

# Virulence QTLs and Genome-wide Recombination Rates in *Cryptococcus*

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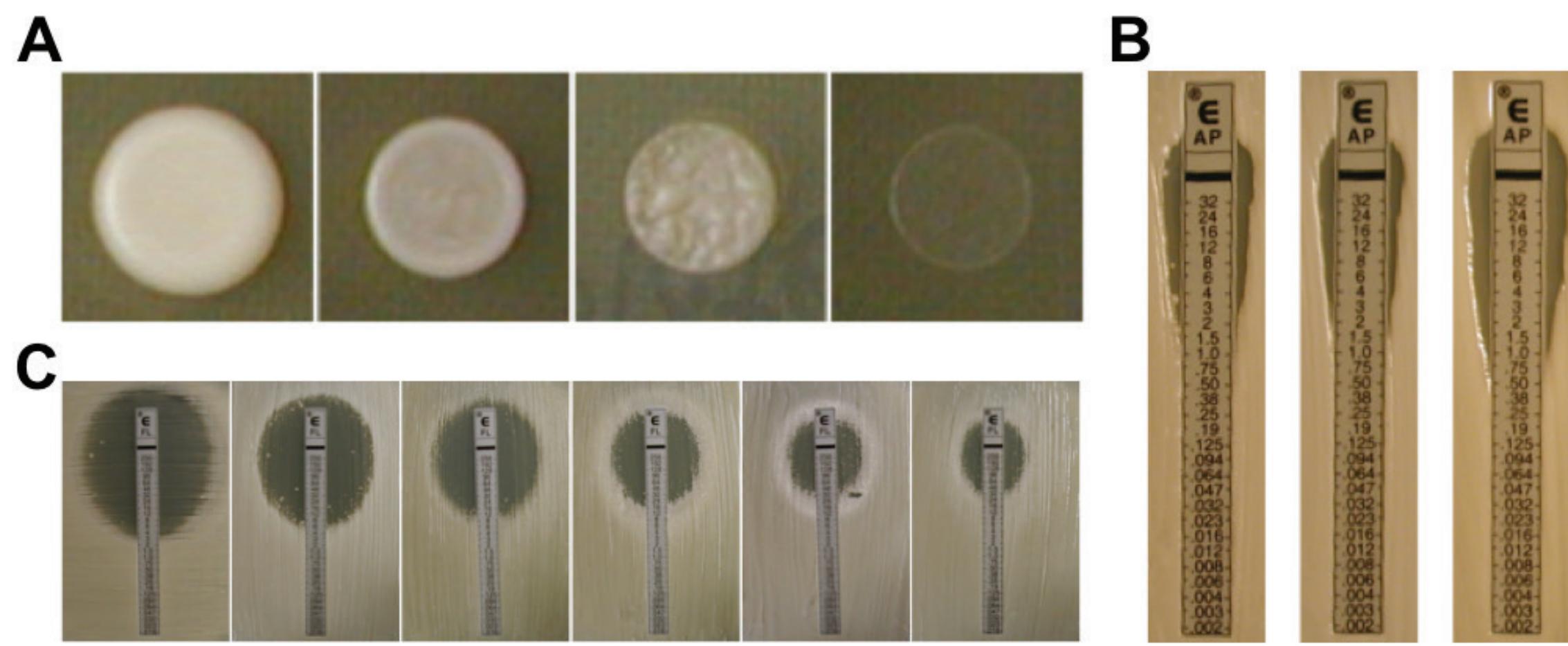
## Introduction

Cryptococcal disease affects ~223,100 people, causing approximately 180,000 deaths, annually<sup>1</sup>. Environmental isolates of *Cryptococcus deneoformans*, vary in their pathogenicity, ranging from benign to hyper-virulent.

**Goal:** Identify the genetic basis underlying the variation in virulence-related traits, (high temperature tolerance and resistance to anti-fungal drugs, amphotericin B and fluconazole) using quantitative trait locus (QTL) mapping in *C. deneoformans*.

