**Introduction**

Aneuploidy occurs when an organism contains an abnormal number of one or a few chromosomes. Familiar examples are those causing human disorders, such as Down’s syndrome (trisomy 21) or Turner’s syndrome (monosomy X) (Hassold and Hunt 2001). While autosomal aneuploidies are generally deleterious in most organisms, presumably because of dosage problems (Chunduri and Storchova 2019), in some species, aneuploidies are surprisingly common, such as in some wild yeast (*Saccharomyces cerevisiae*) isolates (Strope *et al.* 2015). There is debate as to why aneuploidy is tolerated, or even favored, in such populations. Some hypothesize there is an intrinsic mechanism of dosage compensation to buffer the deleterious effects of imbalanced gene dosage (Hose *et al.* 2015; Gasch *et al.* 2016), similar to the mechanism of dosage compensation observed in sex chromosomes (Marin *et al.* 2000). Such autosomal compensation has been observed in *Drosophila* and other species (Birchler *et al.* 1990; Matos *et al.* 2015). In yeast, the presence of such a mechanism has been contested, with some studies concluding that there is no evidence for dosage compensation at the whole-chromosome level (Torres *et al.* 2010). Others suggest that aneuploid wild yeast attenuate protein levels by increasing protease activity or upregulating genes that are part of multiprotein complexes so that the relative dosages are more even (Chen *et al.* 2003; Veitia *et al.* 2008). It has also been shown experimentally that the accumulation or loss of chromosomes can be adaptive in certain environments (Selmecki *et al.* 2006; Pavelka *et al.* 2010; Chen *et al.* 2012; Yona *et al.* 2012; Selmecki *et al.* 2015; de Vries *et al.* 2018). For example, yeast grown in an oxide-rich media accumulate an extra copy of chromosome XI as a response to oxygen stress (Kaya *et al.* 2015), and resistance to fluconazole in *Candida* *albicans* involves acquisition of aneuploidy (Wakabayashi *et al.* 2017; Koo *et al.* 2018).

Understanding dosage compensation is important for several reasons. Aneuploidy cannot be avoided because segregation machinery is not perfect. As such, determining whether there are intrinsic mechanisms of dosage compensation gives insight into the likely consequences of such aneuploidy. Dosage compensation is also critically important during the evolution of sex chromosomes from homomorphic autosomes (Charlesworth 1991). Dosage compensation is thought to play a critical role in the evolution of sex chromosomes because of their different copy numbers in males versus females, a common example being the X-chromosome in XY systems. There are a variety of ways in which dosage compensation occurs in sex chromosomes to make up for differences in gene dosage between the sexes (Chandler 2017). However, the degree to which compensation evolves prior to, during, or after the evolution of dimorphism remains an open question (Gu and Walters 2017).

While dosage compensation has been observed for autosomes in *Drosophila* (Devlin *et al.* 1982; Birchler *et al.* 1990; McAnally and Yampolsky 2009; Chen and Oliver 2015; Hangnoh Lee 2016; Lee *et al.* 2016), it is unknown whether such an intrinsic mechanism exists in yeast. The fact that yeast are often found to be aneuploid in natural isolates (Strope *et al.* 2015) could suggest that aneuploidy causes changes in gene expression that are adaptive, and no DC exists (Kaya *et al.* 2015; Linder *et al.* 2017). Alternatively, yeast may be naturally robust to aneuploidy, so that aneuploid strains do not differ in fitness and thus occur in nature as neutral variants. The second hypothesis, coupled with the occurrence of aneuploid strains at reasonably high frequencies, suggests that yeast may contain an innate mechanism for attenuating or compensating for differences in gene dose and that mutation to aneuploidy is relatively frequent.

To fully understand the effects of aneuploidy on yeast populations, we seek estimates of the rate of aneuploidy and the effects on gene expression. Previous studies have observed the effects of aneuploidy in wild yeast populations, where selection is acting, and in chemically- or mitotically-induced aneuploids, where the rate of production of aneuploids is being manipulated (Linder *et al.* 2017); (Campbell *et al.* 1981; Anders *et al.* 2009; Mulla *et al.* 2014). In this study, we sought to determine the spontaneous rate of aneuploid formation for each chromosome and the effects of aneuploidy on gene expression in two strains of diploid yeast in the absence of selection. In each strain, aneuploid events were captured during a 2000-generation mutation accumulation (MA) experiment (Figure 1) with a single-cell bottleneck every 20 generations (Joseph and Hall 2004a; Zhu *et al.* 2014). By passaging through a single-cell bottleneck the effective population size is kept small (*N*e ≈ 11), which minimizes the effects of selection; only mutations with heterozygous fitness effects (*s*) of approximately 5% or greater (i.e. ) will be efficiently acted on by selection. Using RNA sequencing, we analyzed the gene expression of 20 aneuploid and 18 euploid lines across both strains to find differentially expressed genes and to determine if there was evidence for dosage compensation at the whole-chromosome and individual-gene levels in yeast.

