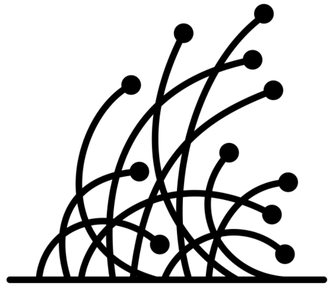


Duplications, ploidy and recombination

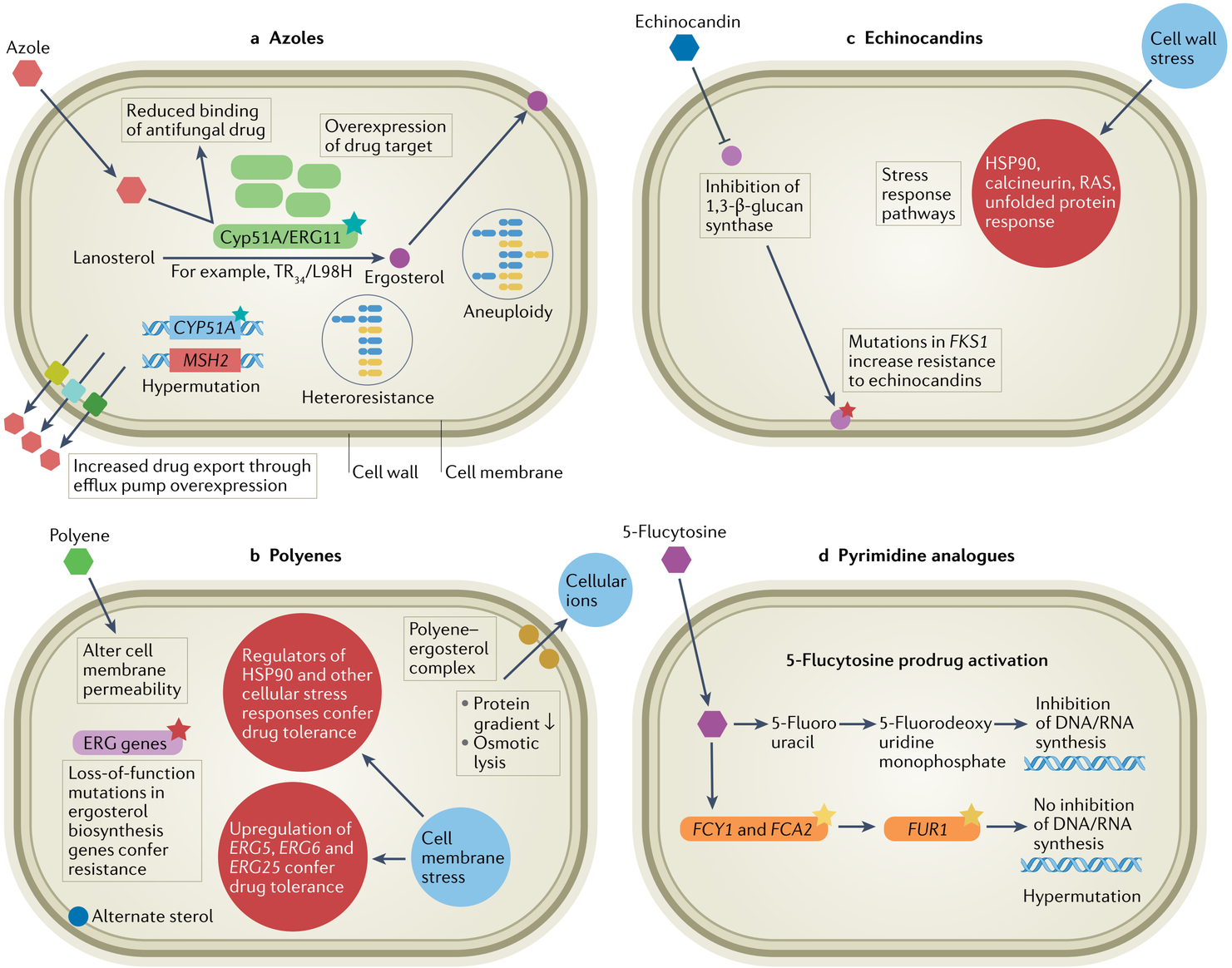
Dr Johanna Rhodes

Learning outcomes

- **What is duplication?**
  - tandem repeats, gene duplications
- **What is ploidy?**
  - Understanding ploidy
  - Aneuploidy and identification
- **What is recombination?**
  - Detection
  - Impact

