Hypotheses:

**Hypothesis**

**1) Does gene expression at the level of the whole chromosome scale with DNA levels for aneuploid chromosomes?**

**If not, is it a significant difference?**

**Do different lines with the same aneuploid chromosome(s) have similar expression levels on that chromosome?**

Analysis

t-test (or non-parametric equivalent) between expected gene expression level and average observed expression level

t-test on gene expression levels between different lines with the same aneuploid chromosome

\*also: use JMP to plot distribution of gene expression levels on aneuploid chromosome – should not be normally distributed

**2) Are there certain genes that are up or down regulated in all aneuploid lines? (ESR?)**

**Which ones are on the aneuploid chromosome and which ones are elsewhere?**

**Are there certain genes shared between ALL lines that are up- or down-regulated, regardless of ploidy? (MA effects?)**

**Are there certain genes on euploid lines that are commonly up- or down-regulated? Are any of these different than the aneuploid lines?**

Using cuffdiff, compare the output files listing DE genes to one another

Only aneuploid lines >50%, >75%, >90%

Sort resulting file by chromosome

then do again using all of the lines

Also separate by experiment and sequencing time

**3) What is the rate of aneuploidy? Is it different between GC and MA, and MA and old MA?**

Look up how to calculate this

**4) What are the fitness consequences of being aneuploid?**

Sam’s part

**5) Are histone genes dosage compensated? Are any ESR genes up- or down-regulated?**

**Other DE genes:**

**What are they? Are there any housekeeping genes?**

Cuffdiff

Do at same time as Hypothesis #2

**Dosage compensation/RNAseq analysis methods/notes:**

Reference genome from SGD – latest update (31-Jan-2015) (S288C)

1. Need to build an index using bowtie

use bowtie2-build (code #1 in text file)

2. Need to use Cufflinks to change .gff transcript file into a .gtf file

<http://cole-trapnell-lab.github.io/cufflinks/file_formats/#the-gffread-utility>

Questions:

What chromosomes are aneuploid? Are there any that are commonly aneuploid? Never aneuploid?

Is there a correlation between type of aneuploidy and fitness?

Is there whole-chromosome dosage compensation? What about individual genes? Environmental stress response genes? Is the “aneuploid stress response” from Torres et al 2010 turned on?

Does the rate of aneuploidy correlate with wild/lab strain status?

In the absence of DC, results are …

Look up the rate of aneuploidy in wild yeast versus lab yeast

Chromosomal instability and heterozygosity?

Mating between 2 haploids, so no lethals to uncover

Framework: How often do aneuploidies arise in the absence of selection?

and what are they, what are their fitness effects?

-Get aneuploids in the wild, but that could be from selection in a potentially stressful environment (ie oxidative stress)

Looking at spontaneous aneuploidies in the absence of selection allows us to get a baseline for how often these spontaneously arise

Looking for if there is a common mechanism of DC in autosomes. Sex chromosomes evolved from autosomes, so they may have brought with them some inherent way of compensating for multiple gene copies.

Are there certain regions on the chromosome that are dosage compensated?

Because my data has so much variation, probably better to look at individual genes

Can look to see if there is expression at a particular site

Chromosome position on x-axis, by color (blue to red, white in the middle). Genes in boxplot(?) colored according to the chromosome position – could tell me if there are any regional compensation effects

Multifactorial statistics?

Can probably ask a lot of questions with this data

Do our aneuploid lines have more gene conversion events?

**Introduction**

Aneuploidy is when an organism contains an abnormal chromosome number, i.e. one not a multiple of the haploid state. There is some debate as to why aneuploidy is often maintained (or tolerated) in populations. Some hypothesize there is an intrinsic mechanism of dosage compensation to buffer the deleterious effects of imbalanced gene dosage [1, 2]. Others contest this argument, claiming there is no evidence for dosage compensation at the whole-chromosome level in S. cerevisiae [3]. An alternate hypothesis is that the accumulation or loss of chromosomes is an adaptive advantage to stressful environments, such as the case with yeast in an oxide-rich media that accumulate an extra copy of chromosome XI [4]. Here, we investigate whether there is an innate dosage compensation response in spontaneously aneuploid yeast that have been put through a 2000-generation mutation accumulation experiment with a single-cell bottleneck every 20 generations [5]. This allows us to examine the effects of spontaneous aneuploidies with little to no selection. The absence of selection allows us to analyze the transcriptome without bias from environmental factors, enabling us to determine the effects of aneuploidy on the genome in a stable condition and without selecting for any particular aneuploidies or mutations.