**Methods**

*Estimating the spontaneous rate of aneuploid mutation*

To determine the rate at which spontaneous aneuploidy occurs in yeast, we analyzed data from two previous mutation accumulation (MA) experiments (Figure 2.1) (Joseph and Hall 2004b). In both, an ancestral strain was copied into multiple MA lines, which were then maintained separately for ~2000 cell generations (*G*) (2063 in homozygous ancestor lines and 2108 in heterozygous ancestor lines) via single-cell transfer every 48 hours ( 1 hour) for 100 transfers. The actual number of generations that passed was more accurately estimated by measuring colony size after 48 hours of growth in a representative sample and then determining cell number by counting using a hemocytometer.

The two diploid ancestral strains differed in their origin and degree of heterozygosity. One strain was obtained from a mating between NCYC 3631, which is a Matα derivative of YPS 606 (an oak strain from Pennsylvania, USA), and NCYC 3596, a Mat*a* derivative of DBPVG1106 (a wine strain isolated from a lici fruit in Indonesia). This highly heterozygous strain had a heterozygous site every ~250 bp and was homozygous for *ho* and *ura3* mutations (MA experiment and strain production by previous graduate student, Megan Behringer).

The other strain was derived from a standard lab strain (S228C) and carried the following mutations: *ho* *ade2, lys2-801, his3-∆D200, leu2-3.112,* and *ura 3-52* (Joseph and Hall 2004a).The strain was obtained by transforming a Mat*a* haploid version of the strain with an *HO URA3* plasmid to generate a diploid version of the strain, followed by counterselection of the plasmid on 5FOA (Joseph and Hall 2004a).This strain was thus homozygous at all loci except the mating type locus.

We used the number of aneuploid chromosomes in the MA lines at the end of the experiment to calculate the rate at which aneuploidy occurs in each of these strains. In brief, if the rate of aneuploidy for chromosome *c* is *µc*, then the probability that a line is not aneuploid for this chromosome is (1-*µc*)G, where G is the number of generations of MA. Thus if *nc* MA lines show aneuploidy for this chromosome, implying that (*n - nc*) do not, where *n* is the total number of MA lines, then we can estimate the rate of aneuploidy per chromosome by solving (1-*µc*)G = (1 - *nc /n*) for *µc*. Similarly, we can estimate the overall aneuploidy rate, *µ*, which is the probability that a cell will become aneuploid for any chromosome in a single cell division, by solving (1-*µ*)G = (1 – *na* /(16 *n*)) for *µ*, where *na* is the number of aneuploid chromosomes across all MA lines.

*Estimating the effects of aneuploidy on gene expression*

To determine the effects of aneuploidy on gene expression, we collected and analyzed RNA sequencing data from a selection of euploid and aneuploid lines from each experiment. For aneuploid samples, we chose all the MA lines that were monosomic for a chromosome (3 lines), those that shared common aneuploidies (21 lines), and those that had more than one aneuploidy (4 lines). From the homozygous ancestor experiment, we selected 10 aneuploid and 12 euploid MA lines. From the heterozygous ancestor experiment, we selected 10 aneuploid and 6 euploid MA lines. Additionally, we collected RNA sequencing data for both ancestral lines, which had been stored at -80ºC since the beginning of the experiment. The homozygous strains were run in two separate RNA sequencing runs, separated by 2 years. In both sequencing runs, we included three replicates of each ancestor. For analysis, we kept these two datasets separate because we found that the ancestor was significantly different across the two sequencing runs. Across both strains and sequencing runs, we obtained RNA sequencing data for 38 strains representing two ancestors, 20 euploid lines and 16 aneuploid lines.

For each strain, we plated cells that had been stored at -80°C, and allowed growth for two days at 30ºC on YPD plates (1% yeast extract, 2% peptone, 2% glucose, 2% agarose), and then initiated 3ml liquid YPD cultures (no agarose) from three separate colonies (biological replicates) of each line. Liquid cultures were incubated on a rotator at 30°C for 24 hours, before being diluted into 50ml YPD and allowed to grow on a shaker at 30°C for 6 hours. Optical density (OD) measurements were taken to ensure all cultures were in the same growth phase. Cells were then pelleted, and RNA was extracted from each replicate using the MasterPure Yeast RNA Purification Kit (Epicentre). Integrity, concentration, and quality of RNA samples were assessed using a Qubit (Thermo Fisher Scientific). Libraries were prepared using the Illumina Stranded RNAseq Kit and were sequenced at the Georgia Genomics Facility on the Illumina NextSeq (75 cycles) single-end 75bp reads High Output flow cell. Samples were multiplexed and split across two sequencing lanes.

Raw reads were processed by the Georgia Genomics Facility to remove sequencing adapters and demultiplex samples. Quality control was performed using FastQC version 1.8.0\_20 with default parameters (available at www.bioinformatics.babraham.ac.uk/projects/fastqc/). Low-quality bases were trimmed using Trimgalore version 0.4.4 using -phred 33, -q 20 (available at www.bioinformatics.babraham.ac.uk/projects/trim\_galore/). RNA samples were aligned to the *Saccharomyces cerevisiae* reference genome (UCSC version sacCer3, available at support.illumina.com/sequencing/sequencing\_software/igenome.html) and transcripts were annotated using Tophat v. 2.1.1 with -i 10 -I 10000 (Trapnell *et al.* 2012). Cufflinks v. 2.2.1 was used to assemble sample transcriptomes using default parameters (Trapnell *et al.* 2012) and Cuffnorm v. 2.2.1 was used with default parameters to normalize reads. Differential expression was determined 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) (Anders *et al.* 2015). Finally, we used Samtools v. 1.3.1 to convert *.sam* files into *.bam* files and sort the resulting *.bam* files (Li *et al.* 2009). Scripts can be found at https://github.com/hollygene/Dosage\_Compensation/tree/master/bash\_scripts.

