Gene-by-environment interactions influence the fitness cost of gene copy-number variation in yeast

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Running title: GxE effects on CNV tolerance

ABSTRACT

Variation in gene copy number can alter gene expression and influence downstream phenotypes; thus copy-number variation (CNV) provides a route for rapid evolution if the benefits outweigh the cost. We recently showed that genetic background significantly influences how yeast cells respond to gene over-expression (OE), revealing that the fitness costs of CNV can vary substantially with genetic background in a common-garden environment. But the interplay between CNV tolerance and environment remains unexplored on a genomic scale. Here we measured the tolerance to gene OE in four genetically distinct *Saccharomyces cerevisiae* strains grown under sodium chloride (NaCl) stress. OE genes that are commonly deleterious during NaCl stress recapitulated those commonly deleterious under standard conditions. However, NaCl stress uncovered novel differences in strain responses to gene OE. West African strain NCYC3290 and North American oak isolate YPS128 are more sensitive to NaCl stress than vineyard BC187 and laboratory strain BY4743. Consistently, NCYC3290 and YPS128 showed the greatest sensitivities to gene OE. Although most genes were deleterious, hundreds were beneficial when overexpressed – remarkably, most of these effects were strain specific. Few beneficial genes were shared between the NaCl-sensitive isolates, implicating mechanistic differences behind their NaCl sensitivity. Transcriptomic analysis suggested underlying vulnerabilities and tolerances across strains, and pointed to natural CNV of a sodium export pump that likely contributes to strain-specific responses to OE of other genes. Our results reveal extensive strain-by-environment interaction in the response to gene CNV, raising important implications for the accessibility of CNV-dependent evolutionary routes under times of stress.

INTRODUCTION

Many unicellular organisms like budding yeast *Saccharomyces cerevisiae* live in environments that fluctuate. Yeast cells can exist in a range of habitats, from fruits and

trees to insects and human-associated niches (1, 2). Many of these habitats vary over time and space. Sudden environmental changes occur frequently in nature and can include fluctuations in nutrient availability, temperature, exposure to toxins, and other conditions (3). Thus, cells have evolved to deal with changing environments including changes that are stressful. Genetic variation has a substantial influence on stress tolerance and response, due in part to neutral genetic drift but also influenced by adaptive changes. A major focus in evolutionary biology has been to understand modes of evolution and the genetic architecture of differences in environmental tolerance. While single-nucleotide changes can influence phenotype, differences in gene copy number provide a driving force, especially in stressful environments (4-8). CNV can impart an immediate effect on gene expression, which in turn can have an immediate influence on phenotype; if the benefits outweigh the costs, CNVs can become fixed (4, 9-13). Yet how the cost and benefit of CNV varies with genetic background is only beginning to emerge.

We previously showed that the fitness consequences of gene overexpression (OE) can vary substantially depending on genetic background. We identified shared and unique responses to OE of ~4,700 yeast genes expressed on a high-copy plasmid in 15 different strains of *S. cerevisiae* selected from diverse niches and locations from around the globe (14). While OE of >400 genes is commonly deleterious to many strains, the majority of fitness effects were seen in only a subset of strains. This reveals that the fitness effects of gene OE vary widely with host genome, indicating extensive gene-by-CNV interactions. This implies that that different genetic backgrounds will have differential access to evolutionary routes that involve CNV; indeed, strains exposed to extreme selection evolve through different mechanisms, including those that leverage CNV and those that do not (15-19).

A major remaining question is how the environment influences genetic variation in the response to CNV. In nature, cells can experience many different conditions and environments; thus, understanding genotype-environment interactions (GxE) on the consequences of CNV is important (20-22). Here we explored this GxE relationship by examining how the response to gene OE varies across strains grown in a stressful condition. We chose sodium chloride (NaCl) as a stress because of the wealth of molecular information on how yeast cells respond to NaCl stress and how cells regulate the response (23, 24). Exposing yeast cells to NaCl causes osmotic stress and ion toxicity, which provoke diverse downstream effects including rapid water efflux, increased Na^+ influx and concentration in the cytosol, production of internal osmolytes among other metabolic changes, and mobilization of transcriptomic changes, including activation of the environmental stress response (ESR) (25-29). Several signaling pathways are known to respond to NaCl stress, including High Osmolarity Glycerol (HOG) pathway, AMP-responsive kinase Snf1, and Calcineurin (24, 30-34). These pathways can mediate defense strategies such as regulating glycerol accumulation, protecting against protein misfolding, and mediating metabolic changes.

To explore how GxE interactions influence the consequences of gene CNV, we expressed the MoBY 2.0 gene OE library (35, 36) in four different yeast strains, each

grown for 10 generations in 0.7M NaCl. While many of the detrimental effects were shared across strains, surprisingly many of the beneficial OE genes were largely strain-specific. Transcriptomic and genomic analysis revealed several important features of strain-specific responses to NaCl and gene CNV, which may translate to strain-specific evolutionary trajectories during times of stress.

METHODS

Strains and growth conditions

Strains used in this study include the laboratory strain BY4743 (MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0), BC187 (37), NCYC3290 (38), and YPS128 (39). Strains were grown in rich YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose) with G418 (200mg/L) for plasmid selection in shake flasks at 30°C, with or without 0.7M NaCl. As described previously (14), strains were transformed with the high-copy MoBY 2.0 library (35, 36) to at least 5-fold replication (~25,000 transformants). Colonies isolated on plates from the transformation were scraped, pooled, and stored at -80°C .