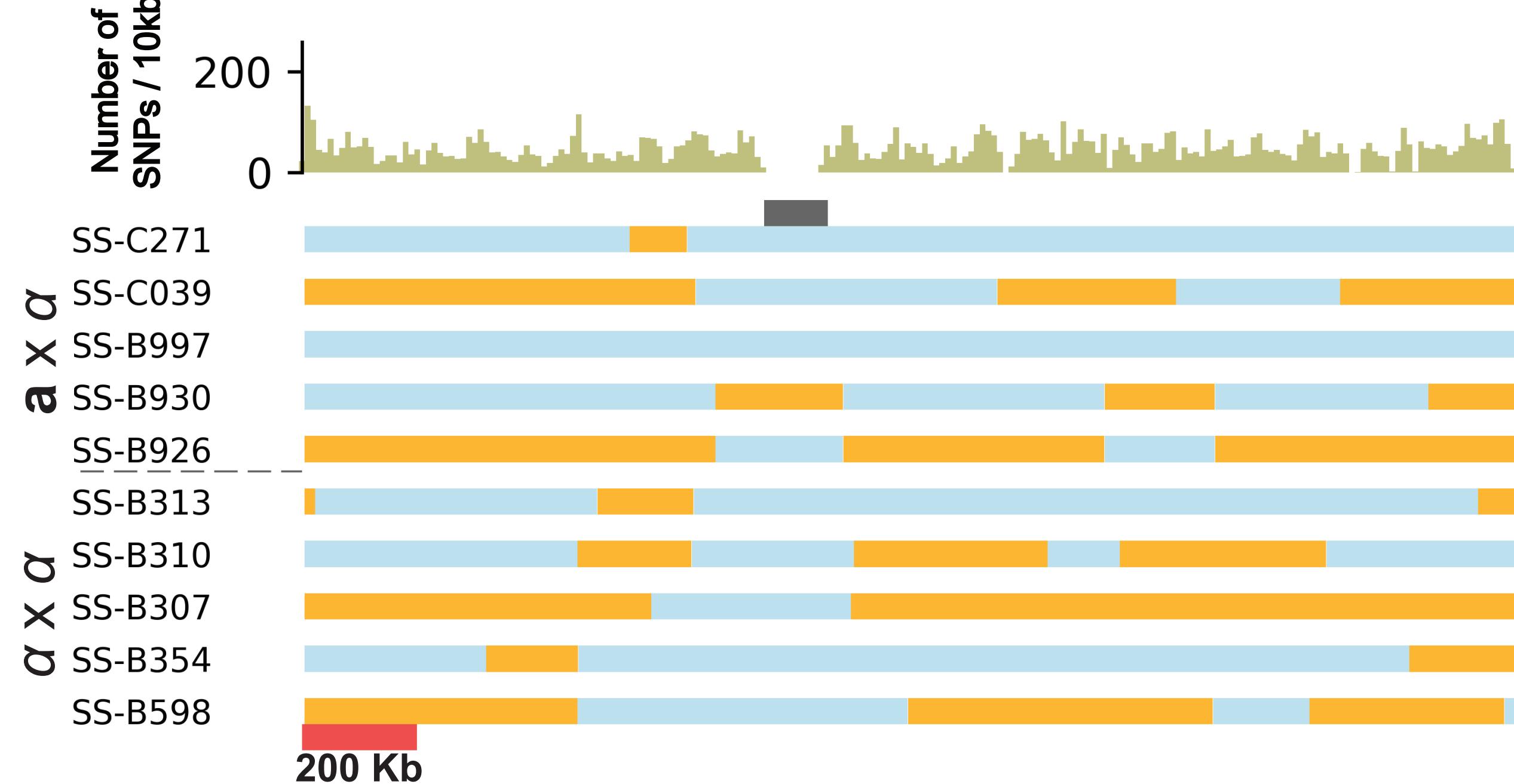
Strains XL280 $\alpha$ , XL280 $\alpha$ , and 431 $\alpha$  were crossed to generate haploid segregants via  $\alpha$ - $\alpha$  bisexual reproduction ( $N = 39$ ) and  $\alpha$ - $\alpha$  unisexual reproduction ( $N = 55$ ). Approximately 87,000 single nucleotide polymorphisms (SNPs) were identified across these segregants using short-read sequencing<sup>2</sup>.