To compare chromosome-level changes in gene expression across strains Cuffnorm v. 2.2.1 (Trapnell *et al.* 2012) was used to calculate FPKM (fragments per kilobase per million reads) for each RNAseq data set. A custom bash script was then generated to join the FPKM values for each strain with the gene annotations file, convert the resulting file into a *.csv* formatted file, remove mitochondrial sequences (as we were not interested in gene expression changes in mitochondrial DNA), and change the chromosome names from Roman numerals to numbers (script can be found at https://github.com/hollygene/Dosage\_Compensation/blob/master/bash\_scripts/DC\_workflow\_April2017.sh). For each gene, the average FPKM across the three replicates for each strain was calculated, followed by the average FPKM ratio (average FPKM in an MA line divided by the average FPKM in the ancestor). We noticed that the FPKM ratio was highly variable across MA line replicates for genes with an average FPKM < 5 across all euploid strains (ancestor + euploid MA lines), so we removed such genes, leaving a total of 6181 genes (Supplemental Figure 2.1, Appendix I). We also removed snRNA genes and tRNA genes, as these are hard to map and can cause issues with data normalization.

To determine whether there was evidence for dosage compensation at the whole-chromosome level, we compared the average FPKM ratio for genes on an aneuploid chromosome to the expectation from gene dose. Thus, a trisomic chromosome would be expected to show a 1.5-fold increase in gene expression and an average FPKM ratio = 1.5 (log2ratio = 0.585). Similarly, monosomic and tetrasomic chromosomes should show average FPKM ratios of 0.5 (log2ratio = -1) and 2 (log2ratio = 1), respectively. We asked whether the observed distribution was consistent with the expected FPKM ratio by calculating the mean and confidence interval of the average FPKM ratio (a one-sample t-test). All analyses were done in RStudio (Team 2013). R scripts are available at https://github.com/hollygene/Dosage\_Compensation/tree/master/R/scripts/R\_scripts.

As we did for chromosome-level gene expression analysis, previous studies have almost exclusively used FPKM to measure gene expression to compare across strains or treatments. However, the use of FPKMs has been criticized because of loss of power due to relatively few replicates (three in our experiments). A possible loss of statistical power for individual genes is not an issue when comparing tens or hundreds of genes for each chromosome as we did in the chromosome-wide gene expression analysis above, and so there we used FPKMs to make our data more comparable to previous data. However, when examining individual genes, power becomes a more serious concern. As such, we used a method, *DESeq2* (Love *et al.* 2014), that models the expression level for a gene in a particular replicate and treatment (in our case ancestor versus MA line). Importantly, the method also models the dispersion of the read depth, assuming the distribution of the read depth can be accurately represented by a negative binomial with genes of similar expression having similar dispersion. This method is thus expected to more accurately predict the actual read depth by explicitly considering the variance in the read depth across replicates. Thus, as a result, an unusually high or low depth for one replicate will not have equal weight compared to the depths for the other replicates. The method should thus be able to better detect genes that are differentially expressed (DE) in an MA line versus its ancestor.

Raw read counts obtained from htseq-count were used as input for DESeq2 (Love *et al.* 2014). Individual *DESeqDataSets* were produced for each strain, due to the high variation found across strains, as determined by principal component analysis (PCA) (Supplemental Figure 2.2, Appendix I). Reads with counts less than 10 in every replicate were removed from further analysis. We used a more stringent cutoff in this analysis to focus on genes for which we have the most power for detecting a change in expression, since we are analyzing individual genes. Removing such genes from the data set resulted in 5532 genes being analyzed (Supplemental Figure 2.1, Appendix I).

The *DESeq()* function was implemented on all datasets with default parameters. Annotations were added using the *S. cerevisiae* database from Bioconductor (Carlson M 2015). The *results()* function in *DESeq2* was implemented with default parameters, using a False Discovery Rate (FDR) of 0.1. Analyses were performed with one MA line and the ancestor at a time, since running all strains together would lead to an overestimate of dispersion because of the numerous aneuploid chromosomes in MA lines (see above). For one of the two batches of the homozygous strain, one of the ancestor replicates was substantially different based on a PCA, and so only 2 of the 3 ancestor replicates were used (Supplemental Figure 2.2, Appendix I). Similar to the whole chromosome analysis, ratio distributions equal to the sample mean divided by the ancestral mean for the normalized counts were obtained from *DESeq2* estimated read counts. To visualize the data, histograms for both cis (present on aneuploid chromosome) and trans (present on remainder of chromosomes) genes were generated using ggplot2 in R (Wickham 2016).