Fitness measurements

The competition experiments were performed as previously described (36). Frozen library-transformed stocks were thawed and placed in 100ml of liquid YPD with 0.7M NaCl and G418 (200mg/L), at a starting OD_{600} of 0.05. Cultures were transferred to fresh media with or without appropriate supplementation after 5 generations to keep cells in log phase. Cells were harvested after 10 generations and cell pellets were stored at -80°C.

Library construction, sequencing, and analysis

Plasmids were collected from each culture aliquot using QIAprep spin miniprep kits (Qiagen, Hilden, Germany). The pool of barcodes was amplified as previously described (2). Samples were pooled, split, and sequenced across three lanes of an Illumina HiSeq Rapid Run using single end 100bp reads. Sequencing data are available in the NIH GEO database under accession number GSE226247.

The data were normalized using library-size normalization as described in (14), with one modification: some barcodes repeatedly rose to very high read count in the wild strains. To avoid these genes skewing the library normalization, we calculated total sample read counts excluding genes with >50,000 reads, then divided all read counts (including these highly abundant counts) by that normalization factor. Some highly deleterious genes completely drop out of the population after NaCl outgrowth; for these genes we imputed missing data similarly to what was described previously (14) as follows: for genes that were well measured at the starting point (>20 normalized read counts in all three replicates) but missing after NaCl outgrowth, we added one pseudocount at generation 10. After library normalization, we scaled all values by 1,000,000 and rounded to the nearest integer for *edgeR* analysis (40). These data (Dataset 1) were used as input to *edgeR* using a linear model with generation and strain as factors. Genes with FDR < 0.05 were considered significant (41)(Dataset 2). Fitness scores

were calculated as the $\log_2$ ratio of normalized read counts after versus before outgrowth. Hierarchical clustering (42) was performed using Cluster 3.0 and visualized using Java TreeView (43). Data for YPD rich media without NaCl were taken from (14).

We considered genes with a fitness benefit as those with a significant positive fitness effect (FDR < 0.05); because many genes selected in BY4743 and BC187 strains had very small effect sizes, we also applied a magnitude threshold, requiring a $\log_2$ fitness effect of at least 0.8564, which was the smallest beneficial fitness effect of significant genes (FDR < 0.05) in NCYC3290 and YPS128. 314 genes met these criteria in at least one of the four strains analyzed (Dataset 3). Functional and biophysical enrichments were evaluated using Hypergeometric tests, taking p-value ≤ 10^{-4} as significant.

Transcriptome profiling and analysis

Yeast strains were grown in biological triplicate in rich YPD medium at 30°C with shaking, for three generations to an OD600 ~0.5; all strains were grown in parallel for each replicate, allowing paired downstream analysis. Cells were collected by centrifugation, flash frozen, and maintained at -80°C until RNA extraction. Total RNA was extracted by hot phenol lysis (44), digested with Turbo DNase (Invitrogen) for 30 min at 37°C, and precipitated with 5 M lithium acetate for 30 min at -20°C. rRNA depletion was performed using the Ribo-Zero (Yeast) rRNA Removal Kit (Illumina, San Diego, CA) and libraries were generated according to the TruSeq Stranded Total RNA kit and purified using a Axygen AxyPrep MAG PCR Clean-Up Kit (Axygen). The samples were pooled, re-split, and run across three lanes on an Illumina HiSeq 2500 sequencer, generating single-end 100 bp reads, with ~7,494,848 reads per sample. Sequencing data are available in the NIH GEO database under accession number GSE226246.

Reads were processed using Trimmomatic version 0.3 (45), and mapped to the S288c reference genome (version R64-1-1) with bwa-mem (version 0.7.12-r1039) (46). Read counts for each gene were calculated by HT-Seq (version 0.6.0) (47) and normalized using the TMM method in *edgeR* (40). We used a linear model in *edgeR* with strain background as a factor and paired replicates, identifying genes differentially expressed in each strain relative to the average of all strains taking FDR <0.05 as significant. Hierarchical clustering (42) was performed by Cluster 3.0 and visualized using Java TreeView (43). Functional analysis and enriched GO categories for each sample were obtained using hypergeometric tests, taking p-value ≤ 10^{-4} as significant. *EdgeR* identified 1,114 genes whose fold-change in expression after NaCl was different in at least one strain compared to the mean fold-change across strains (FDR < 0.05, Dataset 4, Tab 1). Relative expression was represented as the $\log_2$ (fold change) in each strain, comparing normalized read counts 30 min or 3h versus before NaCl treatment in each strain. Comparing basal (unstressed) expression across strains identified 608 genes whose expression was significantly different from the mean (FDR<0.05) in at least one of the strains (Dataset 4, Tab 2).

ENA CNV analysis

We analyzed previously published DNA-seq data generated for these strains (38, 48, 49). To avoid mapping errors to the S288c reference that contains multiple highly-similar ENA genes, we re-mapped reads to a reference sequence consisting of S288c *ENA1* (*YDR040C*) and three representative genes (YDR229W, YGR125W, YJL059W) whose DNA-seq read abundance was close to the median of the genome; these genes span different chromosomes and are expected to be at 1X copy number per haploid genome. We then mapped DNA reads from each wild strain to the reference sequence using bwa-mem (46) and used samtools mpileup function (50) to plot the coverage of each base pair in the reference sequence. Coverage was normalized to the median coverage of the three reference genes. Fig 5 shows the running average of normalized read count over 500bp windows.

