ASSESSING THE IMPACT OF TEMPERATURE ON POPULATION-SPECIFIC GENE EXPRESSION IN A MODEL ESTUARINE CNIDARIAN, *NEMATOSTELLA VECTENSIS*

by

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ABSTRACT

AMY L. KLOCK. Assessing the impact of temperature on population-specific gene expression of a model estuarine cnidarian, *Nematostella vectensis*. (Under the direction of DR. ADAM REITZEL)

Temperature is a critical environmental factor that affects all living organisms. Climate change is resulting in rising global temperatures with marine environments being especially vulnerable. My study aimed to elucidate the temperature-dependent molecular mechanisms deployed by five populations of *N. vectensis* along a naturally occurring thermal gradient along the Atlantic coast of the United States and Canada. Animals were exposed to one of three temperatures over a period of 30 days. Comparisons of transcriptome-wide gene expression indicated that there was not an overall conserved thermal stress response seen across populations or temperatures. Each population displayed unique gene expression profiles suggesting population-specific responses to temperature, even when the temperature differences were identical and the populations were geographically close in proximity. Unexpectedly, populations from the two most distant locations (Nova Scotia and South Carolina) shared more similarity in differentially expressed genes. Together the results from this study support a hypothesis that populations of this sea anemone have evolved largely unique temperature responses when cultured over long time periods. Future research comparing physiological and genomic differences between these locations will assist in understanding how the differences in DNA sequences may contribute to the transcriptional variation and how this variation impacts the ability to respond to broad temperature ranges.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SST	Sea surface temperature
NS	Nova Scotia
ME	Maine
MA	Massachusetts
NC	North Carolina
SC	South Carolina
HSP	Heat shock proteins
DE	Differentially expressed
DEG	Differentially expressed genes
FDR	False discovery rate
GLM	General linear model
PCA	Principal component analysis
MDS	Multidimensional scaling plots
PC1	Principal component 1
PC2	Principal component 2
PC3	Principal component 3
PERMANOVA	Permutational analysis of variance
GO	Gene ontology
MF	Molecular function
BP	Biological process
CC	Cellular component
WGCNA	Weighted gene co-expression network analysis

CHAPTER 1: INTRODUCTION

1.1 Rising Temperatures and Climate Change in Marine Environments

Temperature is a key environmental factor that affects physiology, survival, and distribution of most organisms, especially ectotherms, by affecting the rates of all biochemical and physiological reactions (Hochachka and Somero 2002; Parmesan 2006; Somero 2012). The consensus among climatic models is that temperature is steadily increasing and will continue to do so in the coming decades. Changes in sea surface temperature (SST) are particularly critical in determining the extent of climate change due to water's high thermal capacity requiring a substantial input of thermal energy to increase its temperature. Over the last 100 years, SST has risen anywhere from 0.07°C to 0.11°C (Gil-Alana et al. 2019). At face value, this may not appear to be a big difference but, coupled with increasingly drastic seasonal fluctuations, it is likely to cause detrimental effects within marine ecosystems (Lima and Wethey 2012; Solomon et al. 2007). Hospitable geographic ranges will shift and ultimately shrink resulting in the extinction of thermosensitive organisms that are unable to relocate or acclimatize (Harley et al. 2006; Helmuth et al. 2002; Pörtner et al. 2001).

Episodic high temperature events often result in rapid, acute stress response leading to high fluctuations in stress-related gene expression (Berger et al. 2007; Tomanek 2010; Schoville et al. 2012;), increased mortality rates (Kelly et al. 2012; Lima and Willett 2017;), and decreased growth and reproduction (Parsons 2005; Willett 2010). Phenotypic plasticity is required in organisms that experience large fluctuations in temperature as it allows for quick acclimation to the changing environment on a gene-by-gene basis. On the other hand, small temperature increases can also be physiologically stressful if persistent over time, especially on sessile organisms (Nguyen et al. 2011). For example, a study done by Sleight et al. (2018) found that after chronic exposure to increased temperatures, *Mya truncata* (bivalve mollusk) had differentially expressed genes (DEGs) enriched in processes related to cellular stress and immune response.

Climate change presents a particular challenge for individuals living in "island" habitats, where organisms confront barriers to migration, and therefore must either rely on existing physiological plasticity or genetically adapting to the shifting environment (Parmesan 2006; Visser 2008). In the case of copepod *Tigriopus californicus*, a heat-tolerant phenotype is found at lower latitudes that cannot be achieved at higher latitudes through either acclimation or generational selection. However, this was not the case in a study done by Kenkel et al. (2016) where the coral *Porites astreoides* showed high plasticity when exposed to increasing temperatures. This indicates that plasticity and adaptation have an undetermined and likely species-specific capacity to safeguard isolated populations from going extinct due to increasing temperatures. Understanding the contributions of physiological plasticity and genetic adaptation play in determining thermal tolerance in populations is critical for determining how species will respond to climate change long-term.

1.2 Plasticity and Local Adaptation in Coastal Cnidarians

Coastal cnidarians have provided insight into phenotypic plasticity and local adaptation of populations inhabiting different thermal environments. Responses to temperature and evidence for adaptation have been particularly well studied within this phylum due to the thermal sensitivity of corals and other critical members of shallow water habitats (Maor-Landaw & Levy 2016; Dixon et al. 2020). Resilience to increased environmental temperatures in cnidarians and other marine invertebrates involves various molecular processes including molecular chaperone expression, programmed cell death, and oxidative stress response (Barshis et al. 2012). A recent meta-analysis conducted from 14 previously published studies found that Acropora corals have two distinct stress responses to either high or low intensity stress. High stress was characterized by upregulation of genes involved in cell death, response to reactive oxygen species, immune response, NF- ϰB signaling, protein folding, and degradation. Low intensity stress resulted in a distinct transcriptional response that was nearly reverse from what was previously observed (Dixon, Abbott, and Matz, 2020). Additional research on corals (Barshis et al. 2013; Schoville et al. 2012) has found evidence suggesting some populations may be particularly efficient at adapting to warmer temperatures by increasing constitutive expression of genes responsible for responding to increased thermal stress.

Populations of species, and perhaps tolerant species in general, found in areas where temperatures fluctuate the most are predicted to have numerous genes that are expressed at elevated levels regardless of temperature. These same genes are also predicted to only increase in expression levels within sensitive populations when a thermally stressful event occurs and are consistently highly expressed in tolerant populations ("constitutive frontloading"). The notion here is that some populations will be better adapted to warmer temperatures because they have higher baseline expression levels of genes related to heat stress (e.g., heat shock protein related genes, proteolysis; Barshis et al. 2012). However, studies on other coral species have shown no evidence for frontloading in populations adapted to warmer environments, instead populations may be better adapted through means of genetic plasticity involving shifts in metabolism, cell membrane integrity, and posttranscriptional modifications (Schoville et al. 2012). This diversity of molecular responses to thermal stress and its distribution across natural populations is critical in understanding the mechanism driving the resiliency seen between populations and species.

1.3 Studying Chronic Temperature Stress in a Cnidarian (*Nematostella vectensis*)

The starlet sea anemone, *Nematostella vectensis*, is an excellent model to compare population specific molecular responses to changing thermal environments. *N. vectensis'* native habitat range runs along the Atlantic coast of the United States and Canada (Reitzel et al. 2008a) and experiences a dramatic thermocline spanning nearly 10° latitude (Hand and Uhlinger 1994; Reitzel et al. 2008a). This sessile estuarine anemone is exposed to a range of abiotic factors such as fluctuations in temperatures, UV light exposure, and salinities. This coastal cnidarian often experiences changes in temperature up to 20°C spanning a range of -1.5°C to 41°C (Reitzel, 2013a). Because of the geographic range for *N. vectensis,* different thermal optima for growth and thermal tolerance have been observed dependent on the animal's latitude of origin (Reitzel et al., 2013a). These differences in phenotypes combined with a high degree of structured genetic variation across its range has suggested that *N. vectensis* may exhibit local adaptation to temperature (Reitzel et al., 2013b).

Thus far, studies on the molecular response to temperature across populations in *N. vectensis* are limited. Under acute thermal stress *N. vectensis* has been shown to upregulate genes associated with antioxidant response and heat shock proteins (HSP) (Tarrant, 2014; Reitzel, 2008b; Waller et al., 2018). Due to *N. vectensis'* ease of collection and culturing in a laboratory setting, as well as having a publicly available genome, it has the potential to become a model organism for studying the molecular mechanisms responsible for thermal adaptation and acclimation in cnidarians and other coastal animals.

The aim of my research is to understand the variation in gene expression of *N. vectensis* from different locations under thermal conditions representative of the temperature differences observed across this species' geographic range. Patterns of gene expression may be driven by location of origin, thermal regime exposure, or a combination of the two. Those expression patterns for differentially expressed genes may be driven by conserved temperature stress response genes when exposed to location-based average high temperatures or completely unique sets of genes if adaptation to the thermal environment evolves quickly or independently. I hypothesize that there will be a conventional transcriptional stress response observed in *N. vectensis* when exposed to increasing chronic temperatures depending on location of origin.

CHAPTER 2: MATERIALS AND METHODS

2.1 Animal Collection & Experimental Assay

Adult *N. vectensis* were collected from five locations along the Atlantic coast of North America (Mahone Bay, Nova Scotia (NS); Saco River, Maine (ME); Sippewissett, Massachusetts (MA); Ft. Fisher, North Carolina (NC); and Georgetown, South Carolina (SC)) (Figure 1). They were then cultured in glass dishes under ambient conditions (20°C, 15 parts per thousand (ppt) ASW, fed three times a week with brine shrimp (*Artemia nauplii*), water changed once a week) for a year in order to remove environmental effects and increase animal size. Temperature loggers were deployed at each location to collect average temperatures over the year. These data were recently reported in Sachkova et al. (2020). To assess the effect of thermal stress across populations the temperatures 20°C, 30°C and a location-based average high for the warmest month (July) were selected. In Nova Scotia and Maine this location based high was 28°C while North Carolina and South Carolina were 32°C. As for Massachusetts its location-based average high temperature was 30°C, so a third temperature was not assayed for anemones from this location. Sea anemones from each location were separated out into separate dishes per location and exposed to either 20°C, 30°C or a location-based temperature for 30 days. They were then cultured in glass dishes for each replicate (n=4) under the same salinity and feeding regime (15 ppt ASW, fed three times a week with brine shrimp (*Artemia nauplii*), water changed once a week). Anemones were starved for three days prior to preservation.