Mechanisms of adaptation

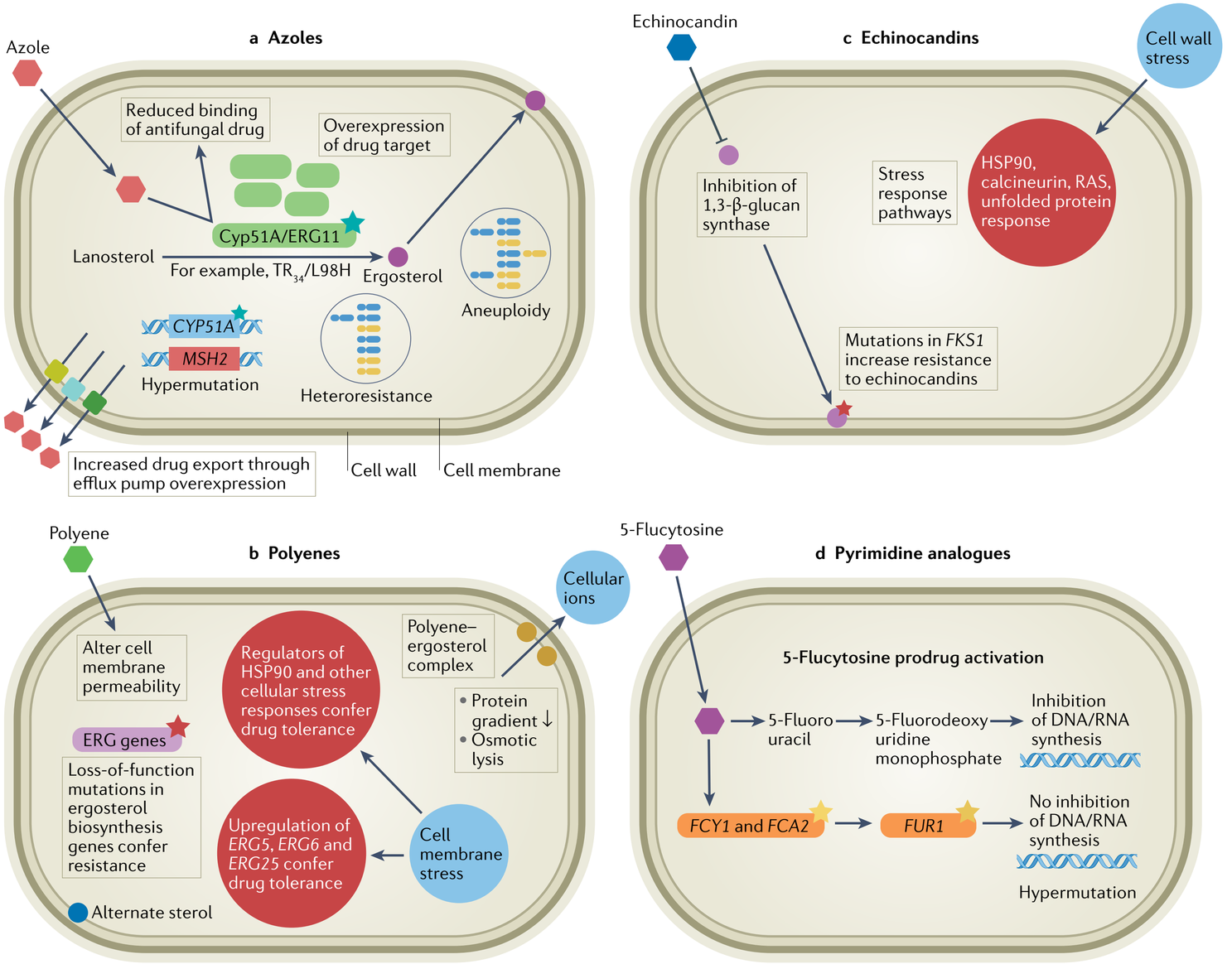
- Gene duplications
  - duplication of antifungal drug target *ERG11*
- Tandem repeats
  - repeats in promoter regions increase gene expression
    - *A. fumigatus* TR_{34} and TR_{46}