In addition to looking at all genes in the genome to identify those that were differentially expressed, we also specifically concentrated on a few classes of genes that have been identified in previous work as either being dosage sensitive (DS) (115 genes, Makanae *et al.* 2013), or particularly likely to alter expression in response to stress. These latter genes include those in the environmental stress response (ESR) pathway (139 genes, Gasch *et al.* 2000) and those thought to play a role in aneuploidy stress response (ASR) (201 genes, Torres *et al.* 2007). ASR genes were previously shown to be significantly differentially expressed in aneuploid but not euploid strains. To identify significant DE for genes from these categories, we tested each gene’s expression against the expected expression for a disomic gene, and determined which genes were significantly different, then parsed those genes into what matches the ESR/DS/ASR genes. We then counted how many times each gene appeared as a measure of its degree of consistent DE across aneuploid MA lines.

In these analyses, we paid particular attention to MA lines carrying the same chromosome aneuploidy to see if they showed similar changes in gene expression of identical genes. We performed gene ontology analysis on genes that we identified in these analyses using the PANTHER GO system (Thomas *et al.* 2003) to determine whether particular functional categories were especially prone to DE.

**Results**

*The rate of spontaneous aneuploidy is nearly twice as high in the heterozygous strain as the homozygous strain*

The number of aneuploidy events by chromosome is shown in Table 2.1. We assume that aneuploidy is caused by mitotic nondisjunction since cells are kept asexual. A single non-disjunction event can produce both a monosomic and a trisomic chromosome in a diploid strain. Thus, two events would be required to obtain the one tetrasomic MA line. The total number of events in the homozygous ancestor strain varied between 0 and 5 per chromosome, which implies a maximal observed rate of nondisjunction for a single chromosome of 1.70 x 10-5 events/division (obtained by solving (1-*µ*c)2063 = 140/145 for *µ*c), and a minimum of zero. The observed rate of an event for any chromosome (i.e. the genome-wide rate) is 6.73 x 10-6 events/division (obtained by solving (1-*µ*)2063 = 1 – 32/(16\*145) for *µ*c). The total number of events in the heterozygous ancestor varied between 0 and 7 per chromosome, which implies a maximal observed rate of nondisjunction for a single chromosome of 1.56 x 10-4 events/division (obtained by solving (1-*µ*c)2108 = 69/76 for *µ*c), and a minimum of zero. The observed rate of an event for any chromosome is 1.51 x 10-5 events/division (obtained by solving (1-*µ*)2108 = 1 - 38/(16\*76) for *µ*c), which is over two-fold higher than the homozygous strain. Examination of the number of euploid versus aneuploid lines indicates that this is a highly significant difference (Table 2.2, Fisher’s Exact test, p < 0.0001).

We note that there were two monosomies and 30 trisomies, a 15-fold difference, in the homozygous experiment and one monosomy and 35 trisomy events in the heterozygous experiment. Since a single nondisjunction event creates both types of aneuploids in the daughter cells, this imbalance implies that monosomies are under-represented in the MA experiments. This finding suggests that monosomies have effects on fitness that are large enough to be seen by selection, even in the low-selection MA framework. Thus, the actual rate of aneuploidy might perhaps be better estimated as twice the trisomy event rate, giving 1.23 x 10-5 and 2.77 x 10-5 events per cell division for the homozygous and heterozygous ancestor strains respectively.

In addition, two chromosomes, 6 and 13, comprise 0 out of 70 observed aneuploidy events across the two experiments. If events occurred at random, each chromosome should have 1/16 of the observed events, or 4.4 each. Under a Poisson distribution, the probability of having a chromosome with no events when the expected number is 4.4 equals *e*-4.4 = 0.013. It thus seems clear that aneuploidy of chromosomes 6 and 13 either cause strongly deleterious fitness effects or are not tolerated. However, they have been seen in aneuploid clinical yeast samples (Zhu *et al.* 2016). The discrepancy between these two findings may be due to differences in genetic background or environmental stresses of the individual strains in question.

To address whether one aneuploidy events increases the probability of another, we asked whether there was an excess of strains carrying two or more aneuploidies. For the homozygous strain, 28 of the 145 MA lines were found to be aneuploid. Of these, four lines contained two aneuploidies (i.e. two separate chromosomes had become aneuploid), which is not significantly different from the Poisson expectation of 3 (Fisher’s Exact Test, p > 0.99). For the heterozygous strain, 29 out of 76 sequenced MA lines were found to be aneuploid. Of these, seven lines contained two aneuploidies, which is the same as the Poisson expectation.

To determine whether chromosome size effects the number of nondisjunction events we captured in our MA experiments, we plotted size versus number of nondisjunction events (Figure 2.2). While there is clearly variation in which chromosomes become aneuploid, there was no significant relationship with size. However, only two chromosomes, 1 and 9, were found to be monosomic and both of these are relatively small chromosomes (1 is the smallest at 230,218 bp, and 9 is the 4th smallest at 439,888 bp). This suggests that while there is no noticeable effect of chromosome length on aneuploidy occurrence, monosomy may be tolerated in smaller chromosomes better than larger chromosomes.