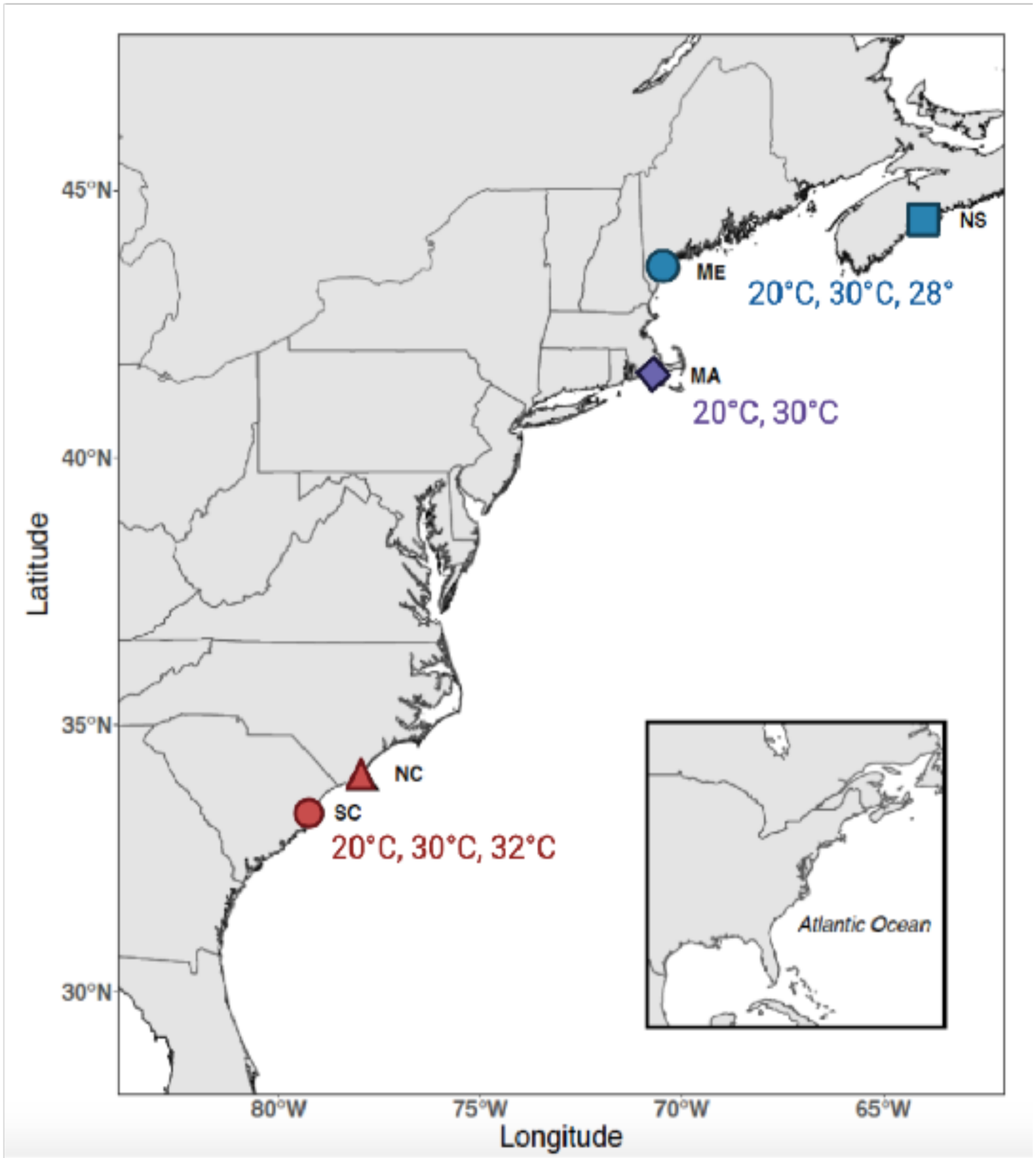


Figure 1: Map of *N. vectensis* collection sites. Sampling sites are denoted by a symbol and chronic experimental temperatures are listed out next to their respective site(s). The color of each location indicates its location-based average high temperature (Nova Scotia and Maine, 28°C; Massachusetts, 30°C; North Carolina and South Carolina, 32°C).

2.2 RNA Extraction & Tag-based RNA Sequencing and Processing

At the end of the 30 days, animals were transported from their dishes into labelled sample collection tubes and snap frozen in liquid nitrogen. Total RNA was isolated from 56 samples (4 replicates of 5 locations with 2-3 temperature points) using the RNAqueous kit (Ambion) according to manufacturer's protocol. Residual genomic DNA was then removed using the DNA-free kit (Invitrogen). Purified RNA was then quantified using a Qubit Fluorometer. Samples were normalized and sent off for tag-based library preparation at the University of Texas at Austin's Genomic Sequencing and Analysis Facility (GSAF) following the 3' Tag-Seq protocol adapted from Illumina HiSeq 2500 (Meyer et al. 2011). This method uses 100 bp Illumina single read sequences (HiSeq2500) and typically generates 2-5 million reads per sample.

Once the raw sequencing data was returned (100 bp, single-end), fastX-toolkit (Pearson et al. 1997) was used to trim and quality filter each read before being mapped to the *N. vectensis* Vienna transcriptome using the Bowtie2 aligner (Langmead & Salzberg 2012). A read-count-per-gene file was generated with reads mapping to multiple genes being discarded. This table of relative gene expression, based on read counts, was generated across all samples and served as the basis for comparing the effect location of origin and temperature have on *N. vectensis'* transcriptomic response. This gene expression table was then imported into the R environment for downstream statistical analysis purposes.

2.3 Differentially Expressed Gene (DEG) Analysis

Data normalization and differential expression analysis was performed using a negative binomial generalized linear model through the R package DESeq2 (Love et al.

2014). Transcript counts with low abundances (mean count < 3) were filtered out to improve the rate of differential gene discovery. Outlier transcripts were then identified and removed using the R package arrayQualityMetrics (Kaufmann, 2009). Normalized gene count data across experimental conditions was then log transformed using the *rlog* function. A pairwise contrast was performed across experimental groups using Wald tests in DESeq2. The Benjamini-Hochberg procedure and false discovery rate (FDR) were used to calculate p-values for each comparison. These lists of significantly differentially expressed genes included adjusted and unadjusted p-values as well as log2 fold change values. These lists were then used for downstream analyses.

A principal component analysis (PCA) of variance-stabilized gene expression was performed using the R package vegan using Manhattan distances (Dixon, 2009). To evaluate the significant findings from the PCA the function a9donis (R package vegan) was used to perform a permutational multivariate analysis of variance (PERMANOVA) again using Manhattan distances to determine if gene expression between experimental groups were significantly different.

A ranked-base gene ontology (GO) enrichment analysis was performed using signed, unadjusted, log-transformed p-values with the GO_MWU package in R across all location and temperature comparisons. This method uses the Mann-Whitney U test to determine if each GO term is significantly enriched in up- or down- regulated genes based on their quantitative shift in rank.

A weighted gene co-expression network analysis (WGCNA) was then employed to identify groups (‘modules’) of genes that show correlated expression across samples in an unbiased fashion. Genes with an adjusted p-value < 0.01 were retained for analysis. How the samples were distributed within experimental conditions was not used in order to ensure that the module eigengenes correlate with gene expression patterns that convey biological processes. A signed co-expression network was built using a soft threshold power of 13. Eigengene modules were determined and given arbitrary color names. Each experimental condition (each location and each temperature) had a corresponding Pearson correlation value as well as a p-value to illustrate each eigengenes expression values as being up- or down-regulated based on location or temperature. Module eigengenes were then functionally characterized using the GO_MWU package, mentioned previously, but using a transcriptome-wide Fisher's exact test for GO term enrichment among modules genes.

CHAPTER 3: RESULTS

Anemones from five locations (Nova Scotia, Maine, Massachusetts, North Carolina, and South Carolina) were cultured for 30 days at two common temperatures (20°C and 30°C) as well as temperatures representative of elevated mean summer high temperature (28°C for Nova Scotia, Maine; 32°C for North Carolina, South Carolina). Anemones in all treatments survived for the full 30 days and appeared healthy (i.e., feeding, tentacles out, transparent physa) at the end of the experiment when preserved for RNA extraction. The number of Tag-Seq reads per replicate after filtering for quality was consistent across samples (average=745,486 reads; range: 338,277-1,241,161) and mapping efficiency was also similar (average= 71%; range: 65% - 81%).

3.1. Multivariate Analyses

I used two approaches to identify the relative impacts for temperature, location, and the intersection of these two variables (i.e., temperature-by-location) on transcriptome-wide gene expression for *Nematostella vectensis*. First, a PERMANOVA found statistical significance between location, temperature, and their interaction on gene expression patterns (Table 1). Location explained the largest proportion of the variation (R^2=0.237, F=4.49, p<0.001). Overall changes in gene expression were also due to temperature differences (F=3.96, p<0.001) but it explained less of the variance (R^2=0.157). The interaction of location and temperature, while significant, explained even less variance (R^2=0.104, F=1.31, p=0.018). Second, a principal component analysis (PCA) was generated to compare the relative relationship between treatments. Principal component 1 (PC1) and 2 (PC2) explained about 27% of the variance (16.5% and 10.5%, respectively), with PC3 explaining 6.3% (Figure 2). Consistent with PERMANOVA results, PCA plots showed separation of particular locations and temperatures. In comparisons with PC1 and PC2, North Carolina and South Carolina are non-overlapping with the higher latitude locations. Also, samples from these two locations were distinct at higher temperatures but overlap at 20°C. For these two lower latitude locations, increasing temperature resulted in a shift to more positive PC1 values. Nova Scotia, Maine, and Massachusetts overlap considerably in comparison with PC1 and PC2 (Massachusetts separates when PC3 is compared with PC2). Samples from higher temperatures, regardless of location, tend to separate along the PC1 axis with more positive values corresponding to higher temperatures compared to 20°C. However, this response was not consistent at the higher temperatures. For example, samples from 28°C, Nova Scotia and Maine, are distributed at more positive PC1 values than 30°C.