## Variation of Virulence Traits



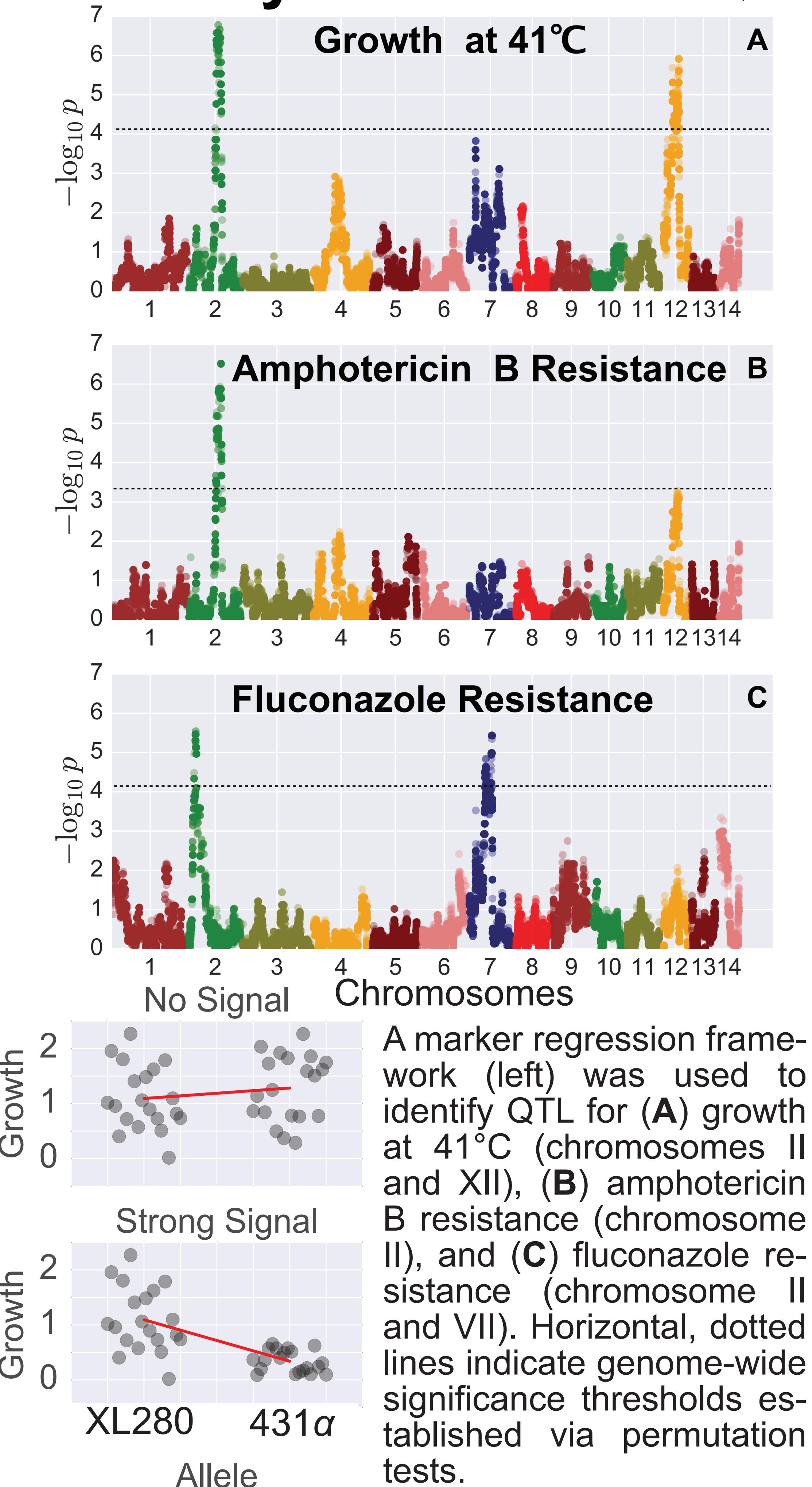
(A) The variation in growth at 41°C on YPD plates is shown for 4 segregants. (B) For amphotericin B resistance, E-strips were used to calculate the minimum inhibitory concentration (MIC). A similar assay was used to calculate (C) the MIC for fluconazole resistance. The results of 6 segregants are shown.