*Little evidence for whole-chromosome dosage compensation in either strain*

We performed RNAseq on 10 euploid and 12 aneuploid homozygous ancestor strain MA lines, and on 6 euploid and 10 aneuploid heterozygous ancestor strain MA lines. Whole-chromosome gene expression was analyzed by calculating the average and 95% confidence intervals of gene expression for each chromosome (Figure 2.3). ANOVAs were also run on each aneuploid sample, comparing the average gene expression from each chromosome to that of the other samples (lm(y~Line), where y is FPKM ratio and Line is the line number). If there were complete dosage compensation occurring on the whole-chromosome level, we would expect no difference between aneuploid and euploid chromosomes, such that ANOVAs would show no effect of chromosome number on gene expression. However, in the absence of dosage compensation, chromosome number would have an effect, with aneuploid chromosomes underlying the significant difference among chromosomes. Further, in the absence of dosage compensation, we would expect gene expression to mirror gene dose such that aneuploid chromosomes would show 0.5 or 1.5-fold increases in expression for monosomic and trisomic chromosomes respectively.

ANOVAs indicated that the effect of chromosome was significant (p < 0.01) in every aneuploid MA line, as expected with no dosage compensation. For chromosomes that did not have any aneuploid lines represented in the dataset, we still found some differential expression in a few aneuploid lines. Specifically, for chromosome III, Line 76, 61, 59, 49, 18, 11 (heterozygous ancestor) are significantly different (p<0.01), suggesting that aneuploidy causes changes in gene expression of genes on chromosome III across aneuploid strains. However, ANOVAs on some euploid lines, also displayed significant p values for certain chromosomes, indicating that some chromosomes show changes in expression even in the absence of changes in dose. This could suggest an impact of the MA framework on gene expression in yeast.

If there is no chromosome-level dosage compensation, then the level of gene expression is expected to be proportional to chromosome copy number. For most aneuploid chromosomes in MA lines this prediction held: expression levels did not differ significantly from the expectation. However, in 4 MA lines (line numbers 18, 49, 59 and 61) from the heterozygous ancestor, the expected level of gene expression was less extreme than expected based on chromosome copy number (Figure 2.3). Chromosome 1 of line 18 had average expression change equal to 1.3-fold, chromosome 5 of line 49 had average expression change equal to 1.35-fold, chromosome 7 of line 59 had average expression change equal to 1.25-fold, and chromosome 7 of line 61 had average expression change equal to 1.39-fold. All these values were significantly different from the expected expression level of 1.5-fold (p < 0.05). However, the cell populations from which we extracted RNA may have been heterogeneous; i.e. composed of both aneuploid and euploid cells due to the possible reversion of cells from aneuploid to euploid state. The vast majority of gene expression changes, 65 of 69, are consistent with a lack of whole-chromosome dosage compensation occurring in either strain, and together these findings support previous work showing no dosage compensation in aneuploid yeast (Torres *et al.* 2010).

*Distribution of gene expression from euploid versus aneuploid chromosomes*

The previous analysis indicates that mean gene expression of aneuploid chromosomes seems to be predicted by gene dose. We next examined whether the mean expression for genes on the non-aneuploid (disomic) chromosomes is altered by aneuploidy. In addition, we examined whether the variance in gene expression for aneuploid chromosomes is the same as for euploid chromosomes in the 20 aneuploid MA lines, and whether the variance in gene expression differs between euploid MA lines and their euploid ancestor. The distribution of FPKM ratios (MA line FPKM / ancestor FPKM) for all genes in euploid samples (Figure 2.4), for genes on the aneuploid chromosome(s) in aneuploid samples (cis genes), and for genes not located on the aneuploid chromosome(s) in aneuploid samples (trans genes) were analyzed (Figures 2.5 and 2.6; Supplemental Figure 2.3, Appendix I).

The expected mean expression ratio in euploid lines is 1. In every euploid line analyzed, the expected distribution had a mean that was indistinguishable from 1 (pval > 0.1, Supplemental Figure 2.3, Appendix I). For aneuploid lines, the expected mean expression for trans genes (those not located on the aneuploid chromosome) is not equal to 1. This is because the aneuploid chromosome will have more (for trisomy) or fewer (for monosomy) reads mapping to it because the chromosome represents a different percentage of the genome in an aneuploid line. In Table 2.3, we indicate the expected mean expression level for trans genes in MA lines carrying a single trisomy. Similarly, for lines with monosomies, the expected mean expression of trans genes is higher. We tested the mean expression of trans genes against the expectation based on the chromosomes for which they were aneuploid and found that in no case were they significantly different (Supplemental Figure 2.3, Appendix I).

To examine whether the variance in gene expression is greater in aneuploid lines, we compared the variance in gene expression of both cis and trans genes to the variance of those same genes in a euploid line using a Levene’s test since the distributions were heavily skewed. For comparisons, we randomly matched a euploid line with each aneuploid line. We determined whether the means and variances of these distributions differed from the expectation (the expectation being that both the means and the variances are equal between aneuploid and euploid lines). The variances of gene expression from cis genes were significantly different from the expectation in every case except for two – the comparison of homozygous line 15 (trisomic for chromosome 9) to homozygous line 5 (euploid) and the comparison of homozygous line 152 (trisomic for chromosomes 1 and 7) to homozygous line 1 (euploid) (Table 2.4). There is nothing immediately notable with these samples, though the ANOVA for chromosome 7 line 1 was significant (p<0.05), however there was no similar connection in line 5 for chromosome 9 (Supplemental Data; Appendix I). Further, the variances of trans genes in the aneuploid and euploid lines were significantly different from each other, which could be due to the discrepancy in gene expression on cis versus trans genes, as discussed above.