Table 1: Multivariate PERMANOVA Results

	df	SumofSqs	MeanSqs	F.model	R2	p-value
Location	4	127753510	31938377	4.49	0.237	0.001
Temp	3	84496684	28165561	3.96	0.157	0.001
Location:Temp	6	55993827	9332305	1.31	0.104	0.018
Residuals	38	270201822	7110574		0.502	
Total	51	538445844			1	

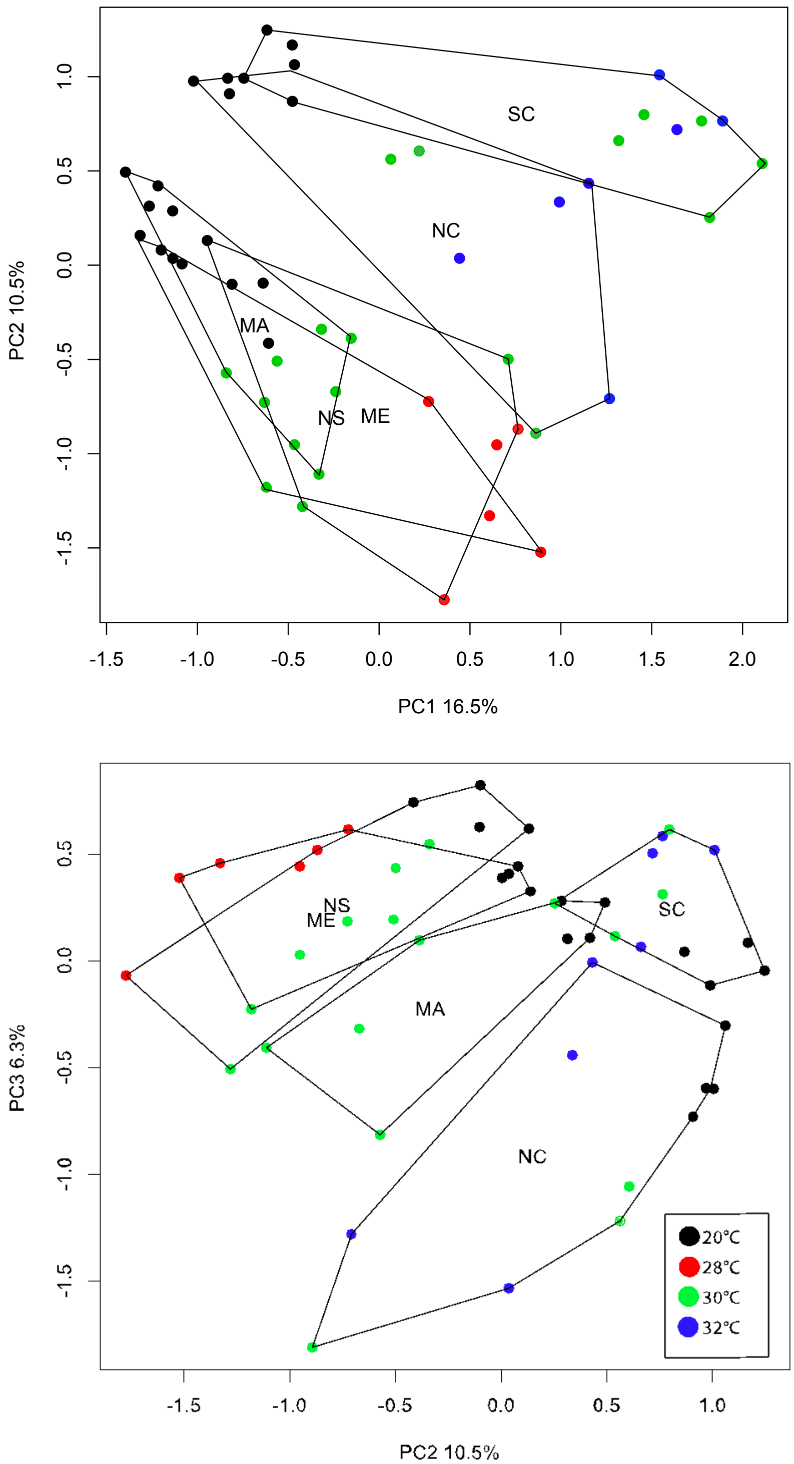


Figure 2: Principal component analysis (PCA) of gene expression across locations and temperatures. Temperatures are denoted by color while locations are grouped within polygons.

3.2 Univariate Analyses

I used two complementary approaches to compare how temperature impacted the number and predicted functions of differentially expressed genes for *N. vectensis* from these five locations. First, I used the negative binomial general linear model (GLM) function in DESeq2 to identify differentially expressed (DE) genes due to location, temperature, and the interaction between them. This comparison was repeated to compare only temperatures shared for all locations (i.e., 20°C and 30°C). These DEGs were then graphically compared. Second, I compared differential expression of genes by focusing on each location and temperature treatment to identify patterns of similar gene expression for these variables. This later approach was necessary because of the unbalanced design of the temperature data (i.e., only Nova Scotia and Maine had a 28°C treatment, only North Carolina and South Carolina had 32°C, Massachusetts had neither temperature). While each approach is essentially representing the distribution and identity of DE genes, each approach helps highlight particular patterns in these multiple comparisons. Where appropriate, I then compared the potential functions for these groups of DE genes using gene ontogeny (GO) analysis to predict particular biology, cellular, and molecular processes.

3.2.1. Shared and Unique DEGs: Location

In this section, I summarize the shared and unique DEGs based on location to show patterns of how the expression of the transcriptome responded to each factor in detail.

3.2.1.1 DEGs at common temperature between locations

Previous research has shown that *N. vectensis* individuals from different locations along the Atlantic coast differ in their growth rate when cultured at identical temperatures.

These differences suggested physiological variation that may differ between locations; however, the potential molecular mechanisms are unknown. Comparisons of gene expression at the same temperatures in my experiment involved four comparisons: all 5 locations at 20°C, all 5 locations at 30°C, two higher latitude populations (Nova Scotia, Maine) at 28°C, and two lower latitude populations (North Carolina, South Carolina) at 32°C.

At 20°C, there were a total of 2189 DEGs (881 unique genes, many genes were DE in multiple comparisons) between comparisons of all locations (p-value <0.05) (Suppl. Table 1). The largest numbers of DEGs were between Maine and the two southern populations: North Carolina (n=429) and South Carolina (n=379), where 36 DEGs were uniquely shared when these lower latitude locations were compared with Maine (Table 2). The comparisons with the next highest number of DEGs was Nova Scotia and the two southern locations where there were 250 and 256 DEGs for North Carolina and South Carolina, respectively, with 15 shared DEGs unique to this comparison. The fewest number of DEGs (n=73) was between North Carolina and South Carolina. On average, 12.5% of the DEGs were unique to the specific comparison; however, this number varied with proportionally higher unique DEGs in the comparisons of Maine with North Carolina (20%) and South Carolina (17.7%). No DEGs were shared across all comparisons and few (n=1-3) were shared between >4 comparisons. The only comparison that shared an

Table 2: Uniquely Expressed Genes across all locations at 20°C

Sites	# Unique Genes	% Unique
MavNC20	200	11.5%
MavNS20	139	11.5%
MavSC20	185	11.9%
MevMA20	128	13.3%
MevNC20	429	20.0%
MevNS20	150	8.0%
MevSC20	379	17.7%
NSvNC20	251	12.4%
SCvNC20	73	1.4%
SCvNS20	256	17.2%
Total	**882**	

appreciable number (>10) of DEGs were those where higher latitudes were compared with lower latitudes. For example, 24 DEGs were uniquely shared in comparisons of (Nova Scotia, Maine, and Massachusetts with North Carolina and South Carolina.

When I focused these comparisons on up- and down-expressed genes instead of total DEGs, I observed a similar pattern of DEGs by location. The specific DEG found between Maine with North and South Carolina (n=36) showed *HSP70D* as being upregulated in both southern locations while several putative immune related genes were upregulated in Maine [*Nodal modulator*, HYR domain, IG like, and NOD-like receptor family CARD domain containing 5 (*NLRC5*)]. When Nova Scotia was compared with North and South Carolina where 15 DEG were identified (UTP18, small subunit (SSU) processome component, homolog (*Utp18*), kinesin family member 14 (*KIF14*), hemagglutinin, amebocyte aggregation factor precursor, tropomyosin, low affinity immunoglobulin epsilon Fc receptor (*FCER2*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), poly (ADP-ribose) polymerase family member, phospholipase D family member, transglutaminase). An additional 9 genes were found to be DE when comparing the two most northern locations (Maine and Nova Scotia) with the two more southern locations (North Carolina and South Carolina). Most of these genes were either uncharacterized or microtubule related, but two were noted: *CALM1* (calmodulin) and *ETFB* (electron-transfer-flavoprotein beta polypeptide) which were both upregulated in the southern locations. Finally, the 24 DEG between the higher and lower latitude populations included 12 genes with annotations. Those upregulated in the lower latitudes include clathrin light chain (*CLTA*), ribosome biogenesis, and fibroblast growth factor receptor; while the higher latitudes had upregulated genes belonging to the PLAC8 family, sphingosine-1-phosphate phosphatase (*SGPP1*), tissue factor pathway inhibitor (*TFPI*), and long-chain enoyl-CoA reductase art 1 (*art-1*).