RESULTS

To investigate the effects of gene OE under NaCl stress, we focused on a subset of diploid strains analyzed in a previous study from our lab, including three strains from defined genetic lineages (West African strain NCYC3290, vineyard strain BC187, and North American oak isolate YPS128) and common lab strain BY4743 as a well-studied reference (38, 51). In addition to genetic differences, these strains display extensive phenotypic diversity in several different environmental conditions, including osmotic stress (49, 51-53). We first measured strain growth rates in 0.3M and 0.7M NaCl to characterize NaCl tolerance. Growth of all strains was reduced in NaCl compared to rich medium (Fig 1). However, there was significant variation in strain responses. West African NCYC3290 and to a lesser extent oak soil YPS128 strains were much more sensitive to the higher dose of NaCl, indicated by their 8.5X and 3.5X reduction in doubling time compared to only ~2X reduction in vineyard strain BC187 and lab strain BY4743. We chose the higher dose of NaCl to interrogate how this environment influences strain-specific responses to CNV.

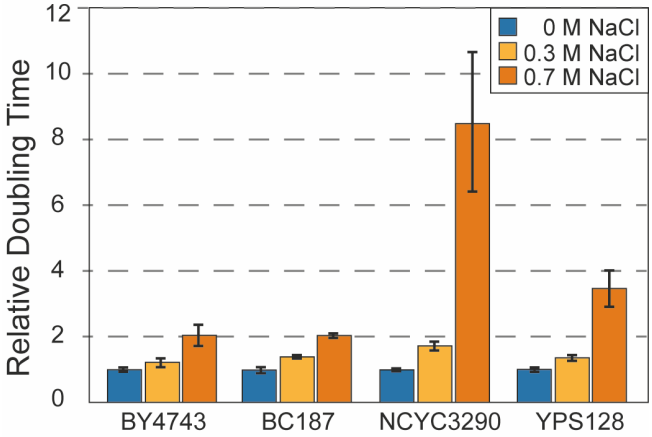

Figure 1: Strains vary in salt sensitivity. Average and standard deviation (n=3) of doubling times of each strain grown in the absence or presence of NaCl according to the key, normalized to the unstressed growth rate of that strain.

Next, we quantified the fitness effects of gene OE across different genetic backgrounds when cells were subjected to a stressful environment of 0.7M NaCl. Each strain was transformed with the library, which includes ~4,700 genes cloned from S288c along with their native upstream and downstream sequences, cloned onto a 2-micron replication plasmid (35, 36). Cells were inoculated into rich medium and an aliquot collected before ('0 generation' sample) and after 10 generations of growth in 0.7M NaCl, in biological triplicate. Gene abundances before and after outgrowth were identified by deep sequencing of plasmid barcodes. Fitness effects were defined as the $\log_2$ ratio of normalized barcode read counts after NaCl treatment *versus* before (see Methods). Genes whose barcodes decreased in frequency over time are inferred to produce a fitness defect relative to the rest of the library, whereas genes whose barcodes increased in frequency are inferred to provide a fitness benefit. We used linear modeling to identify genes with a significant fitness effect in each strain (FDR < 0.05).

Common and unique fitness consequences across strains and environments.

We identified a total of 3,644 genes whose OE produced a fitness effect in at least one strain growing under NaCl stress (FDR< 0.05, Fig 2). Yet the number and magnitude of fitness costs varied substantially. Lab strain BY4743 showed the least impact of gene OE, both in terms of number of deleterious OE genes and their impact on fitness (Fig 2A-B), followed by BC187 which also showed relatively mild fitness effects for most genes. For both of these strains, the distribution of fitness costs was similar with and without NaCl, consistent with their ability to grow well in salt-containing medium (Fig 1). In contrast, the NaCl-sensitive strains showed several key differences. First, both strains were more sensitive to gene OE in the absence of stress, indicated both by the number of deleterious OE genes and their greater effect sizes compared to NaCl-tolerant strains. Second, both strains – but especially the most NaCl-sensitive, NCYC3290 – were much more sensitive to gene OE in the presence of NaCl. For example, the vast majority of OE genes were deleterious in NCYC3290 and with severe fitness costs (Fig 2A-C).

To explore the patterns of fitness costs across strains and conditions, we hierarchically clustered OE genes that had a fitness effect in at least one strain. The resulting heat map illustrates the commonalities and differences across strains and conditions. We identified one cluster of ~200 OE genes (Fig 2C, black bar) that were deleterious in all four 4 strains in both YPD and NaCl stress conditions, to varying degrees. Consistent with past work from our (14), this gene group was heavily enriched for genes involved in translation, including ribosomes and ribosome biogenesis factors, protein folding factors, and genes repressed during stress in the Environmental Stress Response ($p < 10^{-4}$, Hypergeometric tests). But even for these commonly deleterious OE genes the magnitude of the effect varied, especially for the wild strains where the fitness cost was more severe in the presence of NaCl. These results are consistent with the notion that the cost of gene OE is greater in strains already experiencing suboptimal conditions (see Discussion).

