational taxonomic units

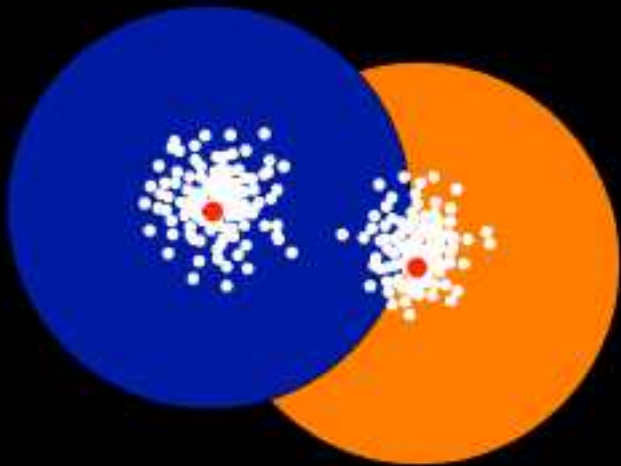
are

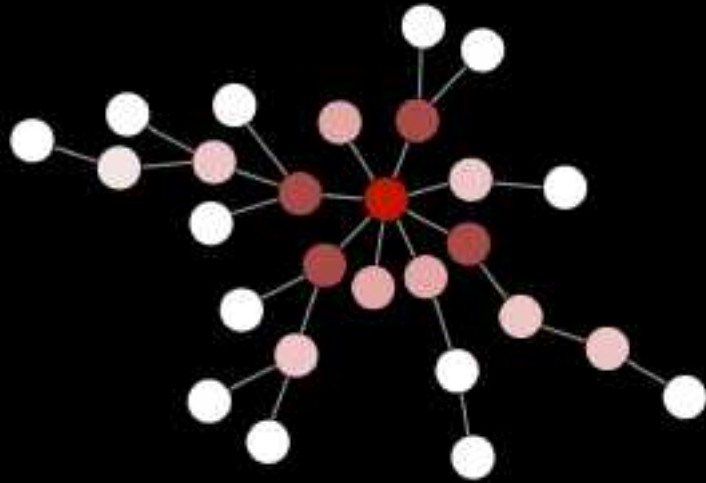
operational criteria for
species delimitation



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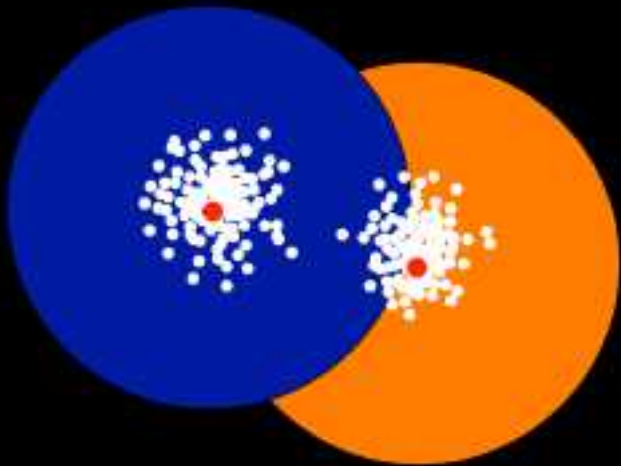
morpho
species