At 30°C, there was a total of 3,935 DEG identified across all comparisons, 1,821 of those genes were found to be uniquely expressed across all locations (Suppl. Table 2). The largest number of DEGs were between South Carolina and the three northern locations (347 DEG from Nova Scotia; 283 DEG from Massachusetts; 52 DEG from Maine) (Table 3). The lowest number of DEGs were found between locations closer in proximity (Maine and Massachusetts (n=89); Maine and Nova Scotia (n=101)). On average 11.8% of DEG were unique across comparisons. South Carolina compared with Nova Scotia (30%) as well as South Carolina with Massachusetts (28%) exhibited the highest percent of unique DEGs found within their respective comparisons. The remaining locations fell in a range from 2.2% and 9.5% of DEG being unique to a single comparison. No DEGs were shared across all comparisons. However, multiple DEGs were shared among several comparisons (i.e. multiple locations sharing sets of genes) and often grouped by location proximity. When Nova Scotia was compared against all other locations at 30°C 15 unique DEGs were identified (Zona pellucida-like domain, short-chain collagen C4, E3 ubiquitin-protein ligase, poly (ADP-ribose) polymerase family, member, dopamine beta-hydroxylase, F-box/WD repeat-containing protein 7 (*FBXW7*), Tryp_SPc, and QKI, KH domain containing RNA binding (*QKI*)). Similarly, when South Carolina was compared against all

Table 3: Uniquely Expressed genes across all locations at 30°C

Sites	# Unique Genes	% Unique
MavNC30	243	12.3%
MavSC30	1004	5.2%
MavNS30	174	28.2%
MevMA30	89	2.2%
MevNC30	187	5.3%
MevNS30	101	8.9%
MevSC30	545	9.5%
NSvNC30	286	10.1%
SCvNC30	154	5.8%
SCvNS30	1152	30.1%
Total	1,821	

other locations 46 unique DEGs were identified. Interestingly when all three northern locations (NS, ME, MA) are compared against the southern locations (NC, SC) 30 unique genes were recognized (Suppl. Table 2). South Carolina alone against Nova Scotia, Maine, and Massachusetts has 155 uniquely expressed genes.

After assessing the overall number of DEG between comparisons I concentrated on genes that were up- or downregulated among multiple comparisons at 30°C. When Nova Scotia, the northern most population, was compared against all other locations 15 DEGs were observed with 8 having functional annotations. Four genes were upregulated at Nova Scotia (zona pellucida-like domain (*ZPLD1)*, short chain collagen C4, E3 ubiquitin-protein ligase, and poly (ADP-ribose) polymerase family, member) while the remaining four genes were downregulated at Nova Scotia (dopamine beta-hydroxylase, F-box/WD repeat-containing protein 7 (*FBXW7*), Tryp_SPc, and QKI, KH domain containing RNA binding (QKI)). This pattern, one location sharing multiple DEGs with all others, was observed again in South Carolina that shared 46 unique DEG with the other locations. From those genes, 31 were upregulated in South Carolina while the other 15 were downregulated. Some of those genes upregulated in South Carolina included cathepsin, chitotriosidase-1, peroxiredoxin-6 (*PRDX6)*, and HSP60. The genes that were downregulated contained several with putative immune-related functions such as proline-rich protein (*PRB3),* ubiquitin-like with PHD and ring finger domains (*UHRF1*), and NACHT domain. Lastly, differential gene expression was compared between the higher and lower latitude locations resulting in 30 uniquely expressed genes; 22 up-regulated in lower latitudes and 8 up-regulated in higher latitudes. From the 22 genes seen to be up-regulated in North and South Carolina, a majority of them are categorized as either uncharacterized or microtubule/structurally related. The 8 genes seen upregulated in NS, ME, and MA contained only four with annotations: cytochrome b (*MT-CYB*), glutamyl-prolyl-tRNA synthetase (*EPRS1*), component of translation initiation and zinc finger SWIM-type containing 8 (*ZSWIM8*).

I cultured anemones from Nova Scotia and Maine at 28°C. There were 155 DEGs, with 76 upregulated in Maine relative to Nova Scotia (Suppl. Table 3). Of those 76 upregulated genes from Maine, only 33 had functional annotations. Three of those genes encode for the enzyme phospholipase A2 which works by cleaving fatty acids within cells (Burke and Dennis 2009). An additional seven genes were directly related to microtubules and cellular structure. Interestingly two genes were upregulated that may play a role in the immunity (mucin-like and Milk fat globule-EGF) (Borisenko et al. 2004). Nova Scotia, on the other hand, had 79 upregulated genes at 28°C with 38 genes having associated annotations. Some of these genes include two associated with epidermal growth factor (*EGF* and *EGF_CA*) and toxin production (ShK domain-like) (Chen et al. 2016, Sachkova et al. 2020).

Last, I cultured anemones from North Carolina and South Carolina at 32°C. There were 100 DEGs, with 49 upregulated in South Carolina and the remaining 51 upregulated in North Carolina (Suppl. Table 4). In South Carolina only 35 of those DEGs had annotations with three of them sticking out as being related to immunity and repair function (NVE16433 Ras-like, estrogen-regulated, growth-inhibitor; NVE24162 pathogenesis-related protein; and NVE672 DNA cross-link repair 1A). In the case of genes upregulated in North Carolina at 32°C, only 26 genes had associated functional annotations. Of note from this list are archain 1 (*ARCN1)* which is found to have a similar function to heat shock proteins and DNA methyltransferase 1 (*DNMT1)* which plays a role in epigenetics and adding methyl groups to newly synthesized DNA (Lyko 2018).

3.2.1.2 Functional Enrichment of Gene Ontologies from DEGS

Following all DEG analysis gene ontology (GO) functional enrichments were performed across all comparisons mentioned previously to evaluate further how transcriptional variation may be overrepresented for particular functions.

At 20°C a pattern of gene expression has been established between the higher and lower latitude populations. Here, I further discuss these patterns through enriched GO terms found between experimental comparisons specifically those seen between higher and lower latitude populations. When Maine was compared to North and South Carolina terms were enriched almost exclusively in the southern locations including terms in biological processes such as 'protein localization to membrane', 'spindle elongation', 'protein localization to endoplasmic reticulum', 'RNA catabolic process', and 'translation initiation' (Figure 6 & Figure 8). Additional enrichments were observed under cellular components ('Ribosome' and 'large ribosomal subunit') and molecular function ('structural molecule' and 'structural constituent of ribosome'). Interestingly, both Nova Scotia and Massachusetts shared very few GO enrichments with North Carolina and no terms with South Carolina (Figure 9 & Figure 3). In nearly all cases, GO terms were enriched relative to the lower latitude locations (NC, SC) rather than the higher latitude locations (NS, ME, MA) (Figures 3-10).

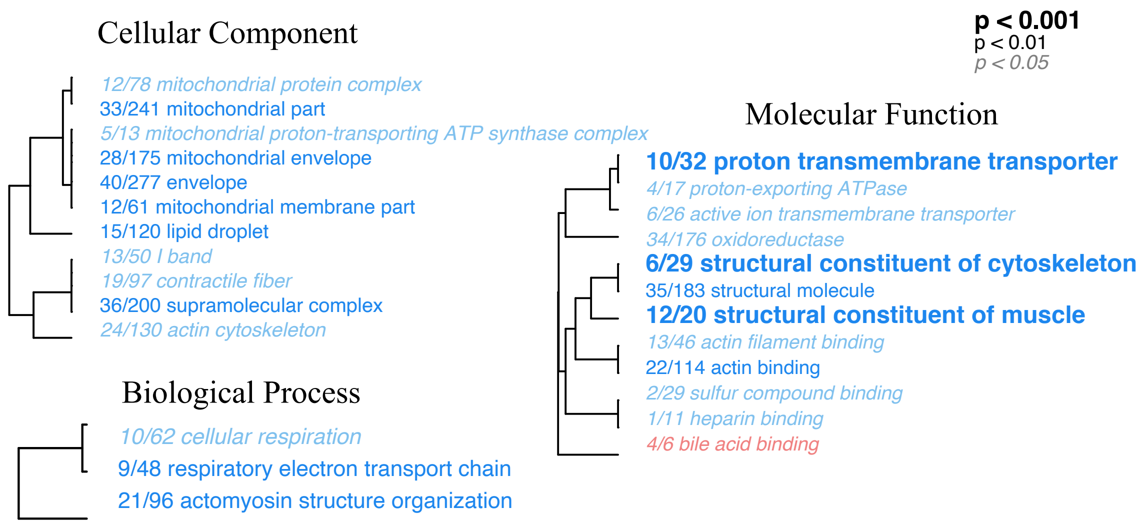


Figure 3: GO enrichment analysis between Massachusetts and North Carolina at 20°C. Terms in red are enriched in Massachusetts while those in blue are enriched in North Carolina.

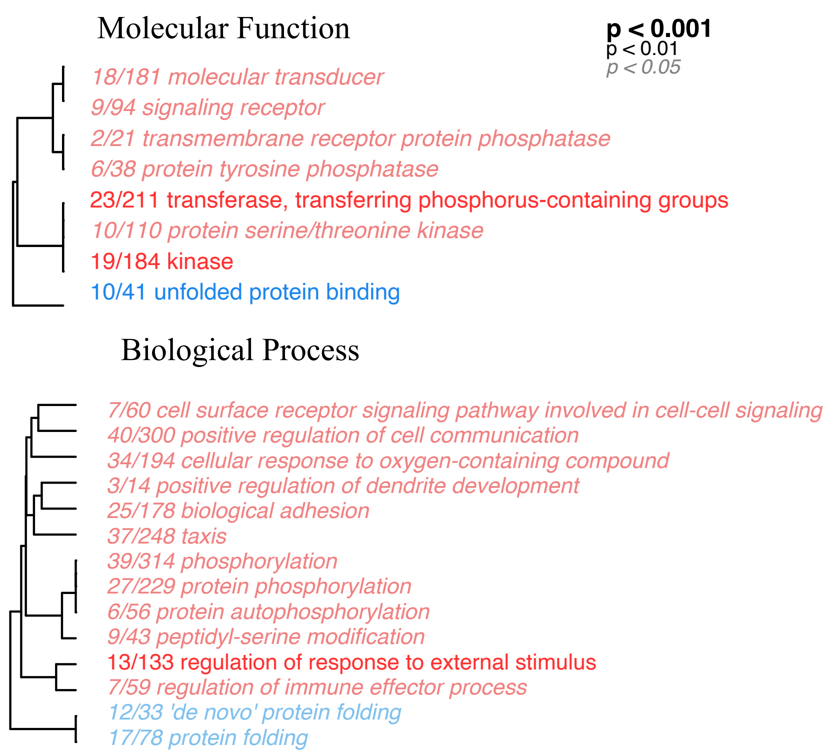


Figure 4: GO enrichment analysis between Massachusetts and Nova Scotia at 20°C. Terms in red are enriched in Massachusetts while those in blue are enriched in Nova Scotia.