## High Density Recombinant SNP Data

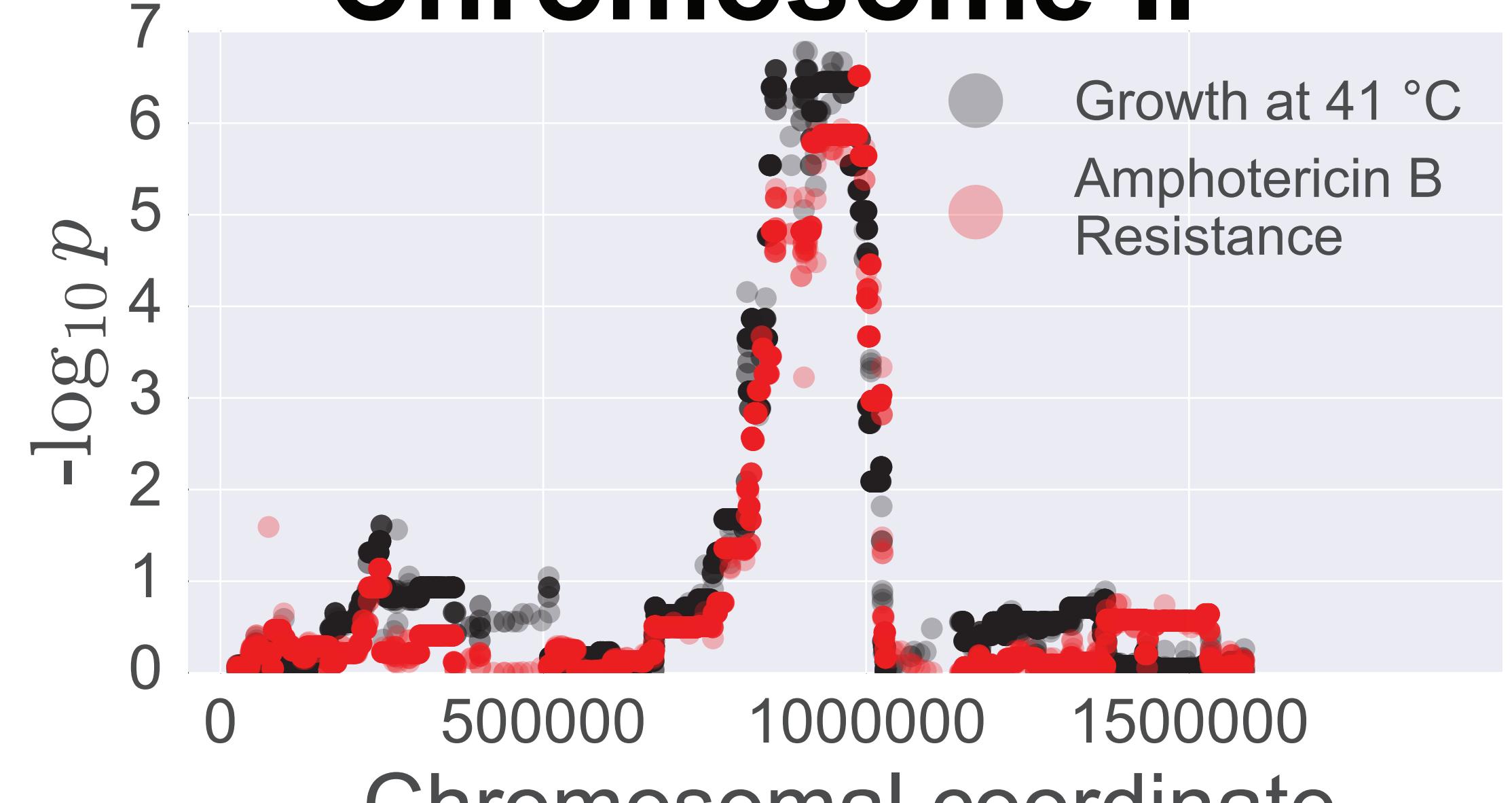


From top to bottom, the SNP density for chromosome 3 (length ~2.1Mb) calculated as the number of SNPs per 10kb (Total: 9779 SNPs), the haplotypes for 5 segregants from the bisexual cross and 5 segregants from the unisexual cross. Haplotypes are inferred from SNP data and displayed as blue if inherited from XL280 $\alpha$  and XL280 $\alpha$  or orange if inherited from 431 $\alpha$ . The position of the centromere is displayed in black.