**Methods**

*Strains, RNA extraction, and sequencing*

The strains were previously described in (zhu et al, megans thesis). Briefly, lines were obtained from previous mutation accumulation experiments, one from a highly heterozygous ancestor (hybrid of NCYC 3631, a Mat alpha derivative of YPS 606 (oak strain, PA, USA), and NCYC 3596, a Mat a derivative of DBPVG1106 (lici fruit, Indonesia, wine clade)). The other strain used was from the Zhu et al mutation accumulation experiment and was of the genotype *ade2, lys2-801, his3-∆D200, leu2-3.112, ura 3-52,* and was homozygous at all loci except the mating type locus(citation).. Both strains from the separate mutation accumulation experiments were diploid, and put through a bottleneck every ~20 generations for ~2000 generations.

30 lines from both hybrid and lab strains (12 aneuploid hybrid, 8 euploid hybrid, 6 aneuploid lab, 2 euploid lab), 3 replicates of each, were inoculated in 3ml liquid YPD (yeast extract 2%, peptone 2%, glucose 2%), and incubated on a rotator at 30° C for 24 hours. RNA was extracted using the MasterPure Yeast RNA Purification Kit (Epicentre). Integrity, concentration, and quality of RNA samples were assessed using the Qubit. Libraries were prepared using the Illumina Stranded RNAseq Kit. They were sequenced at the Georgia Genomics Facility on the Illumina NextSeq (75 cycles) SE75 High Output flow cell. Samples were multiplexed and split across two runs.

Some of the lines were sequenced on a different platform. 45 colonies of the lab strain (3 replicates each of 15 lines) were inoculated each into 3 ml liquid YPD medium, and incubated on a rotator at 30 for 24 hours. After 24 hours, mRNA was extracted using the MasterPure Yeast RNA Purification kit (Epicentre). RNA libraries were constructed using the Illumina Tru-seq mRNA Stranded Kit, amplified using 13 cycles of PCR and sequenced on an Illumina HiSeq 2500.

Because these datasets were extracted by different individuals, libraries were made with different kits, and the samples were run on different sequencing machines, analyses were carried out identically, but separately.

*RNAseq data analysis*

Raw sequence data was obtained from the Georgia Genomics Facility, with adapters removed. Quality control was done using FastQC version 1.8.0\_20 using default parameters (available at <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Low-quality bases were trimmed using Trimgalore v. 0.4.4 using -phred 33, -q 20 (available at https://www.bioinformatics.babraham.ac.uk/projects/trim\_galore/). RNA samples were aligned to the reference genome and annotated transcripts (obtained from <https://support.illumina.com/sequencing/sequencing_software/igenome.html>) using Tophat v. 2.1.1 with -i 10 -I 10000 [6]. Cufflinks v. 2.2.1 was used to assemble the transcriptomes using default parameters [6].Normalization was done using Cuffnorm v. 2.2.1 with default parameters, differential expression was found using Cuffdiff v. 2.2.1 with default parameters, and read counts were found using HTseq v. 0.6.1pl (Python v. 2.7.8) [6]. Samtools (version 1.3.1) was used to view .sam files as .bam files and sort .bam files [7]. Scripts can be found at <https://github.com/hollygene/Dosage_Compensation/blob/master/assembly_script.sh>.

Cuffnorm was used to find FPKM (fragments per kilobase per million reads). A homebrew bash script was used to join the FPKM values for each line with the gene attributes file, turn the file into a .csv, remove mitochondrial sequences, and change the chromosome names from Roman numerals to numbers (script can be found at <https://github.com/hollygene/Dosage_Compensation/blob/master/DC_workflow_April2017.sh>). This data file was then read into the R statistical software [8]. Average FPKM between the replicates for each line at each chromosome was found, followed by the average FPKM ratio (average FPKM of descendent line/average FPKM of ancestral line). Boxplots and ANOVAs were done in R. Comparisons made between aneuploid lines and the expected log2 ratio of trisomic, monosomic, or tetrasomic chromosomes were made using a one-sample t-test with mu = log2(1.5) for trisomic, mu = log2(0.5) for monosomic, and mu = log2(2) for tetrasomic. Two separate, but identical, analyses were done for each of the datasets. R scripts are located at <https://github.com/hollygene/Dosage_Compensation/blob/master/DC_workflow_old_MA.Rmd> and <https://github.com/hollygene/Dosage_Compensation/blob/master/DC_workflow.Rmd>.