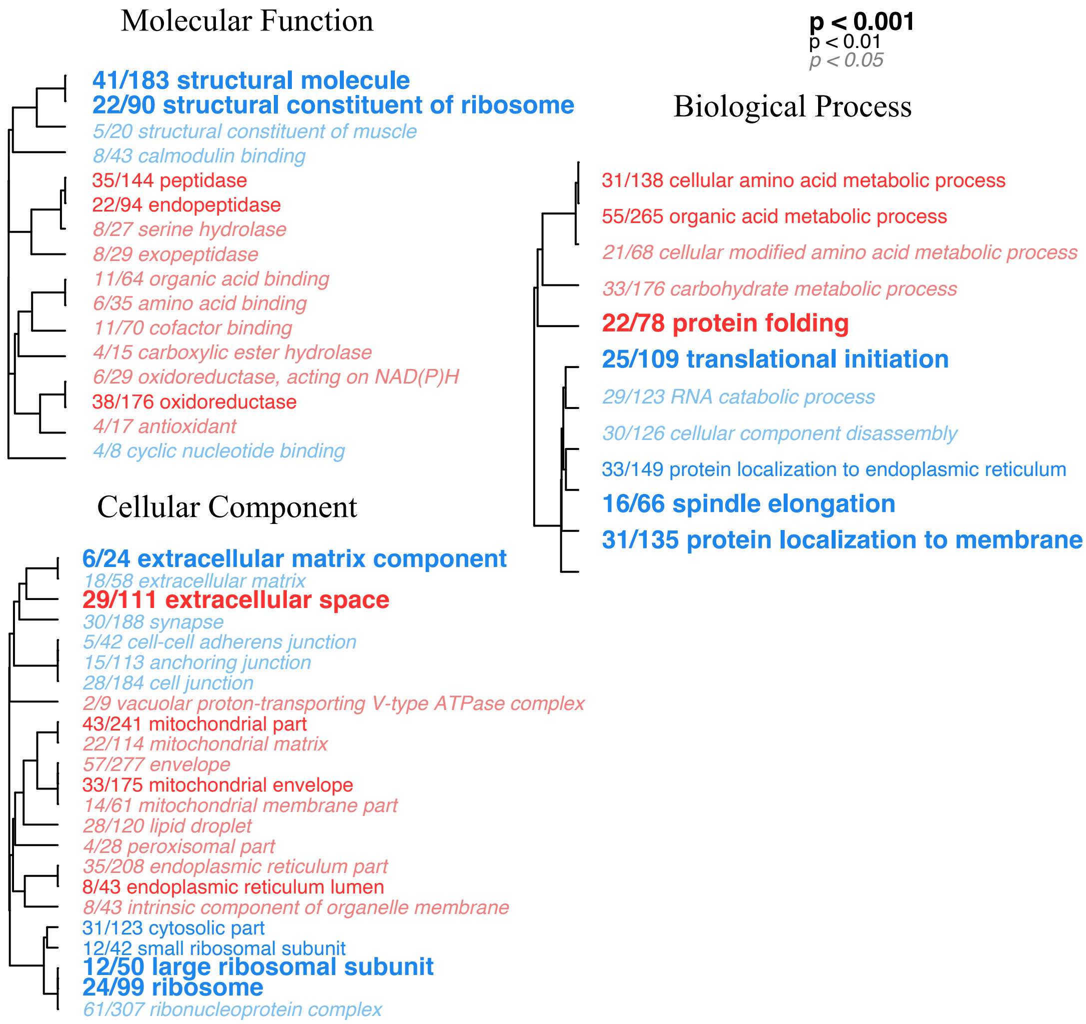


Figure 5: GO enrichment analysis between Maine and Massachusetts at 20°C. Terms in red are enriched in Maine while those in blue are enriched in Massachusetts.

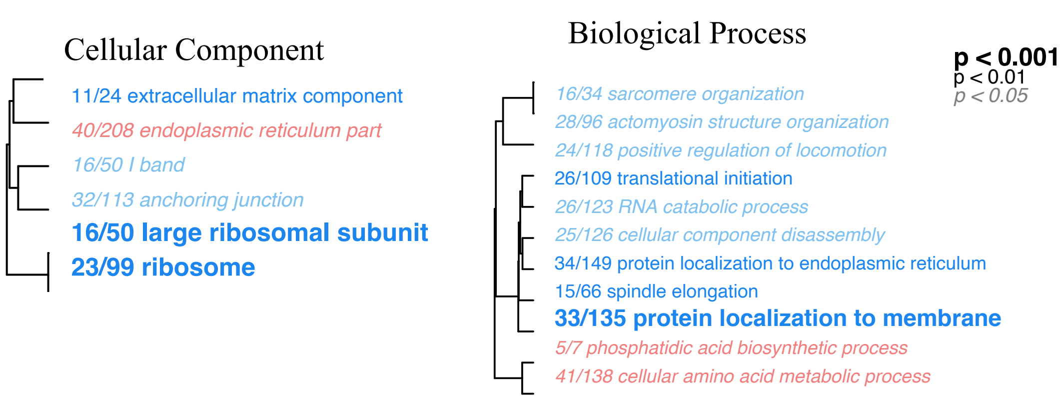


Figure 6: GO enrichment analysis between Maine and North Carolina at 20°C. Terms in red are enriched in Maine while those in blue are enriched in North Carolina.

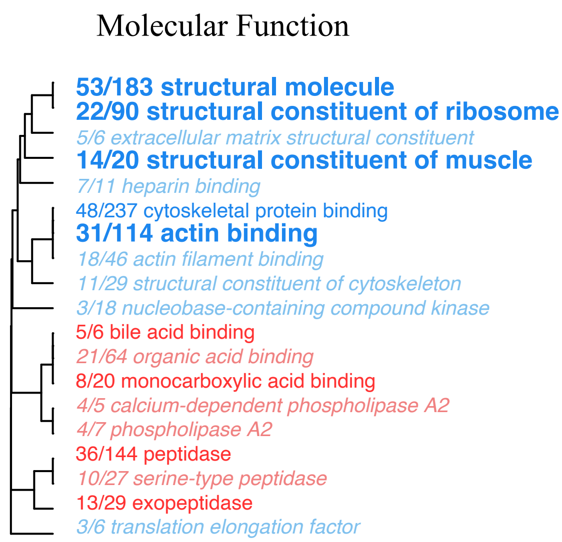


Figure 6 (continued): GO enrichment analysis between Maine and North Carolina at 20°C. Terms in red are enriched in Maine while those in blue are enriched in North Carolina.

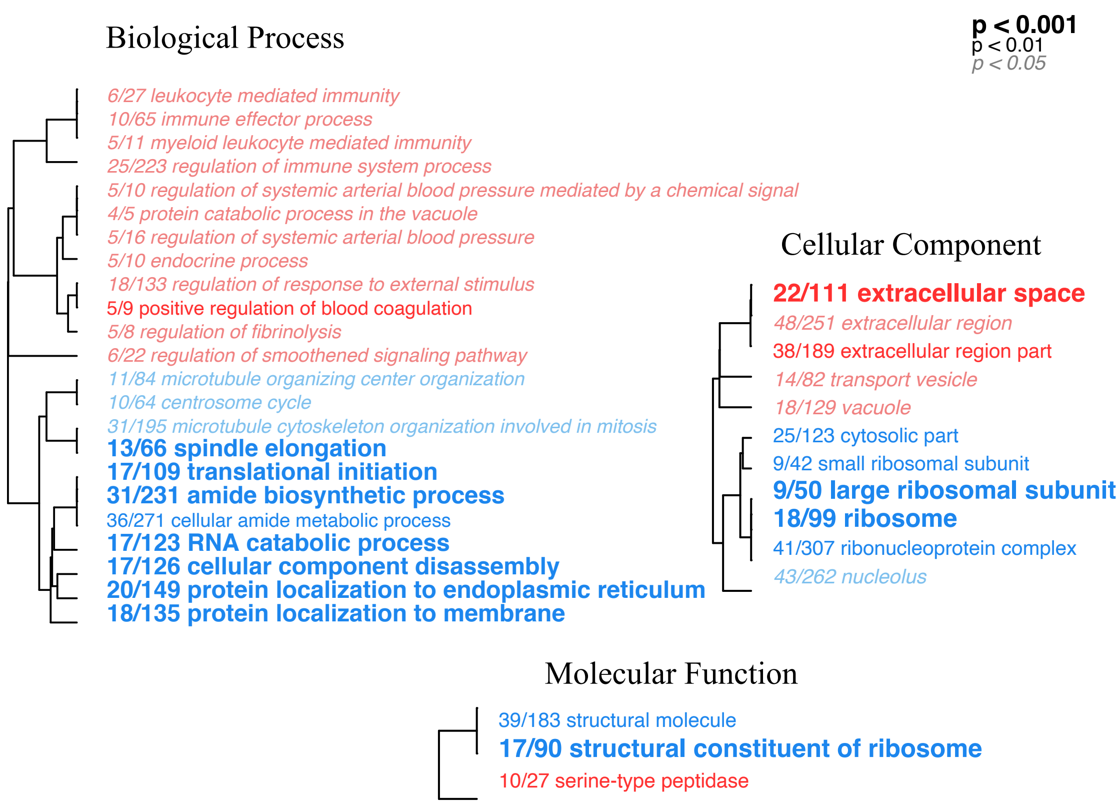


Figure 7: GO enrichment analysis between Maine and Nova Scotia at 20°C. Terms in red are enriched in Maine while those in blue are enriched in Nova Scotia.