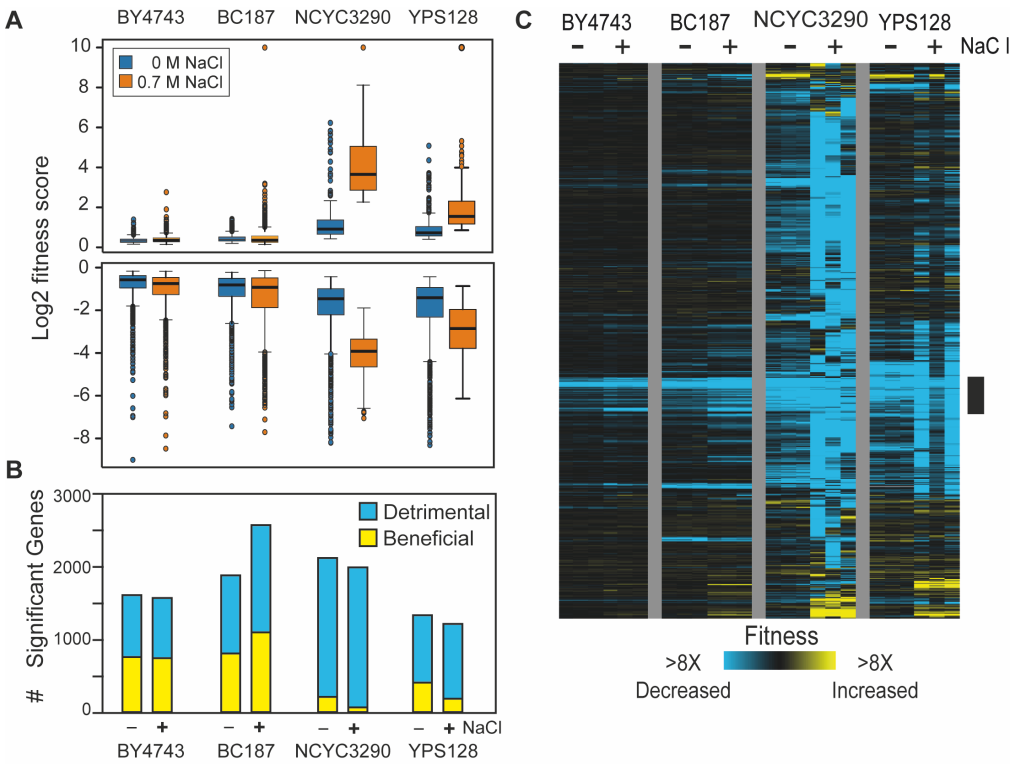

Figure 2: Fitness consequences vary by strain. A) Distribution of average $\log_2$ fitness scores in each strain grown 10 generations in rich medium in the absence of NaCl (blue) or in medium with 0.7M NaCl (orange). B) The number of detrimental (blue) or beneficial (yellow) genes in each strain and media condition. C) Hierarchical clustering of 3,644 genes with a fitness effect (FDR < 0.05) in at least one strain grown in NaCl stress. Each row is a specific gene, each column is a biological replicate of that strain grown in the absence (-) or presence (+) of NaCl. Blue and yellow values represent genes that dropped or rose in abundance during competitive growth, reflecting decreased or increased fitness effects according to the key. The black bar indicates a cluster of genes whose OE is detrimental to varying degrees in all strains and under both conditions. Data from NaCl-free conditions were taken from (14).

Beneficial genes vary widely across strains.

We were particularly interested in genes whose OE provides a benefit under NaCl stress, since these may provide adaptive value. We identified and hierarchically clustered 314 genes whose OE produced a significant benefit in NaCl over an effect threshold in at least one strain (Fig 3A, see Methods). BY4743 and BC187 had a substantial number of genes that passed our statistical threshold but were of very small effect size (hence the use of a magnitude threshold, identifying only 23 beneficial OE genes for BY4743). In contrast, 116, 65 and 183 genes met our criteria of providing a benefit to BC187, NCYC3290 and YPS128, respectively. Interestingly, there was only small overlap in which genes were beneficial during NaCl stress, even for the two NaCl-sensitive strains (Fig 3B). This strongly implies that the genetic basis for NaCl sensitivity is different in the two sensitive strains.

Only four genes were scored as beneficial to all three wild strains but not BY4743 (Fig 3B). These included calmodulin kinase *CMK2*, Snf1-related kinase *HAL5* that

regulates ion tolerance (54, 55), CK2 kinase subunit *CKA1* that has been implicated in NaCl stress (56, 57), and *GCD10* that encodes a tRNA methyl transferase. It is interesting that three of the four genes are kinases that could have a wide range of downstream effects on physiology. Expanding to genes shared between the two sensitive strains identified several other genes involved in cation homeostasis including kinase *SAT4*, cation and drug transporter *QDR2,* and diacylglycerol kinase *DGK1* (itself a target of CK2 (58)). This group was also enriched for genes regulated by the HOG-regulated osmotic stress transcription factor Sko1 ($p=5x10^{-4}$, Hypergeometric test), which is interesting in the context of the NaCl sensitivity of these strains. Interestingly, the total set of genes whose OE was beneficial to YPS128 were enriched for genes involved in mRNA P-body and stress granule assembly and ergosterol biosynthesis ($p<1x10^{-4}$, Hypergeometric test). BC187 also benefited from OE of many RNA binding proteins, several of which were shared with YPS128. In contrast, genes beneficial to NCYC3290 were enriched for those involved in glycosyl-group transferase activity and flocculation. Together, these results indicate that the fitness consequences of beneficial genes are largely strain specific, in some cases causing opposing fitness effects in different strains (Fig 3, clusters a, c, and d).