**Result***s*

proportion of lines with aneuploidies:

MA experiment: 31/145

29 whole-chromosomal duplications

2 whole-chromosome losses

GC experiment: 30/93 lines

proportion of lines with segmental duplications:

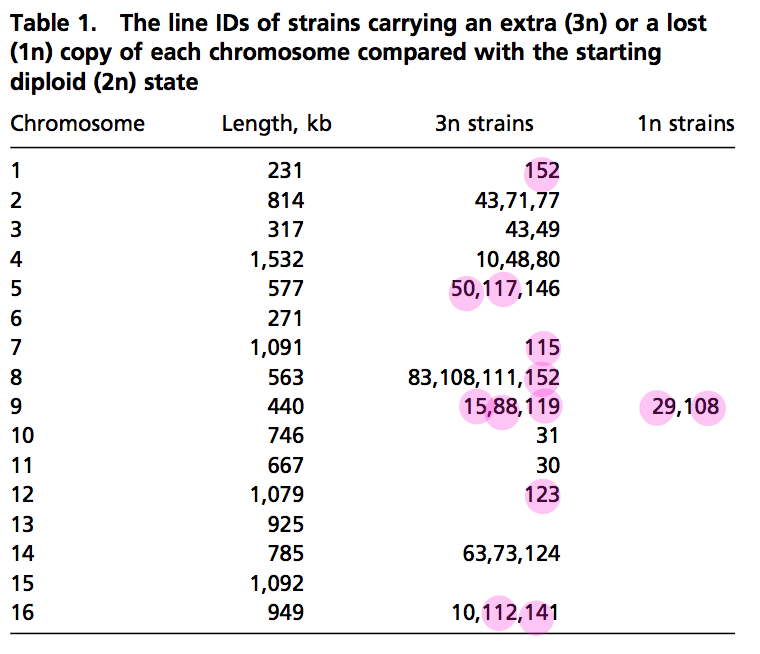
MA: 0

GC: 2

proportion of lines with multiple aneuploidies:

MA: 4 MA lines multiple aneuploidies

GC: 7 lines GC with multiple aneuploidies



Chromosomes with Multiple Aneuploid Lines: (calculated from MA data only)

2: 3

3: 2

4: 3

5: 3

8: 4

9: 5

14: 3

16: 3

2 Lines had multiple events: 43: 2&3 trisomic, 108: 9 monosomic, 8 trisomic

more tetrasomies (1) in GC than MA experiment

(monosomies and trisomies) and 2 events (tetrasomies)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| # events | GC | MA | total |  |
| 0 | 57 | 116 | 186 |  |
| 1 | 37 | 27 | 66 |
| 2 | 1 | 2 | 1 |  |

Given rate, should be able to calculate the expected numbers

Rate: # trisomies + # monosomies OR rather, #trisomies\*2 since when you get a trisomy you also get a monsomy (but if you don’t end up seeing them, implies strong selection against a monosomy)

GC:

observed – 37

expected – 74

MA:

observed – 27

expected – 54

rate

Equation 1

The rate of aneuploidy was calculated using Equation 1. For the lab strain, the rate of aneuploidy was found to be 1.06 x10-4. The probability of getting one aneuploid event (i.e. a single monosomy or trisomy in a single line) was calculated using equation 2. For the lab strain this probability was found to be 0.178. Multiplying this by the number of lines in the lab strain gives 25.8, indicating we expect 25 lines to have one aneuploidy event. Our data shows we found 27 lines with one event. The probability of two aneuploid events was calculated from equation 3. In the lab strain we found the expected number of two aneuploid events to be \*\*\*. The probability of more than two aneuploid events was found using Equation 4. This probability in the lab strain was found to be 0.022, indicating that we would expect to see 3.19 lines with more than two events. Zero lines with more than two events were found, indicating strong selection against this type of aneuploidy.