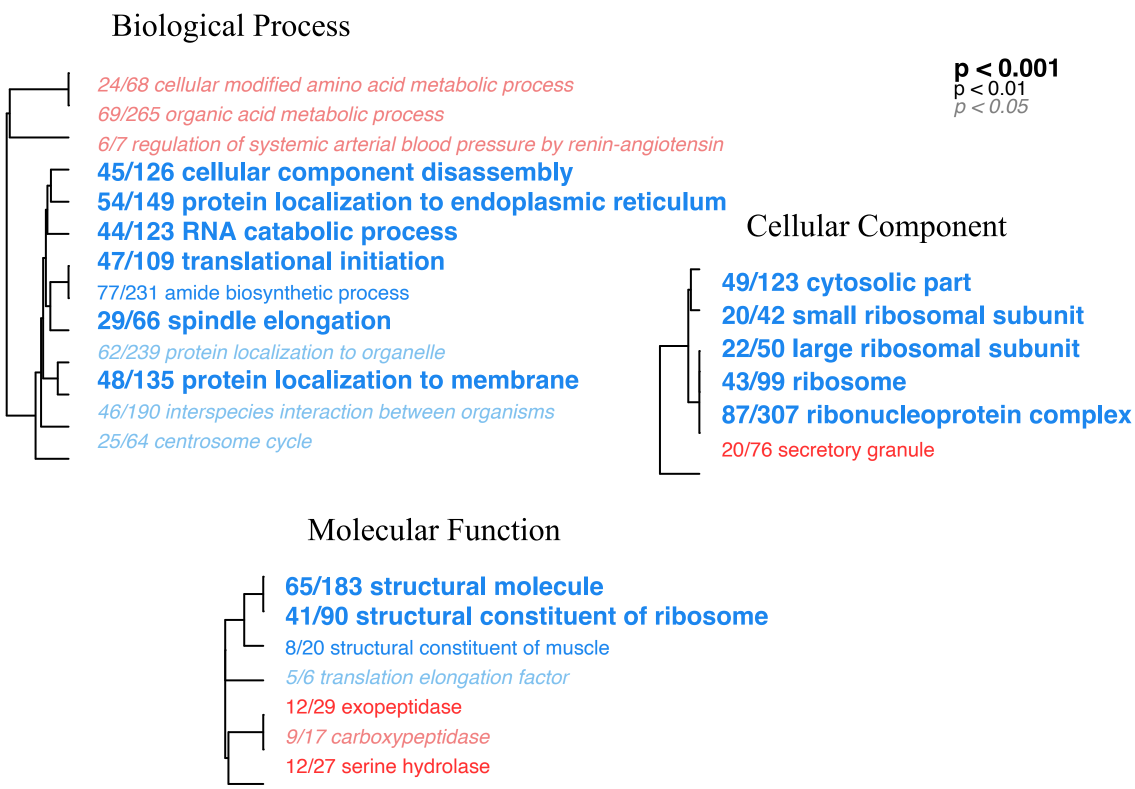


Figure 8: GO enrichment analysis between Maine and South Carolina at 20°C. Terms in red are enriched in Maine while those in blue are enriched in South Carolina.

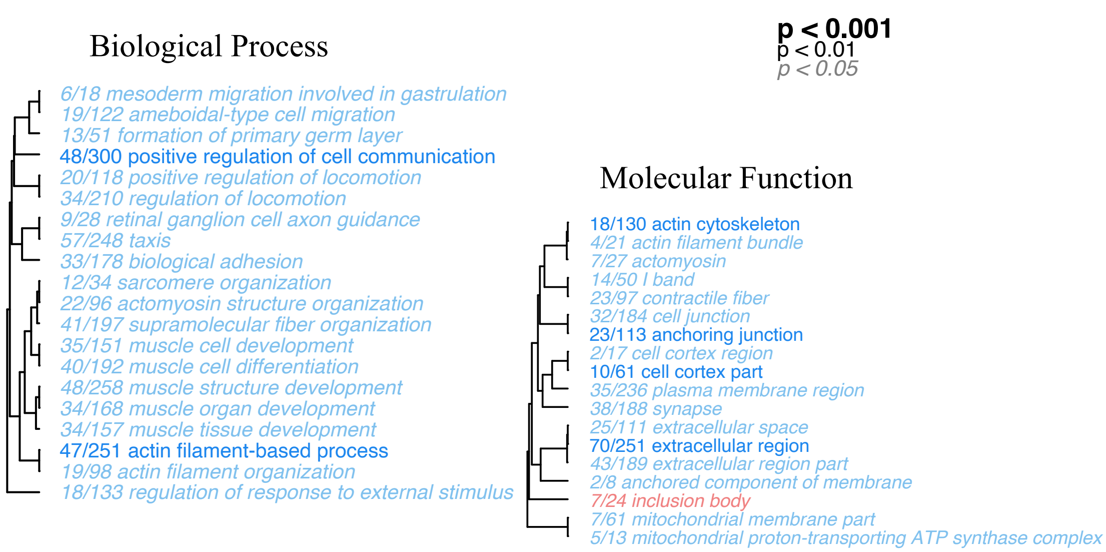


Figure 9: GO enrichment analysis between Nova Scotia and North Carolina at 20°C. Terms in red are enriched in Nova Scotia while those in blue are enriched in North Carolina.

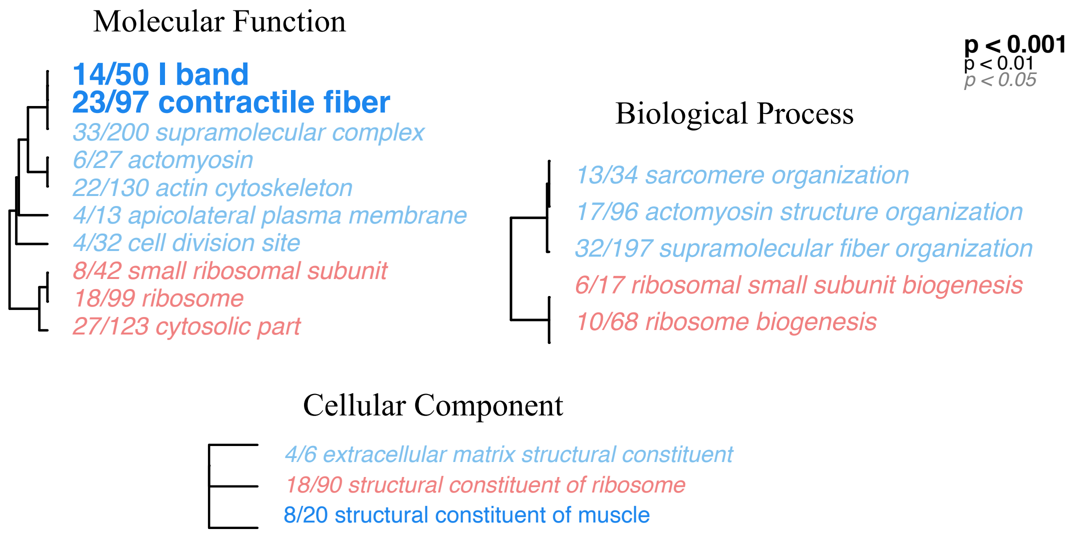


Figure 10: GO enrichment analysis between South Carolina and North Carolina at 20°C. Terms in red are enriched in South Carolina while those in blue are enriched in North Carolina.

Functional enrichment at 30°C did not discern any further patterns from the data, unlike that seen from 20°C. Nova Scotia was examined compared to the other four locations (Figure 12, Figure 15, Figure 16, and Figure 19) and under biological processes four terms were enriched in Maine and South Carolina, relative to Nova Scotia (Figure 15 & Figure 19) (oxidation-reduction process, cellular respiration, respiratory electron transport chain, and generation of precursor metabolites and energy). From here, I looked at South Carolina against the other locations and found no shared GO terms under biological processes, but when South Carolina is compared to only the northern locations a couple of shared enrichment terms are observed: oxidation-reduction process and cellular respiration. GO terms enriched in South Carolina against the other locations included several related to ribosomes and mitochondrial make-up (cellular component) as well as oxidoreductase (molecular function) (Figure 13, Figure 17, Figure 18, and Figure 19).

Finally, the higher latitude locations were compared to the two lower latitude locations, but no discernable differences were found among GO enrichment terms seen across comparisons (Figures 12-19).

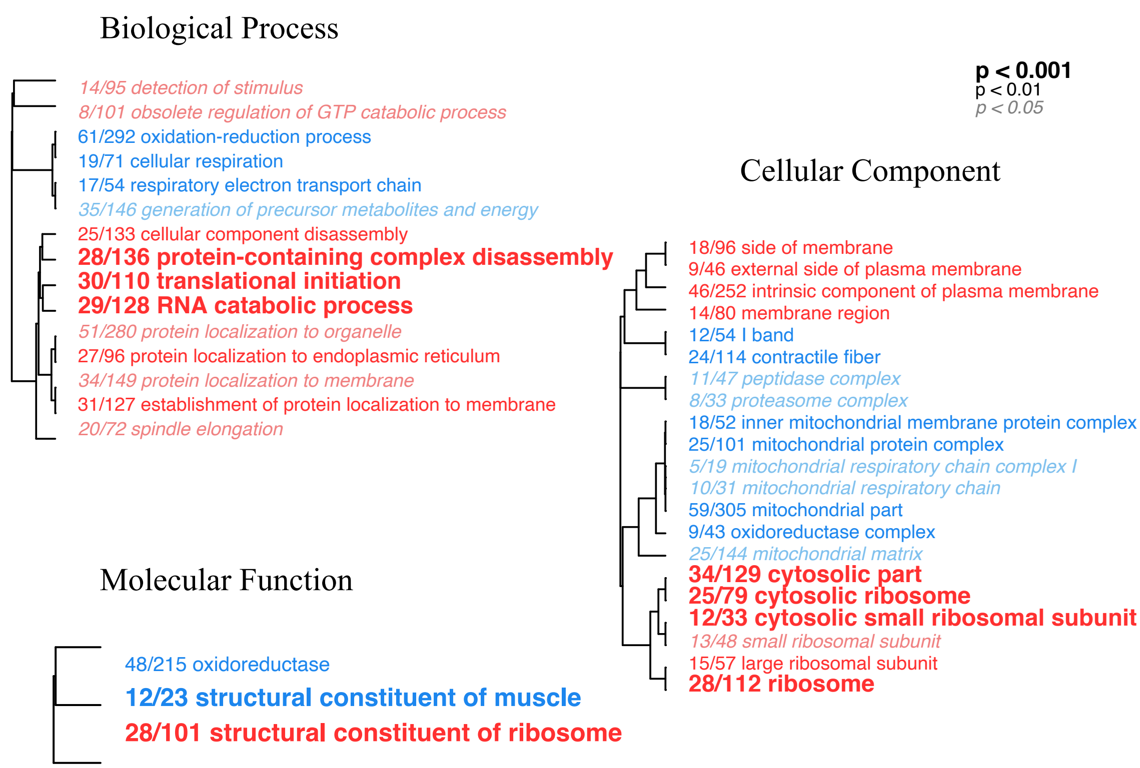


Figure 11: GO enrichment analysis between Massachusetts and North Carolina at 30°C. Terms in red are enriched in Massachusetts while those in blue are enriched in North Carolina.