Fisher *et al.* 2022

Mechanisms of adaptation

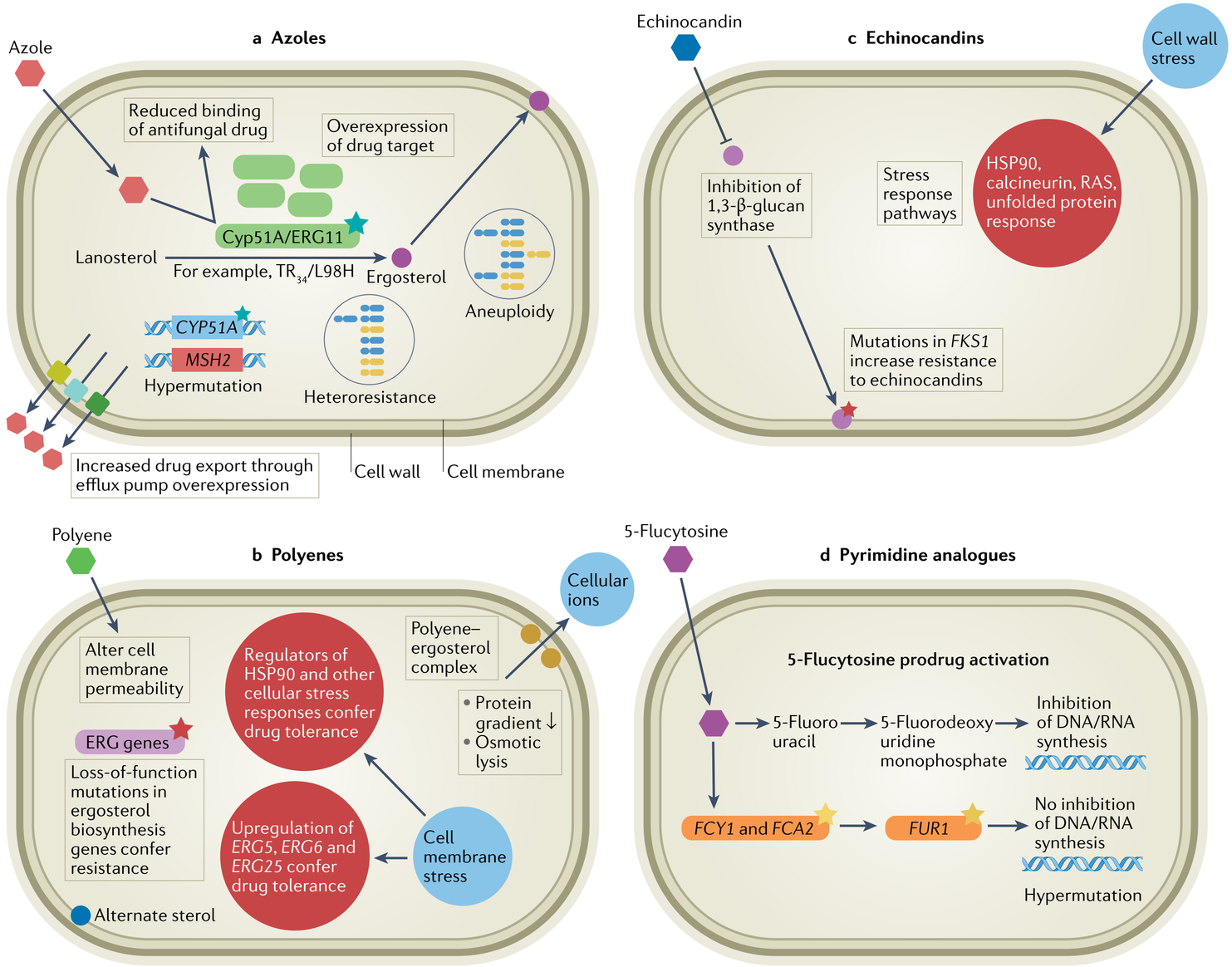
- Most eukaryotic species have two (diploid) or >2 (polyploid) sets of chromosomes
  - result of ancient whole genome duplication or hybridisation events
- Many clinically relevant fungi undergo ploidy changs during adaptation
  - to adverse/novel environments
    - some fungi exist as stable haploid, diploid, polyploid, undergo ploidy changes and revert back