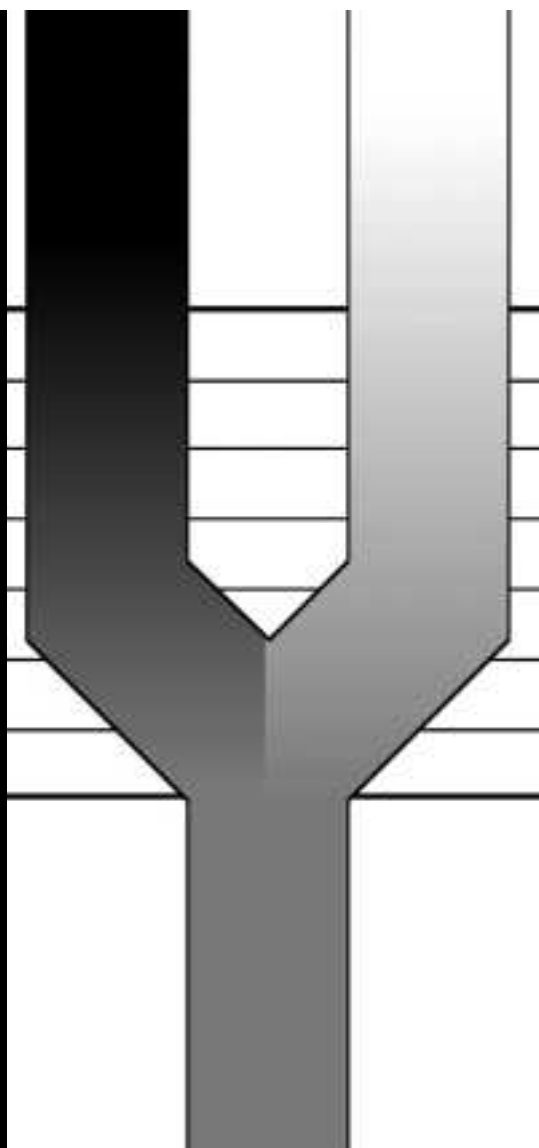
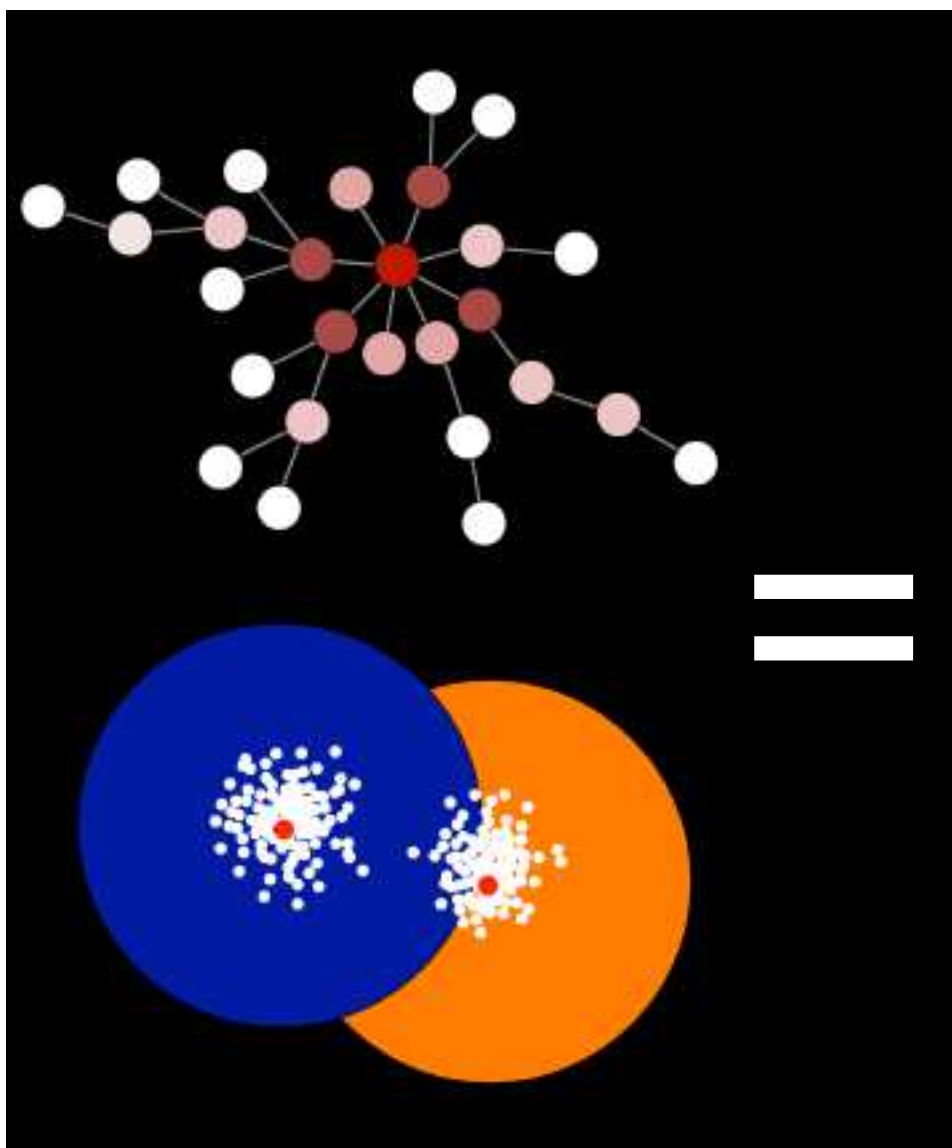