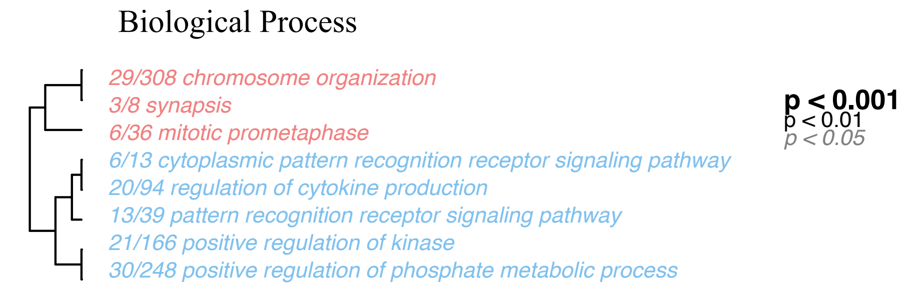


Figure 12: GO enrichment analysis between Massachusetts and Nova Scotia at 30°C. Terms in red are enriched in Massachusetts while those in blue are enriched in Nova Scotia.

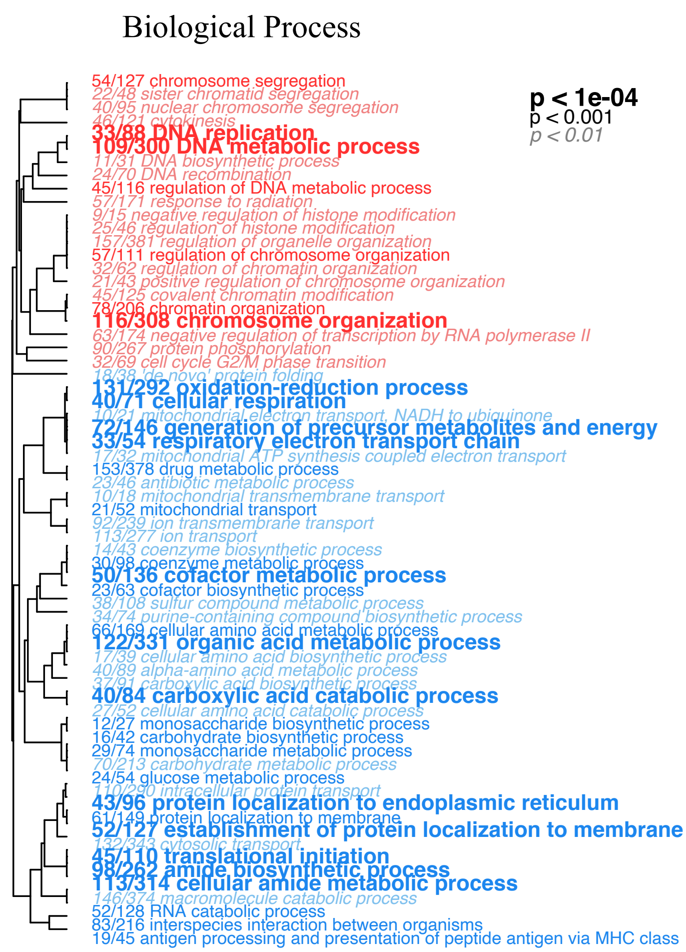
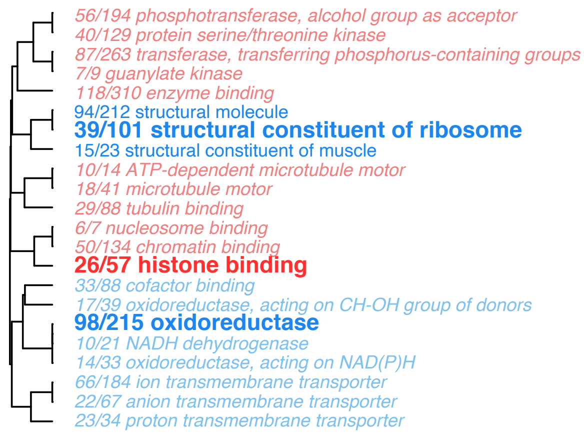


Figure 13: GO enrichment analysis between Massachusetts and South Carolina at 30°C. Terms in red are enriched in Massachusetts while those in blue are enriched in South Carolina.

Cellular Component



Molecular Function

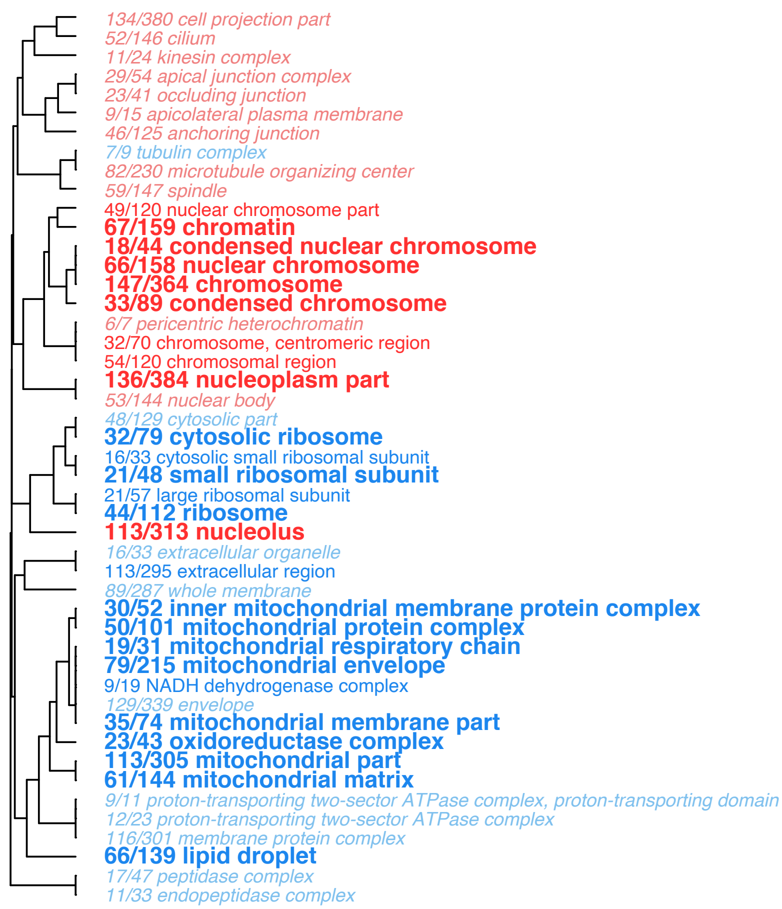


Figure 13 (continued): GO enrichment analysis between Massachusetts and South Carolina at 30°C. Terms in red are enriched in Massachusetts while those in blue are enriched in South Carolina.

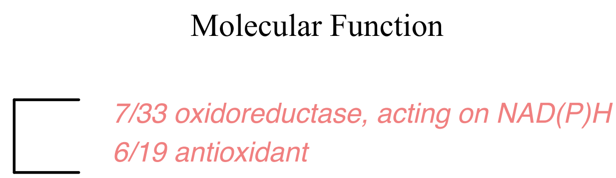


Figure 14: GO enrichment analysis between Maine and North Carolina at 30°C. Terms in red are enriched in Maine.