Fisher *et al.* 2022

Mechanisms of adaptation

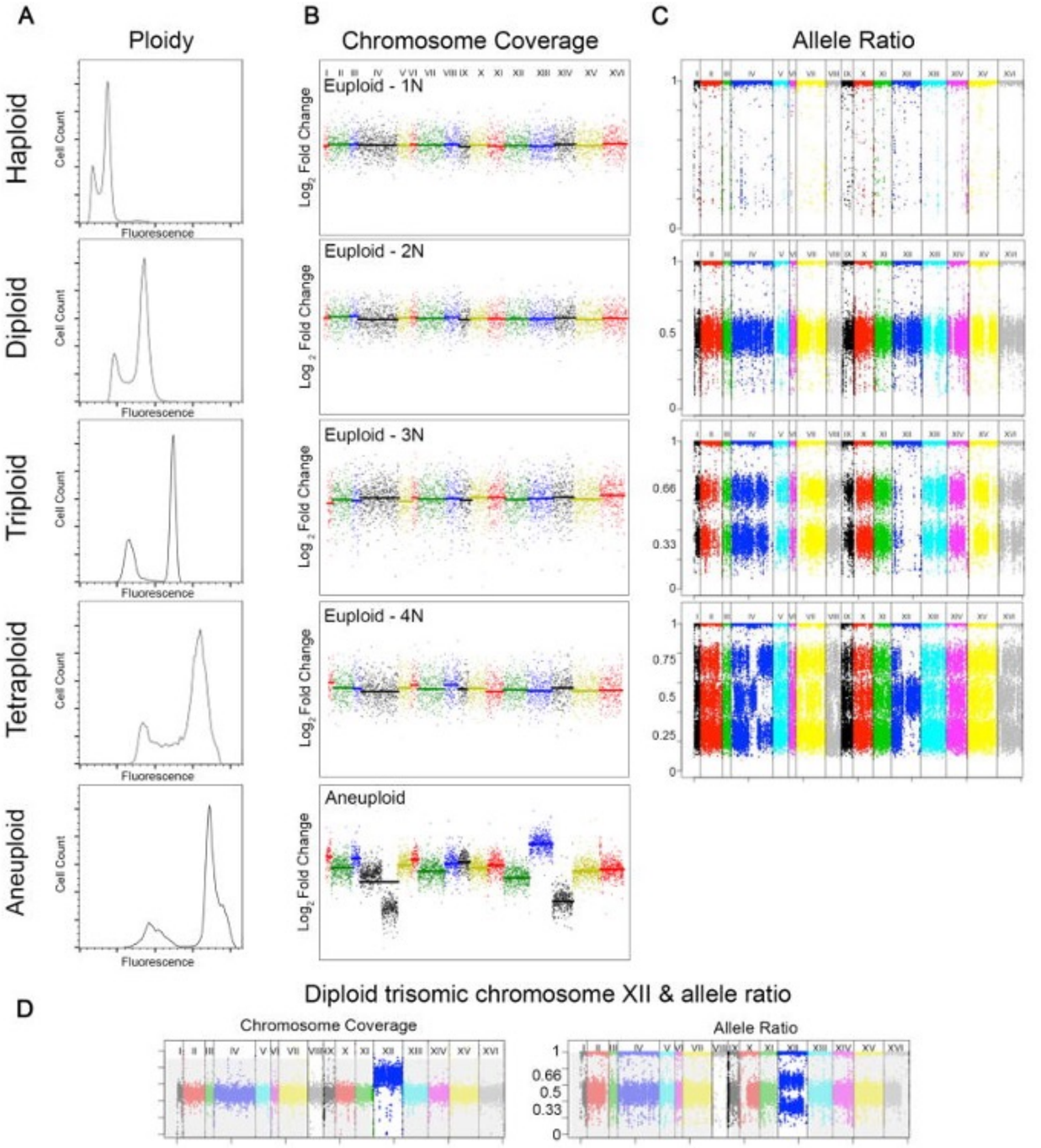
- Aneuploidy = abnormal chromosome number
  - observed in novel environments
  - periods of cellular stress
  - during ploidy level changes