## Discovery of Virulence QTL

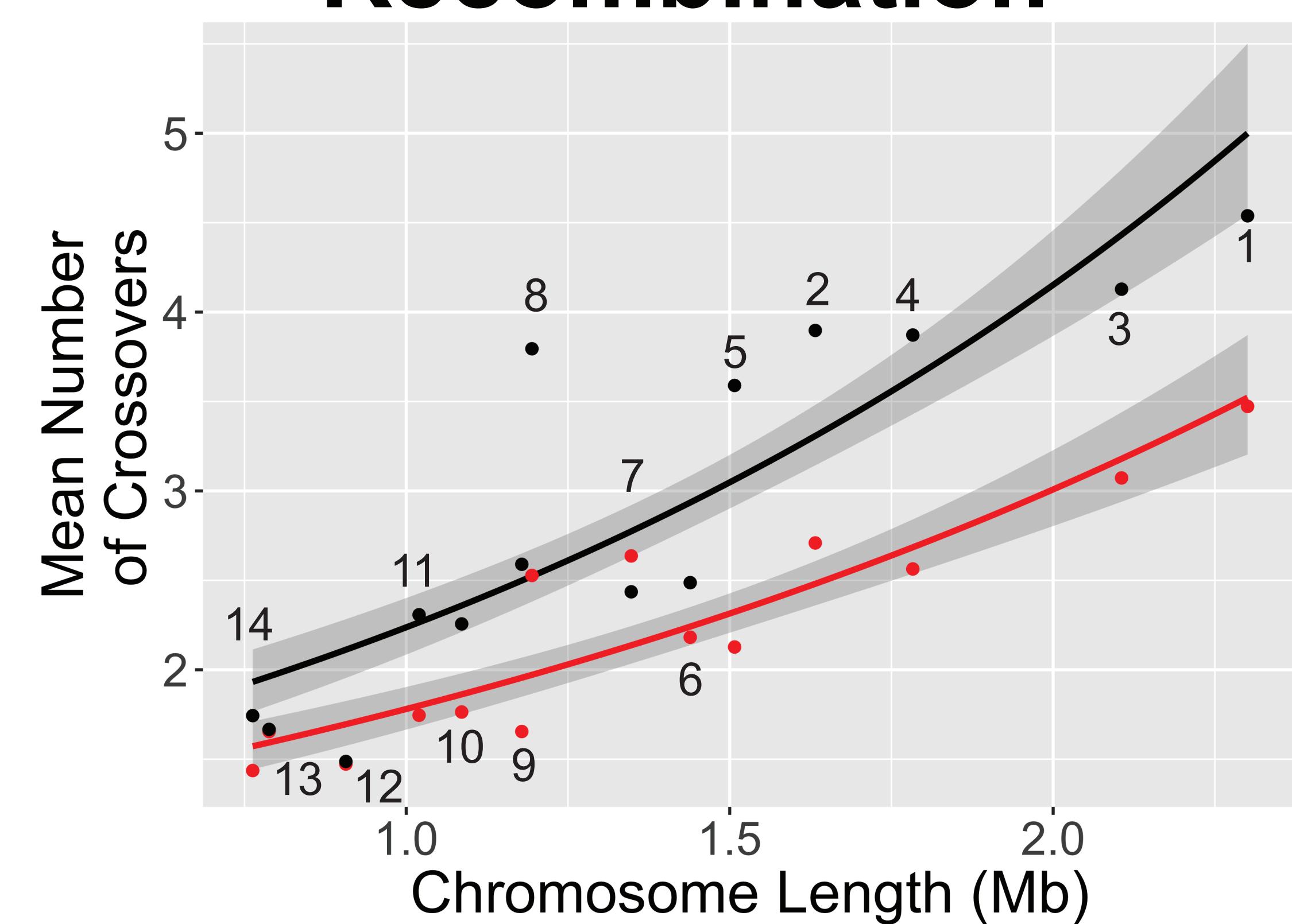


## Pleiotropic QTL on Chromosome II



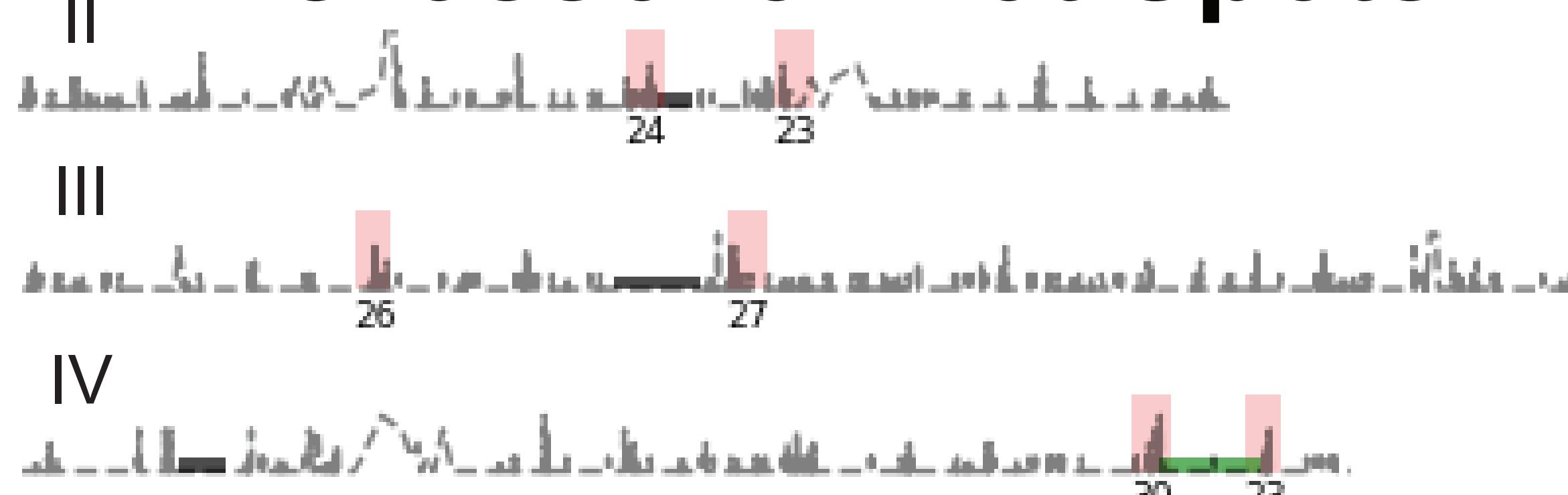
A QTL implicated in high temperature growth and amphotericin B resistance. A total of 48 genes lie in the QTL, two (*HRT1* and *KTR3*) are known to play a role in temperature sensitivity<sup>3,4</sup> and one (*SSK1*) is known to cause amphotericin B sensitivity in *C. neoformans*<sup>5</sup>.