Hybrids of two yeast species have been shown to systematically lose all or part of one parent’s genome early in mitosis [9]. It is possible that our hybrid of distantly-related S. cerevisiae strains may show a milder version of genome incompatibility as exemplified by the higher rate of aneuploidy compared to the homozygous lab strain.

Chromosome IX was aneuploid most often in (at least MA, maybe GC lines?). A previous study found that chromosome IX was highly unstable in terms of chromosome loss in both diploid *S. cerevisiae* and diploid hybrid *S. cerevisiae-S. bayanus* strains [10] .

Chromosome V is lost spontaneously in S cerevisiae at a rate of 2-8 x 10-6 cell generations [11].

Equation 2

Equation 3

Equation 4

pro 2 or more events (separate events, ie monosomy and trisomy, two trisomies)

1 – prob(0) – prob (1)

1 – ( 1-u)^2100 – 2100 \* u(1-u)^2099

= 1- (54/93) - .3158 = 0.104 🡪 expect 9 lines that have >1 event

Average rate of events per chromosome: 4 would expect this for every chromosome but don’t get that, also implies selection against certain aneuploid chromosomes

*Segmental Duplications Arose x% of the time*

Are the segmental duplications where you would expect them to be? (i.e. the ratios?)

\*\*How would you calculate the expected # of segmental duplications?

For line 76, where is the breakpoint? Presumably the section has to contain the centromere. Is the breakpoint in a gene?

*Fitness effects of aneuploidy*

some aneuploidies have beneficial fitness effects, compared to ancestor

these higher fitness effects, could they be analogous to cancer? somehow these aneuploidies made the cells proliferate even quicker than WT.. especially the line 11 one with two aneuploidies??

do lines with beneficial fitness effects show the same amount of differential expression as lines with no or detrimental fitness effects compared to the ancestor?

*No evidence for whole-chromosome dosage compensation in either lab or hybrid strains*

Figure: for each chromosome, make a boxplot like the other ones but include all of the lines on it

Table: p-values of each aneuploid line compared with euploid lines (highest p value of those comparisons). comaprisons of lines aneuploid for the same chromosome to one another

Comparisons using Tukey’s Honestly Significantly Different Test (cite) were made between euploid and the aneuploid lines of interest (i.e. for chromosome 1, only those lines aneuploid for chromosome one were analyzed against each of the euploid lines). All aneuploid lines analyzed showed significant differential expression against each euploid line for the chromosome of interest. Most, but not all, aneuploid lines had nonsignificant p-values when comparing the gene expression on the aneuploid chromosome to the expected value of gene expression of a monosomic/trisomic/tetrasomic chromosome. Together, these observations support the conclusion that there is no whole-chromosome dosage compensation occurring in either the hybrid or lab strains. These findings are supported by previous work showing no dosage compensation in aneuploid yeast [12].

\*Maybe include a plot of the correlation between DNA copy number and RNA copy number in aneuploid chromosomes.

even though some lines have statistically significantly different gene expression levels, it could be because there is more RNA present to be degraded in trisomic lines, and less RNA present to be degraded in monosomic lines

Although autosomal dosage compensation has been observed in higher eukaryotes (cite some drosophila autosomal DC papers), it may be that these loci are more susceptible to dosage imbalances, or the phenomenon and mechanism of autosomal dosage compensation has evolved later in eukaryotes. Perhaps yeast do not require a mechanism of autosomal DC as they are so numerous and have a short generation time, so selection can act quickly to get rid of segmental or whole chromosome aneuploidies.

**Individual Genes**

Comparisons across all aneuploid/euploid/GC/MA lines

ESR genes?

Histone gene compensation?

What genes are up/down regulated in aneuploid lines with higher fitness than the ancestor?

Discussion

Acknowledgements

Figures

Figure 1: Monosomies are underrepresented in aneuploid lines. Only chromosome 9 had a monosomy event show up. This suggests there is strong selection against monosomies at the chromosomes that have trisomies but no monosomies. Also, chromosomes 6, 13, and 15 did not have any aneuploid events at the end of MA, suggesting strong selection against aneuploids of any kind for those chromosomes. This has been found previously, as chromosomes 6 and 13 are not obtained in diploid yeast through chemical-induction of aneuploidy (CITE).

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