Fisher *et al.* 2022

Aneuploidy detection

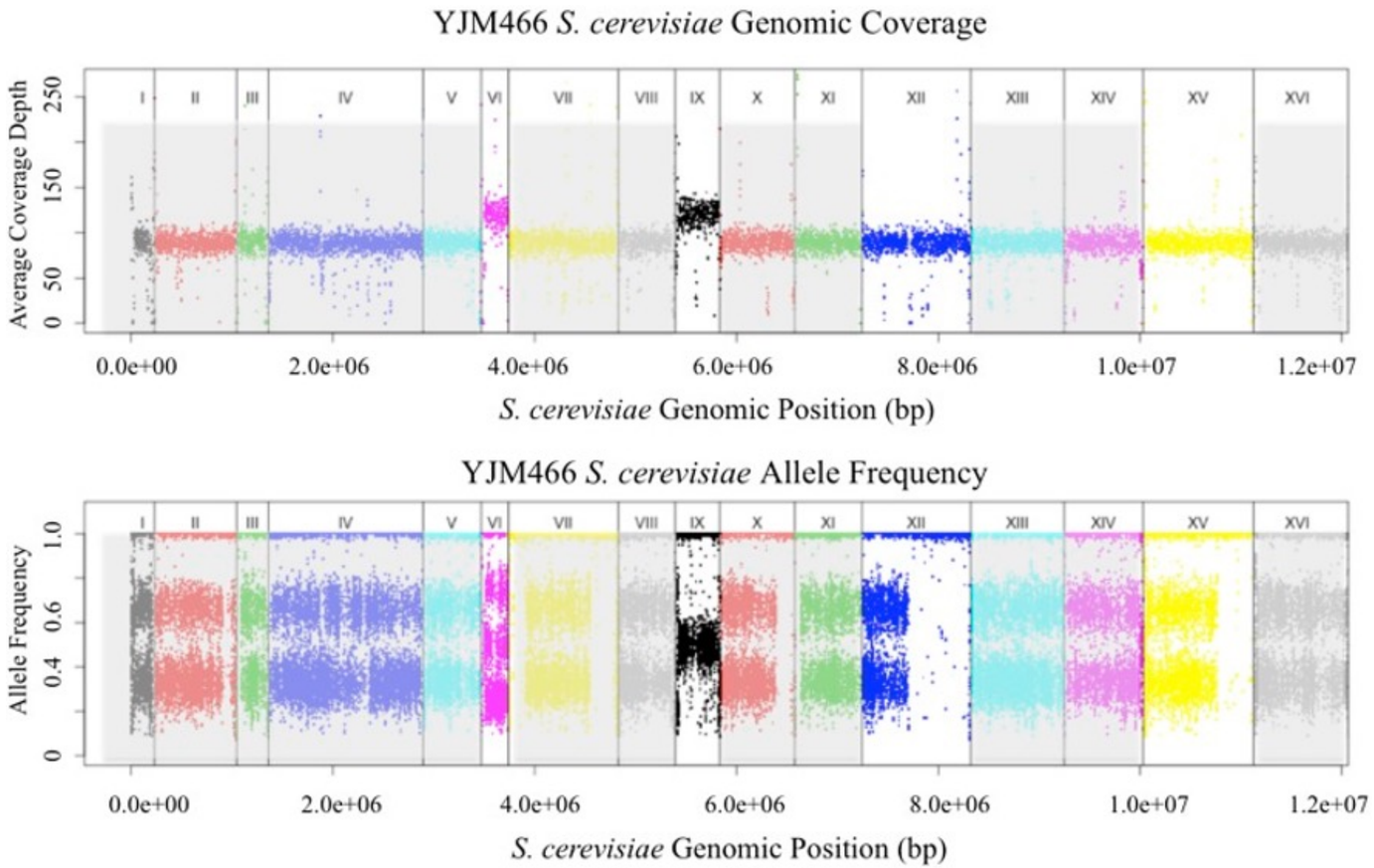
- Flow cytometry (A)
  - disadvantages:
    - isolates with single aneuploid chr may not be significantly different in fluorescent signal compared to known ploidy control
    - isolates with multiple aneuploidies may not show different DNA fluorescence because they cancel each other out
- Whole genome sequencing (B)
  - copy number of each chromosome relative to entire genome based on read depth
    - aneuploidy is increase or decrease in read depth relative to entire genome
    - can also pick up segmental chromosome aneuploidies and gene duplications/deletions
  - allele frequencies determine baseline ploidy
    - haploid (1N) genome will have allele frequency of 1, diploid (2N) will have an allele frequency of 0.5 and 1 etc (C)
    - limited to strains with significant heterozygosity