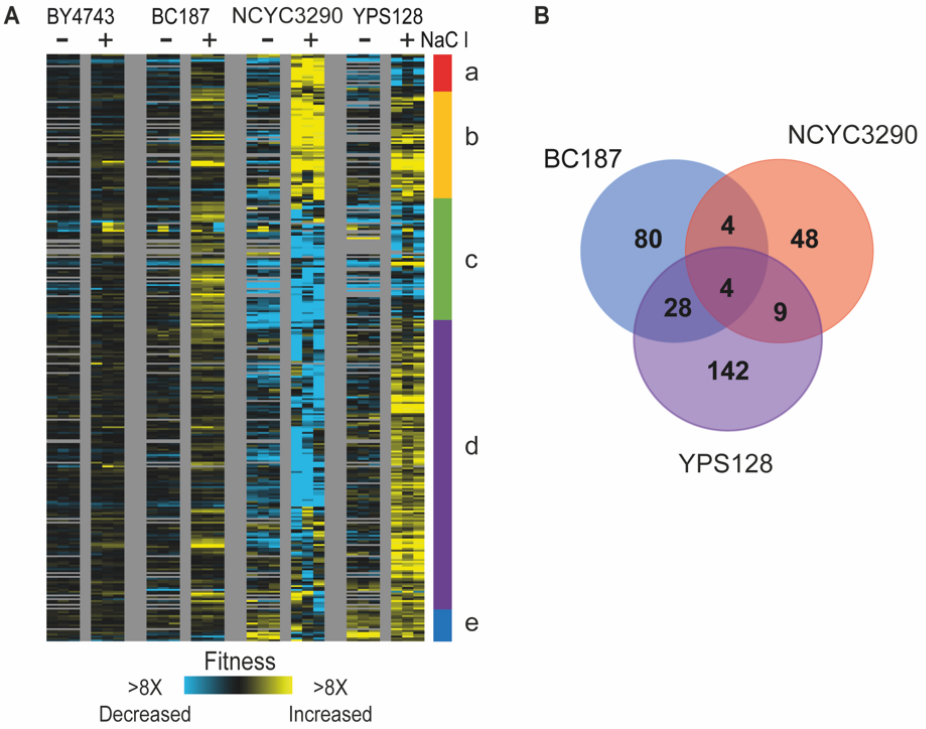

Figure 3. Beneficial genes vary widely by strain. A) Hierarchical clustering of 314 genes that were beneficial in at least one strain (see Methods), as described in Figure 2; grey values indicate missing values. Several clusters were enriched for functional groups ($p<1x10^{-4}$, Hypergeometric test) including genes involved in a) cell-cycle entry, c) negative regulation of heterochromatin, d) cytoplasmic mRNA processing body assembly, snoRNA processing. B) Overlap of beneficial genes identified in the three wild strains.

338 ***Transcriptomic analysis implicates genetic modifiers***
339
340 The low overlap in beneficial OE genes among the NaCl sensitive strains suggests that
341 NaCl sensitivity is explained by different genetic or physiological limitations. In attempt
342 to better understand background-specific effects that could influence these differences,
343 we characterized each strain's transcriptomic response to NaCl shock. Cells grown in
344 rich medium were shifted to media with 0.7M NaCl, and samples were collected before
345 and at 30 minutes and 3 hours after the shift, in biological triplicate (see Methods). We
346 expected that the sensitive strains may show substantial differences in transcriptomic
347 response to the shock, reflecting their increased NaCl sensitivity; however this was not
348 the case. Using a linear model to identify strain-by-environment interactions, we
349 identified 1,141 genes whose expression to NaCl differed across strains at one or both
350 time points after shock (FDR < 0.05, see Methods). The four strains showed fairly
351 similar gene expression changes at both induced and repressed genes (Fig 4A) – there
352 were no large gene groups that were uniquely induced or repressed in any of the
353 strains. Furthermore, the magnitude of many of those gene expression changes were
354 similar across strains. This was especially surprising for the sensitive strains, since we
355 expected that they may exhibit larger magnitude changes if they experience a stronger
356 stress from 0.7M NaCl treatment.
357
358 Despite these global similarities, on closer inspection we noticed a difference in the
359 timing of the response, especially compared to the BY4743 laboratory strain. The lab
360 strain had much larger changes in expression at 30 min after NaCl shock than the other
361 strains, and many of these expression differences were already subsiding by 3 hours.
362 Genes induced and repressed in the ESR provide a good representation (Fig 4B): both
363 gene sets show much larger and earlier expression changes in the laboratory strain. In
364 contrast, all three wild strains showed delayed expression changes, of ESR genes (Fig
365 4B) and more broadly (Fig 4A). Much of the transcriptome response is regulated by the
366 Hog1 kinase that responds to osmotic stress. Interestingly, it is well known that the
367 timing of Hog1 signaling varies with the dose of ionic stress: at higher doses of stress,
368 signaling is delayed and produces a delayed transcriptomic response (59-62). This
369 suggested that wild strains could all be experiencing a higher level of NaCl stress that
370 delays the transcriptomic response (yet does not grossly change the magnitude or
371 genes in the response, see Discussion).
372
373 Although the transcript changes to NaCl were not wildly different across strains aside of
374 the timing, we wondered if basal expression differences in the strains, before NaCl
375 exposure, could be informative. We therefore identified 608 genes whose expression
376 was significantly different in at least one strain compared to the mean (FDR < 0.05, see
377 Methods). Here, expression differences were more noticeable across the strains (Fig
378 4C). In fact, the laboratory strain was a clear outlier compared to the three wild strains:
379 one large group of genes was expressed significantly higher in BY4743 in the absence
380 of stress, and this group was enriched for stress-defense genes, oxidoreductases,
381 amino acid biosynthesis genes, transporters, and genes encoding proteins localized to
382 the membrane and vacuole (p<1e-4, Hypergeometric test). This result suggests that
383 BY4743 is already prepared for stress even before exposure (see Discussion). A

second group of genes was expressed significantly lower in the lab strain, and these were heavily enriched for genes encoding respiration factors and ergosterol biosynthesis genes and targets of Hap1 (Fig 4C). Some of these differences may result from known polymorphisms in S288c-derived strains that affect the regulation of those genes (including *MKT1*, *MIP1*, *HAP1* along with auxotrophic markers that affect mitochondrial and respiratory functions and/or ergosterol-gene expression (63-65)).