*Individual Dosage-Compensated Genes*

Our analyses so far indicated that at the whole-chromosome level aneuploidy leads to changes in gene expression predicted by gene dose, such that there was no evidence for dosage compensation, and minor (or no) effects on expression of the rest of genome. Next, we investigated individual genes. We sought to group genes present on aneuploid chromosomes into five categories based on their gene expression, using similar metrics as a previous study (Malone *et al.* 2012): 1. Not dosage compensated: these genes have expression levels not significantly different as those predicted by their gene dose. 2. Partially dosage compensated: these genes show less extreme gene expression changes than predicted by their dose. 3. Fully dosage compensated: these genes show no change in expression in response to changes in gene dose. 4. Over-dosage compensated: these genes show changes in expression that are in the opposite direction of the change in gene dose. 5. Anti-dosage compensated genes show more extreme changes in expression (in the direction of the aneuploidy – i.e. monosomic genes would have lower gene expression than predicted by monosomy) than predicted by the change in gene dose (Table 2.5). Any gene that had expression levels different from the ancestor and different from the expectation based on gene dose was assigned to one of the categories depending on their level of expression. For the aneuploid strains we analyzed, we found several genes in each of these categories (Table 2.6). Since we are testing many genes (5587), power becomes limited due to the need to correct for multiple testing. For this reason, it is important to test for expression that is consistent both with respect to the ancestor and to the expectation based on gene dose. Many genes do not differ from either, in which case we cannot conclude the degree to which they are compensated – these genes were assigned as category 0 genes, or “unknown” compensation. Our analyses revealed that we lack the power to distinguish whether a gene exhibits dosage compensation or not; the vast majority of genes are in category 0 (Table 2.6). Because of this, the genes that we are (most) able to confidently assign are those in categories 1, 2, 4 and 5. For those genes in category 4 and 5, we find that there is little agreement between different strains in terms of the percentage of genes in these categories (Figure 2.6).

We compared the trans genes of aneuploid samples with those of samples with a different aneuploid chromosome(s) to determine if there was a common response to aneuploidy, as has been shown in previous studies (Gasch *et al.* 2000; Zillikens *et al.* 2017a). We found that in lines from the heterozygous ancestor, at most, 8/10 aneuploid samples shared 15 DE trans genes (genes that were not located on an aneuploid chromosome) (Figure 2.9). In lines from the homozygous ancestor, at most 6 aneuploid lines shared 8 DE trans genes (Figure 2.14).

We then examined if euploid lines shared a common gene expression response and found that in lines from the homozygous ancestor, at most 5 euploid samples shared 8 common differentially expressed genes (Figure 2.15). In the heterozygous ancestor, at most 5 lines shared 54 DE genes (Figure 2.16). This result suggests a shared effect of the mutation accumulation experimental design on gene expression, particularly in the heterozygous ancestor samples.

*Histone Genes*

Histone genes H2A and H2B are known to possess a mechanism of dosage compensation in *S. cerevisiae* (Osley and Hereford 1981; Medici *et al.* 2014). Our analyses did not include samples with aneuploidies on these chromosomes (II and IV), but we do have aneuploid samples for chromosomes containing other histone genes: XIV, XV, and XVI (containing histones 3,4, and linker, respectively). Six lines across both experiments are trisomic for chromosome XIV, 1 line is trisomic for chromosome XV, 13 lines are trisomic for XVI, and 1 line is tetrasomic for XVI. Previous studies have found that these genes do not display dosage compensation and we did also not find evidence for compensation (Peter R. Eriksson 2012) (Supplemental Table 2.1, Appendix I).

*Stress Response Genes*

Yeast are known to undergo what is known as the environmental stress response (Gasch *et al.* 2000; Zillikens *et al.* 2017a), when conditions are unfavorable due to various factors, including temperature stress, oxidative stress, and nutrient limitation. We analyzed genes previously found to relate to the environmental stress response and found that our aneuploid samples did differentially express most of these genes (figure 2.16-2.19), though there was no significant trend of shared ESR genes between samples.

It has been found that similarly, aneuploid yeast undergo what is referred to as the “aneuploid stress response (ASR),” in which certain trans genes are differentially expressed (Torres *et al.* 2010). A majority of these genes are also differentially expressed during the environmental stress response. To determine if we found the same pattern of differential expression in our spontaneously aneuploid samples, we investigated these ASR genes (201 genes total) and found that in samples from the heterozygous ancestor, at most 7 lines shared 3 DE ASR genes. In the homozygous ancestor aneuploid lines, at most only 4 lines shared just 1 DE ASR gene (Figures 2.20 & 2.21). As expected, the euploid lines in both datasets did not show many DE ASR genes and similarly did not share many DE ASR genes (Figures 2.22 & 2.23).