Zhu *et al.* 2016

Aneuploidy in fungi

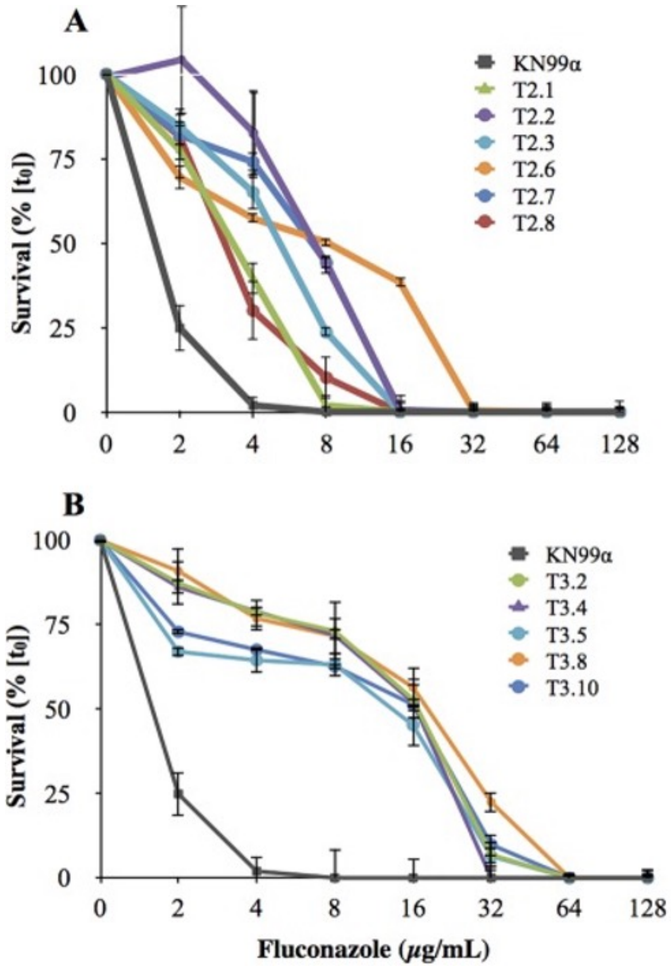
- *S. cerevisiae*
  - environmental isolates include stable haploids, diploids and polyploids
  - clinical and industrial isolates show aneuploidies
  - adaptive - stable aneuploid chromosomes found in sherry and ale strains



Zhu *et al.* 2016

Aneuploidy in fungi

- *S. cerevisiae*
  - environmental isolates include stable haploids, diploids and polyploids
  - clinical and industrial isolates show aneuploidies
  - adaptive - stable aneuploid chromosomes found in sherry and ale strains
- *Cryptococcus neoformans*
  - Aneuploidy, and amplification of Chr 1 is linked to antifungal drug stress and resistance
- *Candida albicans*
  - aneuploidy seen as adaptation to growth on alternative growth sources, exposure to drugs, high temperature, host interactions



Strain	Aneuploidy	MIC_{50}	MIC_{90}
KN99α	---	2	4
T2.1	+Chr6, -Chr13	4	8
T2.2	+Chr1	8	16
T2.3	+Chr1	8	16
T2.6	+Chr1, -Chr9	16	32
T2.7	+Chr1,8	8	16
T2.8	-Chr13	4	8