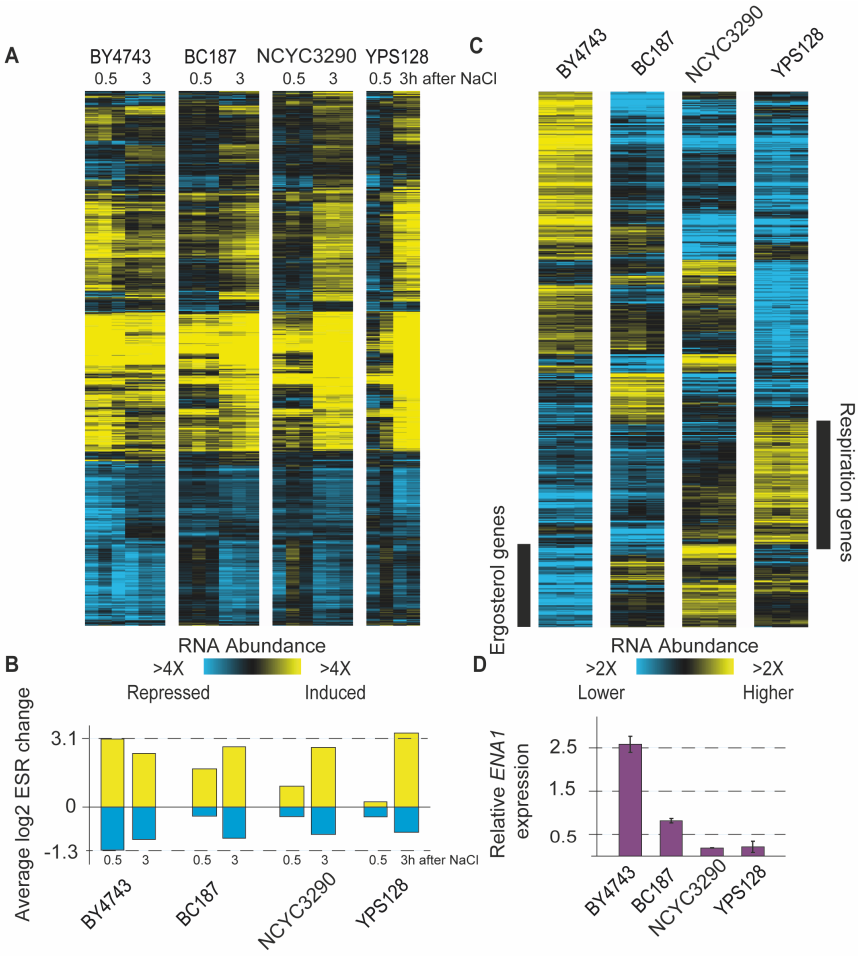

Figure 4. Gene expression differences implicate strain-specific physiology. A) Hierarchical clustering of 1,141 genes whose $\log_2$(fold change) in response to NaCl was different from the mean in at least one of the four strains (FDR < 0.05). As shown for Figure 2, except that here values represent relative mRNA abundance in stressed samples versus the prestressed sample from that strain. Each column represents one of three biological replicates (or two in the case of YPS128). B) The average $\log_2$ fold-change in expression of genes from A that are part of the induced (positive $\log_2$ changes) or repressed (negative $\log_2$ changes) ESR, at 30 minutes and 3 hours after NaCl treatment in each strain. C) Hierarchical clustering of 608 genes whose basal expression before NaCl is different from the mean in at least one of the four strains (FDR < 0.05). Each column is one of three biological replicates of cells growing in rich medium in the absence of stress, where $\log_2$ values represent expression relative to the mean of all strains according to the key. D) Average and standard deviation of *ENA1* expression in the absence of stress relative to the mean of all strains.

Remarkably, among the genes with much higher basal expression in BY4743 were several linked directly to Na+ transport, including *ENA1*, *ENA5*, and *NHA1*. In fact, *ENA*

genes coding for P-type ATPase sodium pumps are known to have undergone tandem duplications in different strains including S288c, which harbors three copies per haploid genome, each with high sequence homology (66-69). *ENA* genes are important components in the saline detoxification (although they are not present in the MoBY 2.0 library). We plotted the relative abundance of RNA-seq reads mapping to *ENA1* as a representative and found that expression varied wildly across strains. The two sensitive strains showed very low expression of *ENA1* relative to the other strains; expression in vineyard strain BC187 was ~4X higher than NCYC3290, while expression in lab strain BY4743 was 14X higher (Fig 4C). These differences raised the possibility of underlying gene copy-number differences.

To investigate if underlying expression differences were due to natural CNV of these genes, we interrogated DNA-seq reads mapped to a single ENA gene copy relative to three representative genes present in single copy per haploid genome (see Methods). NCYC3290 and YPS128 both showed per-base coverage that was on par with the single copy genes, barring some fluctuation in read depth that may be due to polymorphism-dependent mapping errors, indicating that these strains harbor a single *ENA* copy per haploid genome (Fig 5A). In contrast, BC187 showed read distribution that was 2.5-3X higher across most of the gene, excluding the very amino terminus. The reduced coverage at the very front of the gene could be due to incomplete gene duplication, or it may reflect substantial polymorphisms between BC187 and the reference genome that obscure copy number. The latter possibility is supported by the known high rate of evolution of ENA genes, including at least one case of introgression from *S. paradoxus* (52, 67, 70, 71). We conclude that BC187 has 2.5-3 ENA gene copies per haploid genome, although the functionality of all copies remains to be explored.