*Dosage-Sensitive Genes*

Previous studies have found that certain genes are more sensitive to changes in gene dose than others. Using the “genetic tug-of-war” method, Makanae et al 2013 found the copy-number limits of overexpression in all 5806 protein-coding genes in *S. cerevisiae*, and found 115 genes whose copy number limits were 10 or less (more than this amount caused cell death) (Makanae *et al.* 2013). Curious as to whether our samples exhibited a compensatory response for these dosage sensitive genes, we looked at the same set of genes and parsed out those that were significantly differentially expressed in our aneuploid samples. Most aneuploid samples had few differentially expressed dosage sensitive genes, indicating that our samples were employing a level of compensation to combat the deleterious effects of high expression of these genes (Figure 2.24 – 2.25). As expected, the euploid lines in both experiments had very few DE dosage-sensitive genes (Figures 2.26 – 2.27).

The genes of most interest were those contained on the aneuploid chromosomes, as these genes were found in differing copy number compared to the rest of the genes in the genome. Most samples showed a high level of compensation of dosage-sensitive genes on the aneuploid chromosome and elsewhere in the genome. However, samples with a trisomy for chromosome 9 appeared to be more tolerant of the duplication (likely due to individual gene compensation) than other chromosomes – samples ranged from 0 to 33% compensation (Table 6). Previous studies have found that the increase in a partner gene can rescue the sensitivity of a strain to another increased dosage. This may be occurring in the samples that had little to no compensation of the dosage sensitive genes on the aneuploid chromosome.

**Discussion**

*Rate of aneuploidy*

We calculated the rate of aneuploidy based on data from two previous yeast mutation accumulation experiments: one with a heterozygous strain and one with a homozygous strain (Figure 2.1). We found that the rate of aneuploidy is higher in the heterozygous strain than the homozygous strain (p<0.0001, Fisher’s exact test, Table 2.2). The heterozygous ancestor strain MA lines had a total of 29 aneuploids and 47 euploids, whereas the homozygous ancestor MA lines had a total of 28 aneuploid and 117 euploid lines. Previous studies have found that hybrids of two yeast species have been shown to systematically lose all or part of one parent’s genome (Marinoni *et al.* 1999). It is possible that the mating of distantly related *S. cerevisiae* strains to produce the heterozygous strain showed a milder version of genome incompatibility as exemplified by the higher rate of aneuploidy compared to the homozygous lab strain. However, the heterozygous strain did not show any growth defects (which could have indicated a phenotypic effect of genome incompatibility) compared to the homozygous strain. Further, since our strains were not isogenic, it is possible that our findings are instead the result of differences in the genetic background of the strains. To prevent this, future experiments could create diploids from each of the parents used to make the heterozygous strain, put these through a mutation accumulation experiment, and determine the amount of aneuploidies that arise to determine if aneuploidy levels decrease in more homozygous strains.

In our experiment, we found 3 and 6 events for the homozygous and heterozygous ancestor involving chromosome V nondisjunction, implying a rate of 9.67 x 10-6 and 3.90 x 10-5 events per cell division, respectively. (Mulla *et al.* 2014)This previous study used a laboratory strain (A364A), which was highly homozygous – this likely explains the discrepancy in rates between the previous study and our heterozygous ancestor strain rate and is consistent with the homozygous strain rate of aneuploidy of chromosome V found in our study.

We found a difference in aneuploidy rates on the individual chromosome level as well as overall. In the heterozygous ancestor strain MA lines, we found 10 trisomies of chromosome XVI, compared with 3 in homozygous ancestor MA lines (Table 2.1). Previous studies have found a similar discrepancy between diploid and diploid-hybrid strains of yeast, with the hybrid strains showing a higher rate of aneuploidy at chromosome XVI (Kumaran *et al.* 2013). These results suggest that heterozygosity influences either nondisjunction rate or tolerance of certain aneuploidies and that certain chromosomes are either more likely to become aneuploid or are better tolerated after becoming aneuploid, or both.

Due to the diploid nature of our initial MA ancestors, we were able to analyze trisomics, monosomics, and a tetrasomic to study the rate and effects of whole-chromosome aneuploidy. Contrary to most previous studies, we were able to observe the spontaneous rate and effects of monosomy, which is drastically less common than trisomy in our samples (Table 2.1). Considering nondisjunction events result in both a trisomy and a monosomy, we would expect to see an equal number of each in our data. The lack of monosomies implies that there is strong selective bias against such events, implying that less gene copies is more deleterious than more gene copies.