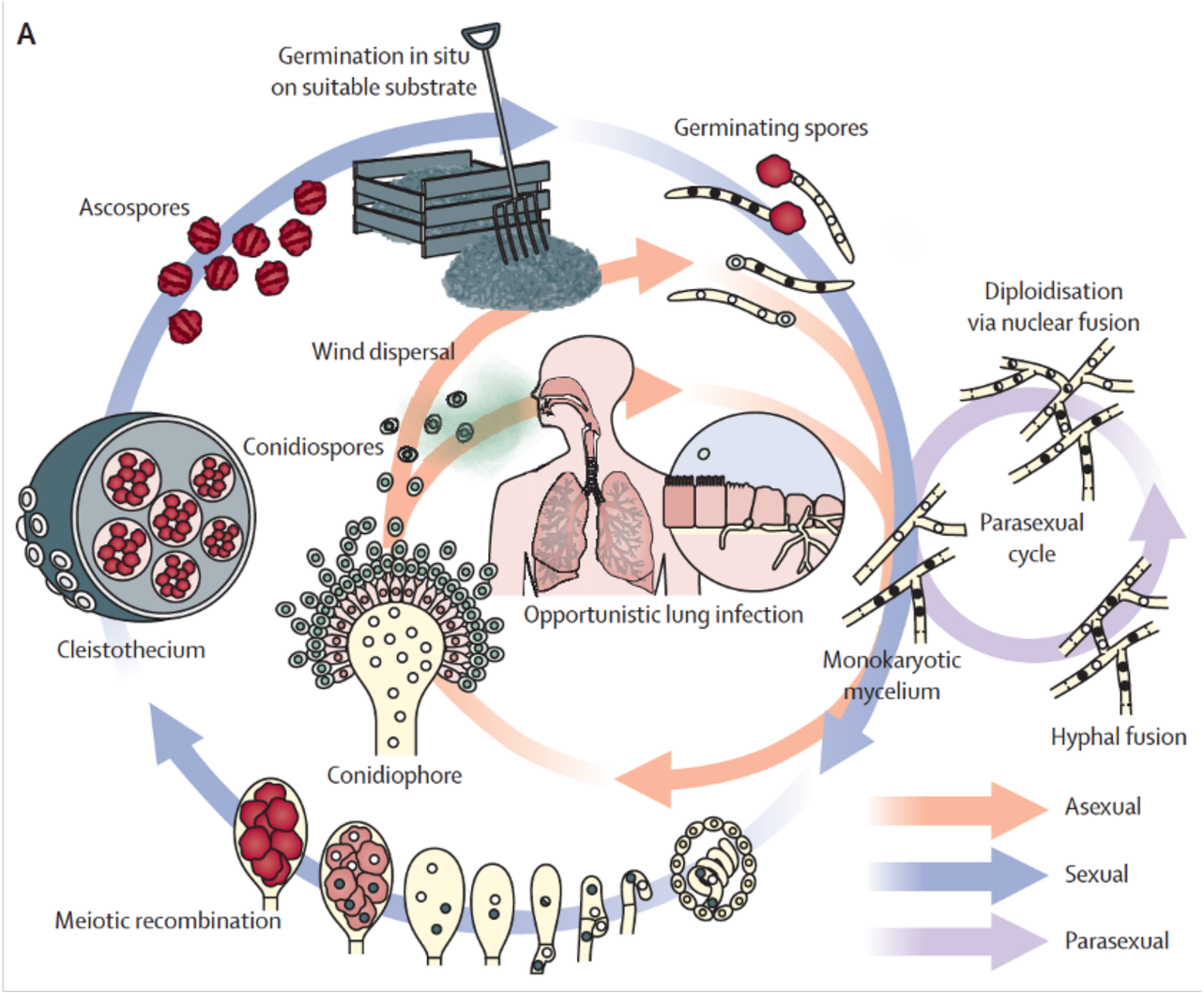
Strain	Aneuploidy	MIC_{50}	MIC_{90}
KN99α	---	2	4
T3.2	+Chr1	16	32
T3.4	+Chr1	16	32
T3.5	+Chr1, 4	16	32
T3.8	+Chr1	32	64
T3.10	+Chr1, 4	32	32

Gerstein *et al.* 2015

Recombination

Generating diversity

- Breaking and reassembling pieces of DNA to create new combinations of alleles.
- Sexual recombination:
  - involves meiosis —> crossing-over —> exchange of DNA
  - need two compatible mating types
  - creates genetic variation in the offspring