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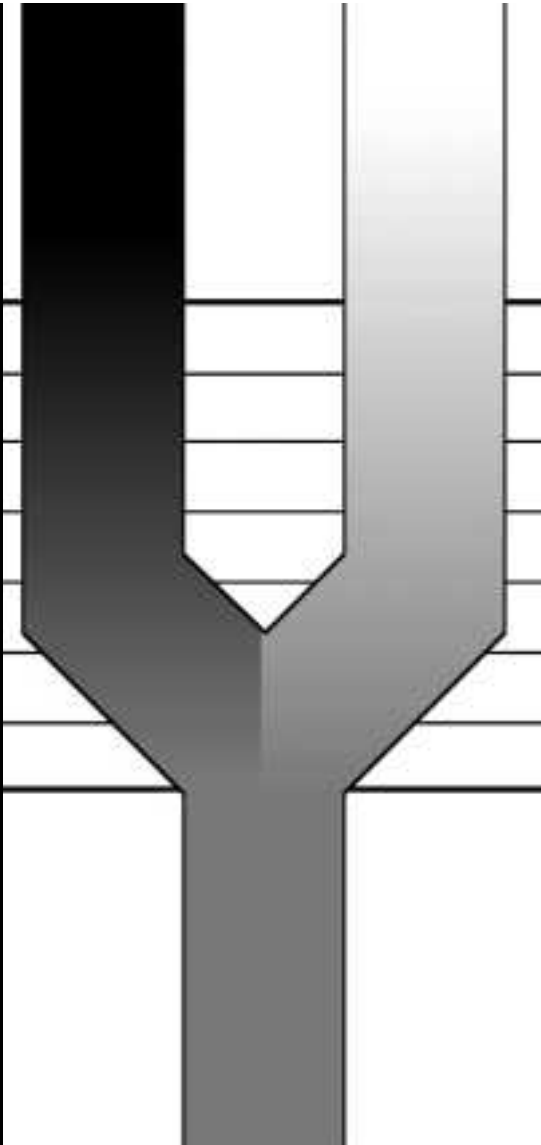
biological
species