*No evidence for whole-chromosome dosage compensation at the transcript level*

Our results suggest that there is no general mechanism for dosage-compensation in aneuploid yeast, either at the whole-chromosome or individual gene level (Figure 2.4, Table 2.6). This mirrors previous findings that RNA level scales with DNA copy number (Torres *et al.* 2010). In haploid yeast that were disomic for one or more chromosomes, it was found previously that some samples actually exhibited a fitness gain (Torres *et al.* 2007). However, the gene expression of genes on the aneuploid chromosomes in these strains was as high as expected for a disomic chromosome. It has also been found that aneuploid yeast samples utilized posttranscriptional methods of lowering protein levels and that no RNA-level compensation was occurring (Torres *et al.* 2010). It is likely that previous studies which claimed to have found whole-chromosome dosage compensation effects were using heterogeneous samples of yeast that were both aneuploid and euploid (James Hose 2015; Audrey P Gasch 2016), causing gene expression ratios to be intermediate between what is expected for aneuploid and euploid DNA copy levels. Similarly, the apparent compensation we observed in our study may also be caused by heterogenous samples. To avoid this, future studies should employ the use of fluorescent activated cell sorting (FACS) to separate the aneuploid cells from the euploid cells and use only the aneuploid culture for RNA extraction. However, evidence suggests that certain individual genes on the aneuploid chromosomes are partially compensated. It is possible that genes that are more deleterious in high numbers but are on chromosomes that also contain genes that are beneficial in high copy number are up- or down-regulated on a gene-by-gene basis in order to deal with the extra or missing chromosome, implying a robust stress response.

*Aneuploidy effects on trans genes*

Previous studies have proposed that there is an effect of aneuploidy on the remainder of the genome, by looking at the peaks of the distributions and claiming that the apparent skew to the left of 1.00 indicated that the aneuploid chromosome was causing other expression effects in the genome (Hou *et al.* 2018). We investigated this in our data and found that genes not on aneuploid chromosomes show the expected level of gene expression when compared to the ancestral strain (Figure 2.7, Table 2.3), implying that aneuploidy does not cause a global gene expression change. However, we were curious if aneuploid lines shared any differentially expressed genes not located on aneuploid chromosomes. We compared gene expression data between aneuploid samples and found in our heterozygous dataset 15 commonly differentially expressed genes among 8 of our aneuploid lines. Similarly, in the homozygous ancestor lines, we found 8 commonly differentially expressed trans genes among 6 of the aneuploid lines.

Previous studies in yeast have found evidence of a transcriptional response to environmental stress as well as a transcriptional response to aneuploidy; these two were termed the “environmental stress response” genes and the “aneuploidy stress response” genes (Gasch *et al.* 2000; Torres *et al.* 2007; Zillikens *et al.* 2017b). We investigated the environmental stress response (ESR) genes found in previous studies and found that most ESR genes were differentially expressed in our aneuploid samples, suggesting that the state of aneuploidy has similar effects on the transcriptome to various environmental stresses including high salinity, high temperatures, and highly oxidative-species rich environments. It would be interesting to know if the yeast samples exposed to these environmental stresses had any copy number changes in their genomes – this would further add evidence to the hypothesis that aneuploidy is an adaptive state to changes in the environment or a consequence of stress. We were curious if our results reflected the same findings as previous studies of an “aneuploidy stress response,” similar to the environmental stress response in yeast Indeed, our data do corroborate these findings (Figures 2.20-2.21). However, our aneuploid strains do not have very many commonly DE ASR genes, suggesting that each aneuploidy confers a different stress and therefore a different transcriptional stress response.

*Conclusions and future directions*

This study demonstrated that heterozygosity is correlated with a higher aneuploidy rate, that there is no evidence for whole-chromosome dosage compensation in aneuploid yeast, and that aneuploid chromosomes do not significantly influence the gene expression patterns among the rest of the genome. We did find evidence for compensation at the individual gene level for genes that are particularly toxic in high copy numbers, suggesting that cells are able to employ transcriptional compensatory mechanisms to tolerate aneuploidy at least at the individual gene level. Further, our analyses demonstrated evidence for indivudal aneuploid samples to differentially express environmental and aneuploidy stress response genes. There were no significant commonalities among aneuploid lines in this regard, however, implying that each aneuploid line deals with its aneuploidy in a unique manner.

Our finding of no global effects of aneuploidy on gene expression is in direct opposition to a recent paper claiming this – however, we proved mathematically that the apparent skew of trans genes is actually due to sequencing bias from reads mapping to more (or less) copies of the aneuploid chromosome(s). Our analyses and evidence bring insights into the effects of aneuploidy on gene expression in budding yeast and can be applied to other species as well. The finding that trans genes are not affected by aneuploidy can be useful when treating human diseases caused by aneuploidy (i.e. Down’s Syndrome, Trisomy X). Further, our findings provide insight into the evolution of sex chromosomes and dosage compensation – the likelihood of a whole-chromosome mechanism of dosage compensation being applied to every chromosome in the genome is less likely to arise early in evolution than individual genes, especially those that are particularly deleterious, being compensated individually.

More insights into how wild yeast tolerate aneuploidy are required. A recent study found that the SSD1 gene in yeast is linked with aneuploidy tolerance in wild strains versus lab strains (Hose *et al.* 2020). This gene is a translational repressor and is functional in wild yeast isolates but not in laboratoruy strains. This implies that wild aneuploid yeast strains can tolerate aneuploidy by attenuating translation of the duplicated genes. This reflected previous work in aneuploid yeast that showed compensation at the protein, but not RNA, level (Noah Dephoure 2014). Our analyses provide further evidence for this, and future studies could use SSD1 knockout strains of yeast for mutation accumulation studies and determine rates and tolerance of aneuploidy in a similar manner as this study.

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