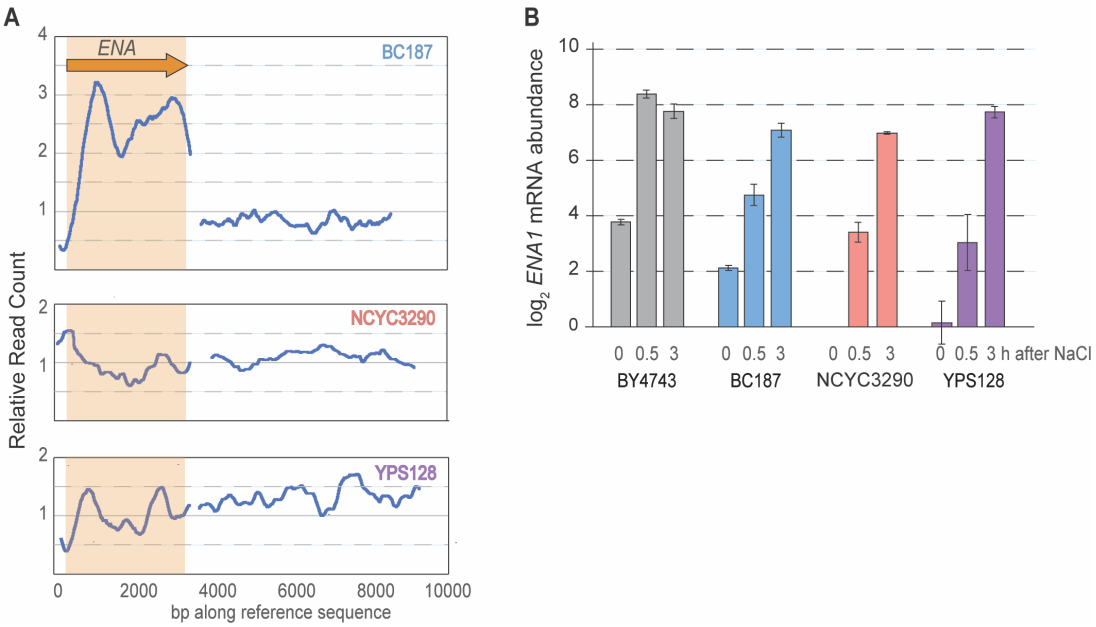

Figure 5. Strains vary in *ENA* copy number and expression. A) Relative DNA-seq read count across a representative *ENA* gene (left trace) and three representative single-gene copies (right trace) in each of

439 the three wild strains (see Methods). B) Average and standard deviation (n=3) of log_2 fold-change in
440 *ENA1* mRNA abundance before and after NaCl treatment, normalized to NCYC3290 basal levels at time
441 0 min.
442
443 Strains with higher abundance of sodium pumps are well known to have increased
444 tolerance to sodium stress (71, 72). Interestingly, however, strain-specific differences in
445 *ENA* transcript abundance were not fully explained by differences in ENA copy number:
446 although BY4743 and BC187 both harbor 2.5-3 *ENA* copies per haploid genome, basal
447 expression in the lab strain was 3-fold higher than BC187 (Fig 4C); likewise, basal
448 expression in BC187 was >4-fold higher than the sensitive strains. These results
449 suggested that the response to NaCl could be influenced both by variation in *ENA* gene
450 copy number and by *ENA* gene regulation. ENA genes are also transcriptionally
451 induced by NaCl (73-75). We plotted *ENA1* mRNA abundance before and after NaCl in
452 each strain normalized to NCYC3290 basal levels. While BY4743 harbored the highest
453 *ENA1* expression in the absence of stress, the strain further induced *ENA1* another 25-
454 fold within 30 minutes after 0.7M NaCl exposure. Remarkably, the other strains all
455 induced *ENA1* expression to within 2-fold of BY4743 mRNA levels, albeit with delayed
456 kinetics. While induction of *ENA1* may help with the acclimation to continued salt stress
457 (76), it is likely that the basal expression levels influence the immediate survival after
458 rapid-onset salt stress. Thus, we propose that although strains retain the ability to
459 induce *ENA1* expression after stress, the low starting mRNA levels likely contribute to
460 variations in the ability to survive the initial NaCl exposure – this likely also explains
461 differences in the fitness cost of other OE genes, including OE of other sodium
462 transporters and ion-response regulators whose duplication provides a major benefit to
463 wild strains but has no effect in BY4743 (see Discussion).
464
465 **DISCUSSION**
466
467 Our results show that environmental stress has a major influence on the fitness
468 consequences of gene OE, and those effects vary substantially across genetically
469 distinct individuals. That environmental stress influences the cost of gene OE may not
470 seem surprising from a physiological point of view, and this is consistent with
471 longstanding models on the effects of stress on mutational tolerance (77-80). However,
472 our results have major implications for how strain and environment affect the fitness
473 costs of gene CNV, which is a major route to rapid evolution. We propose that Strain-
474 by-Environment-by-CNV interactions are prominent and could produce substantial
475 variation in the evolutionary trajectories accessible to different individuals and over
476 space and time.
477
478 Simply duplicating a gene's copy number can increase its expression, at least for genes
479 that are not dosage regulated (13, 48, 81, 82). Amplification of many genes is
480 deleterious even in the absence of stress, likely due to the increased burden of
481 producing extra DNA, RNA, and protein but also due to internal imbalances caused
482 therein (12, 83). NaCl treatment exacerbated the deleterious effects of many of those
483 genes, as might be expected (77-80). But even for the same concentration of NaCl,
484 strains more sensitive to NaCl showed both more deleterious responses and larger
485 effect sizes during NaCl and compared to more tolerant strains (Fig 2). This is