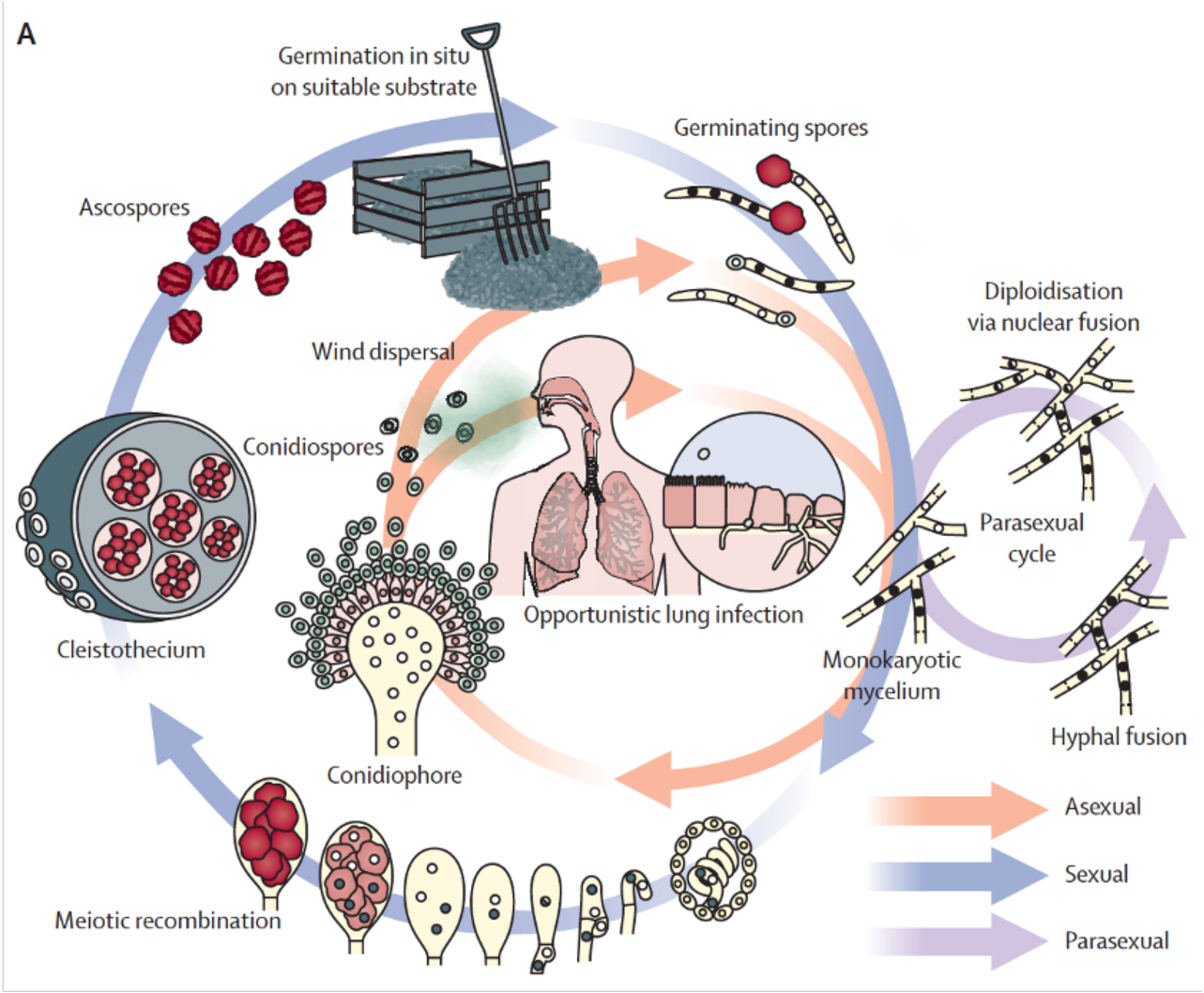


Zhang *et al.* 2021

Recombination

Generating diversity

- Breaking and reassembling pieces of DNA to create new combinations of alleles.
- Parasexual recombination:
  - no meiosis
  - undergo fusion to form heterokaryons, and chromosomes exchange segments before returning to a haploid state.



Zhang *et al.* 2021

Recombination

Generating diversity

- Gene conversion:
  - DNA repair mechanisms may favour one gene sequence over another, leading to unequal representation/new allelic combinations
- Mitotic recombination
  - gene shuffling in asexual fungi

Recombination

Generating diversity

- Adaptability
  - Environmental adaptation e.g. temperature, nutrient availability
- Drug resistance and/or virulence
  - see *A. fumigatus* - highest number of meiotic crossovers
- Speciation
  - Recombination contributes to genetic divergence, and formation of new species