## Genome-wide Recombination



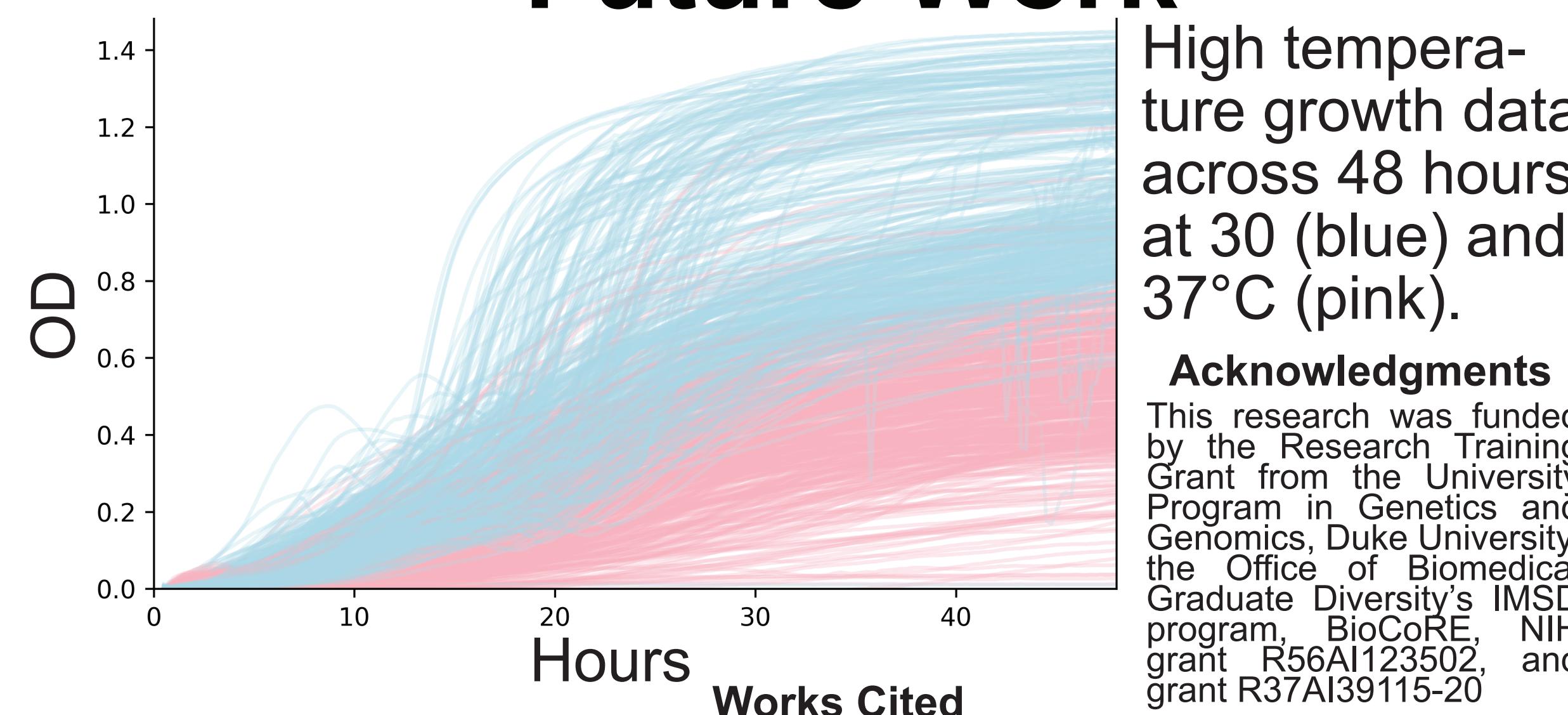
Genome-wide recombination rates were estimated using Poisson regression. This model predicts an obligatory ~0.98 crossovers per chromosome for offspring from the  $\alpha$ - $\alpha$  unisexual crosses (red) and ~1.30 crossovers per chromosome for offspring from the  $\alpha$ - $\alpha$  bisexual crosses (black). The expected number of crossovers increases by a ratio of ~1.768 per Mb increase in chromosome size (slopes of red and black lines). Shaded regions are 95% confidence intervals of the regression estimates. Numbers indicate chromosomes.

## Crossover Hot Spots



The crossovers along chromosomes II, III, and IV are shown. Each chromosome was overlaid with 50kb bins to identify crossover hot spots (highlighted red) using the expected number of crossovers from our analysis above. The number of crossovers within hot spots are labeled underneath. The *MAT* locus and centromeres are displayed as green and black bars, respectively.

## Future Work



High temperature growth data across 48 hours at 30 (blue) and 37°C (pink).

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