486 consistent with the idea that the cost of CNV is generally worse when cells are already
487 taxed, in accordance with previous implications from our work (14). In many cases, the
488 increased cost of gene OE may be independent of the gene's function (11, 12, 14) and
489 could simply represent compounded burdens of producing extra protein during an
490 energy-consuming stress response. In other cases, specific gene functions may be
491 counterproductive during the NaCl acclimation and thus uniquely deleterious in that
492 environment. For example, increased expression of functional aquaporin water
493 transporters is detrimental during osmotic shock, due to passive water loss that
494 exacerbates stress-induced water efflux (84). Although increased aquaporin expression
495 is beneficial in other environments, the cost of that increase is harder to overcome in
496 high-osmolar conditions The implication is that different evolutionary routes will be more
497 or less accessible depending on the environmental context.
498
499 Adaptive benefits of CNVs are well known during environmental stress (4-8), and thus
500 we expected to find some genes whose OE is uniquely beneficial during NaCl exposure
501 compared to standard conditions. The surprise was that there was little overlap in
502 beneficial genes across strains, including the more sensitive strains. Part of the low
503 overlap results from inclusion of the laboratory strain, BY4743. This strain showed the
504 fewest beneficial genes, and most of those genes produced only mild benefits. This
505 along with growth responses shown in Fig 1 are consistent with the notion that BY4743
506 is fairly tolerant of NaCl, and thus there is little room for improvement. Our genomic
507 analyses raised several possible explanations. Unstressed BY4743 shows higher
508 expression of many genes directly related to stress defense (Fig 4C), including genes
509 linked to osmotic and other stress defenses. Furthermore, BY4743 displays very high
510 expression of ENA genes encoding sodium efflux pumps even in the absence of stress,
511 likely due to both natural ENA gene amplification and higher expression from one or
512 more copies. Together, these results strongly suggest that BY4743 is already prepared
513 for NaCl stress before exposure, requiring less acclimation effort upon treatment. This
514 preparedness could also explain the accelerated transcriptomic response to NaCl shock
515 (Fig 4A,B): higher basal stress tolerance coupled with immediate Na+ efflux could
516 result in a lower effective dose of NaCl, which is known to produce a faster signaling
517 response (59-62). Whether these expression differences have been selected due to
518 laboratory domestication or merely accumulated due to neutral drift, these results are
519 consistent with the notion that there is little adaptive benefit for most OE genes in the
520 lab strain under these conditions.
521
522 The results in the lab strain are perhaps not surprising, since laboratory strains are often
523 outliers in their responses (48, 85-87) – but we were surprised to see the low overlap in
524 beneficial OE genes across wild strains, especially NaCl-sensitive NCYC3290 and
525 YPS128 (Fig 3B). The beneficial gene sets for each strain were enriched for distinct
526 functions; the exception was shared enrichment of osmo-responsive Sko1 targets
527 among the beneficial genes shared by the two sensitive strains. Yet there were several
528 genes common to two or all three of the wild strains (albeit with different effect sizes,
529 often greatest in the sensitive strains, Dataset 2). These included genes linked to cation
530 stress, including kinases Hal5 and Sat4 and transporter Qdr1 that are known to

531 modulate ion tolerance (55, 88, 89). It is interesting that these genes had virtually no
532 benefit in the lab strain that is already well equipped for Na+ stress.
533
534 We propose that the significant fitness benefit of these genes to wild strains but not
535 BY4743 could be impacted by variation in abundance of *ENA* ATPase Na+ pumps. The
536 sensitive strains harbor only one copy of *ENA* per haploid genome, whereas BC187
537 carries 2.5 to 3 copies that remain lower expressed than in BY4743 (Fig 5). Given the
538 importance of ENA expression in NaCl tolerance (71, 72), we propose that differences
539 in *ENA* copy number and expression influence which other OE genes will be beneficial.
540 This would explain why strains with lower *ENA* mRNA abundance greatly benefit from
541 OE of other genes directly involved in sodium efflux, whereas BY4743 receives no
542 benefit from over-expression of those genes. Interestingly, several other genes
543 uniquely beneficial to one or more wild strains transcriptionally up-regulate *ENA1,*
544 including CK2 whose OE benefited all strains and *CRZ1* that was highly beneficial to
545 BC187 (33, 74, 90). These possibilities highlight the potential for genetic interactions
546 between CNVs, since the impact of one gene's amplification is dependent on another
547 gene's copy number.
548
549 In all, this study adds to the body of evidence that the impact of a mutation, in this case
550 gene OE to mimic the effects of CNV, depends on not only the genomic context but also
551 environment. Ultimately, the combined effect of genomic and environmental variation
552 can perhaps best be considered as Gene-by-System interactions (91), where the impact
553 of a gene's CNV depends on the physiological state of the cell. A remaining challenge
554 is developing statistical models that can both represent Gene-by-System interactions
555 and uncover the biology behind them.
556
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558
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562
563
564 **DATASET LEGENDS**
565
566 **Dataset 1: Normalized and imputed barcode read count.** Normalized and imputed
567 data from MoBY 2.0 selection experiments, as described in Methods.
568
569 **Dataset 2: *EdgeR* output for MoBY 2.0 selections.** Each block represents edge R
570 results for each indicated strain, see *edgeR* manual for details.
571
572 **Dataset 3: Beneficial genes.** Each tab represents *edgeR* output for genes
573 determined to be significant at FDR < 0.05. Tabs for BY4743 and BC187 labeled
574 'threshold' are those with FDR < 0.05 and meeting the effect size threshold as
575 described in Methods.
576

Dataset 4: Normalized log2 relative gene expression from RNA-seq experiments. Tab 1) log_2 values represent the fold change in expression in each denoted strain at 30 min or 3 h versus the unstressed sample for that strain. Tab 2) log_2 values represent the fold difference in expression in each denoted unstress strain relative to the mean expression of all four strains from that paired replicate.

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