operational
criteria

time ↑



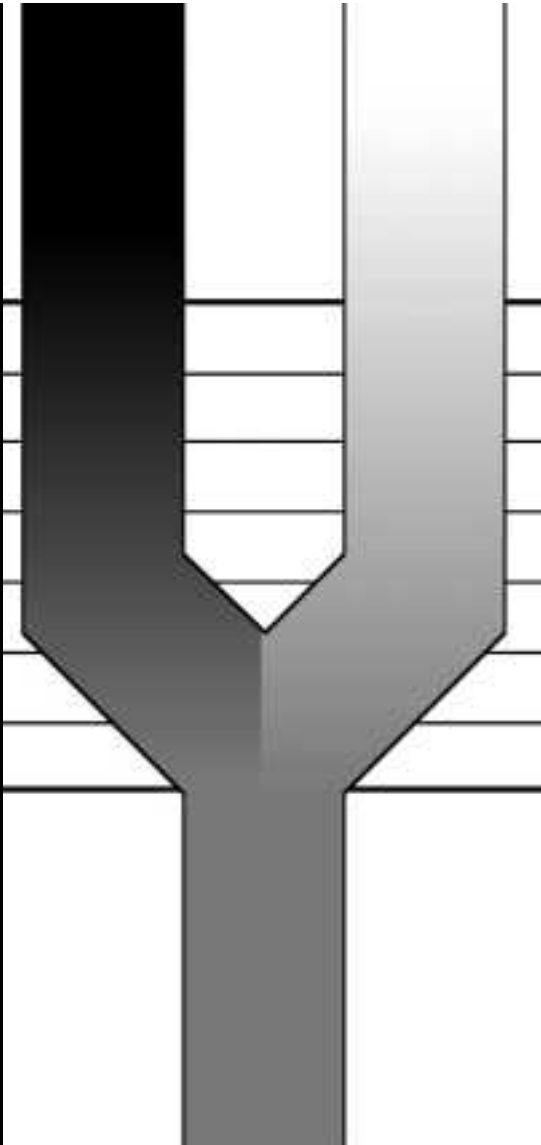
operational criteria

Phylogenetic

Morphological

Biological

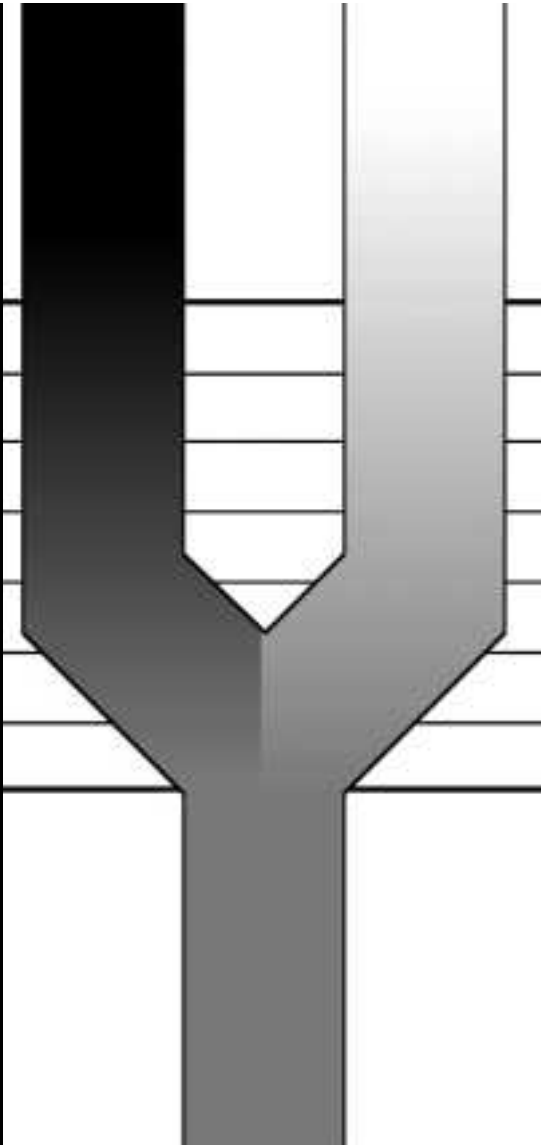
time ↑



operational criteria

Phylogenetic
Multi-rate PTP
Morphological
Swarm
Biological
DADA

time ↑



operational criteria

Phylogenetic
Multi-rate PTP

Morphological

Swarm

Biological

DADA

in conclusion

OTUs $\stackrel{?}{=}$ species

OTUS ? separately
evolving
= metapopulation
lineages

thanks to:

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