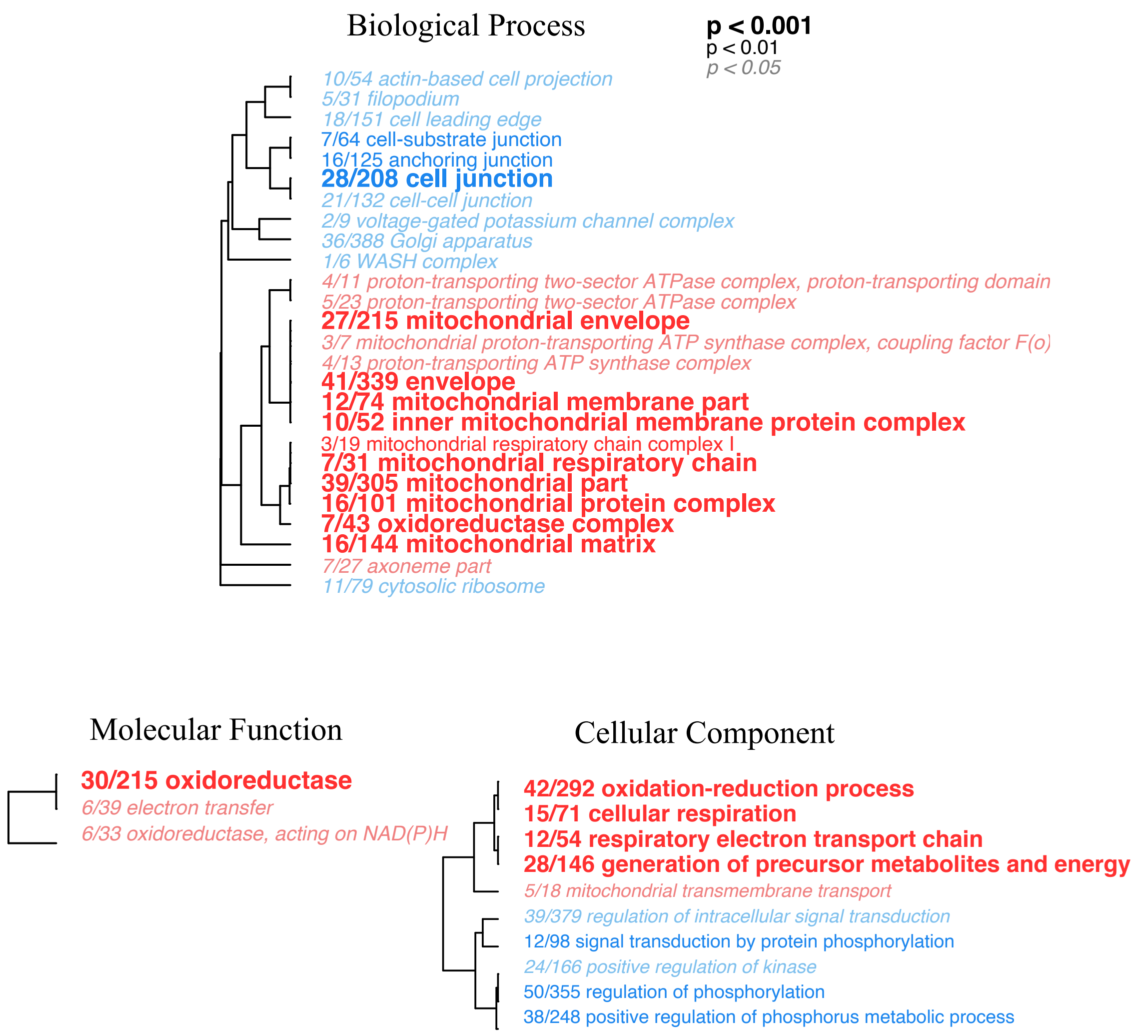


Figure 15: GO enrichment analysis between Maine and Nova Scotia at 30°C. Terms in red are enriched in Maine while those in blue are enriched in Nova

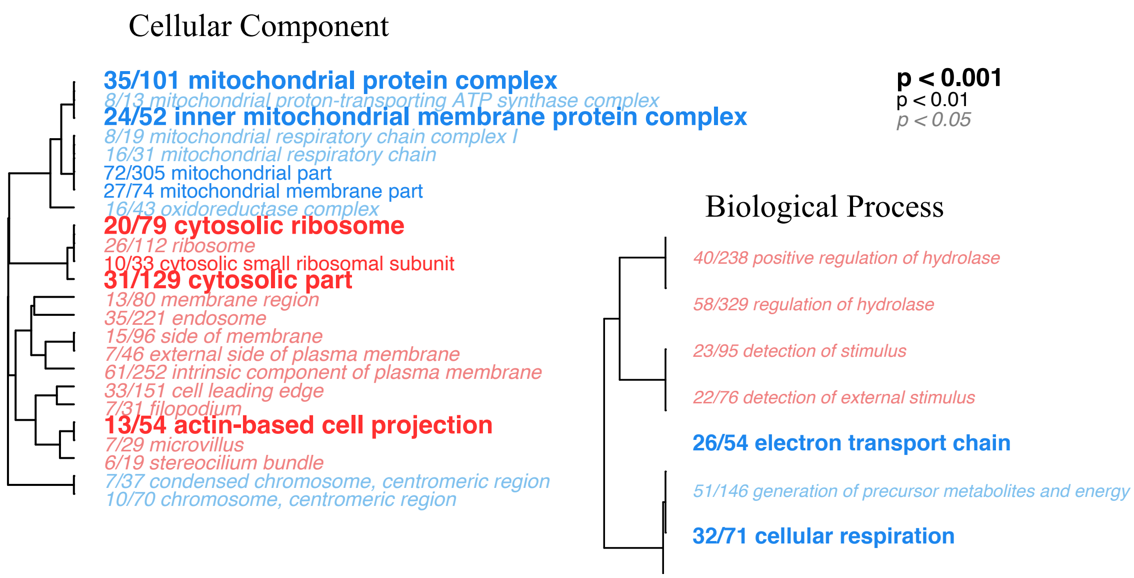


Figure 16: GO enrichment analysis between Nova Scotia and North Carolina at 30°C. Terms in red are enriched in Nova Scotia while those in blue are enriched in North Carolina.

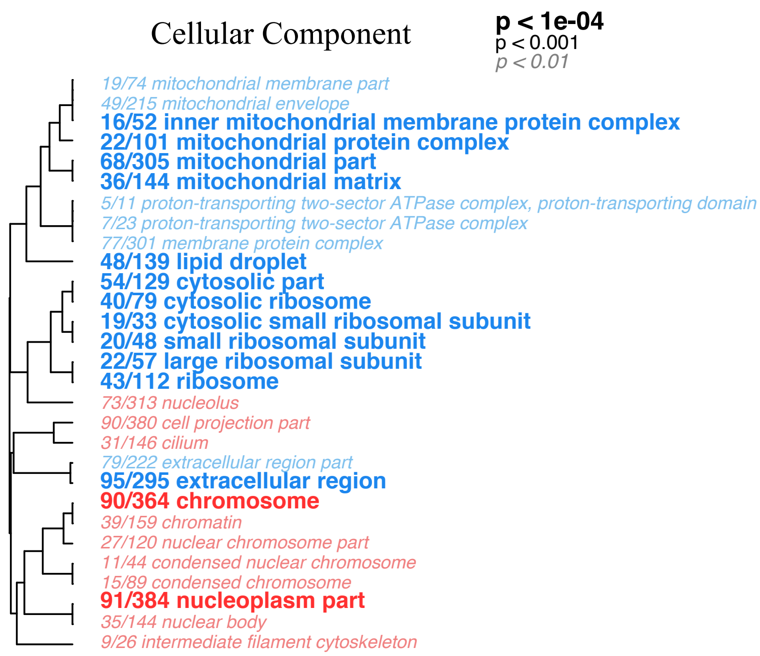
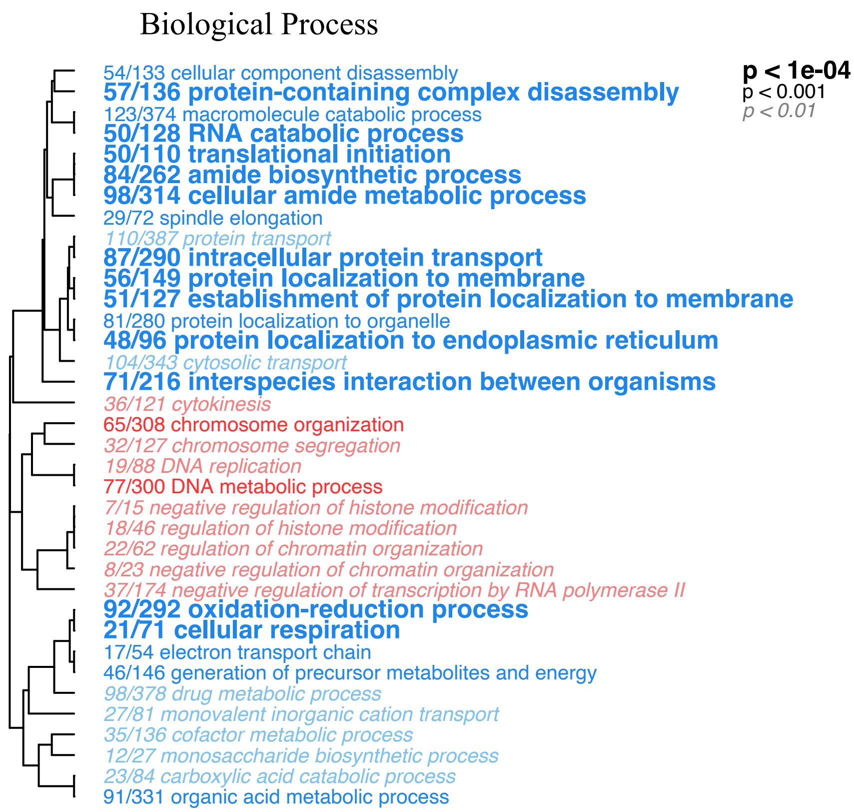


Figure 17: GO enrichment analysis between Maine and South Carolina at 30°C. Terms in red are enriched in Maine while those in blue are enriched in South Carolina.



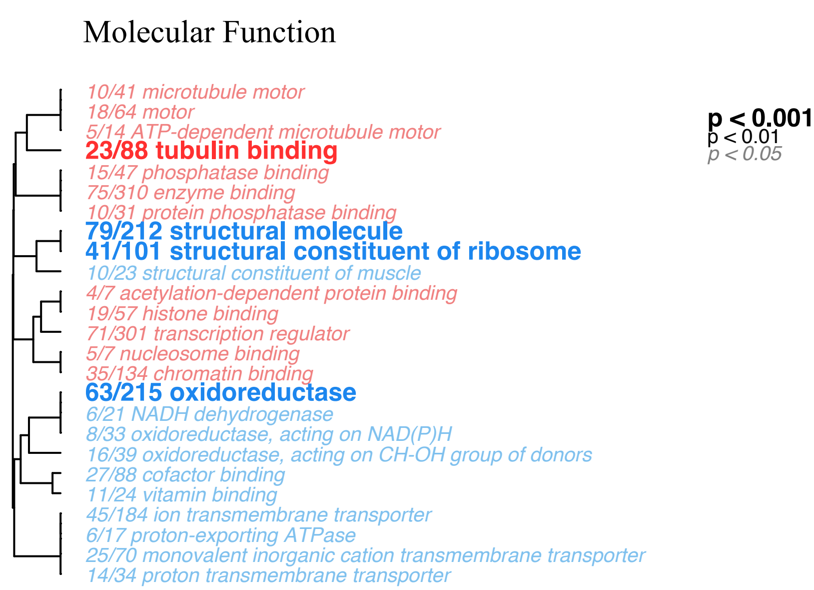


Figure 17 (continued): GO enrichment analysis between Maine and South Carolina at 30°C. Terms in red are enriched in Maine while those in blue are enriched in South Carolina.

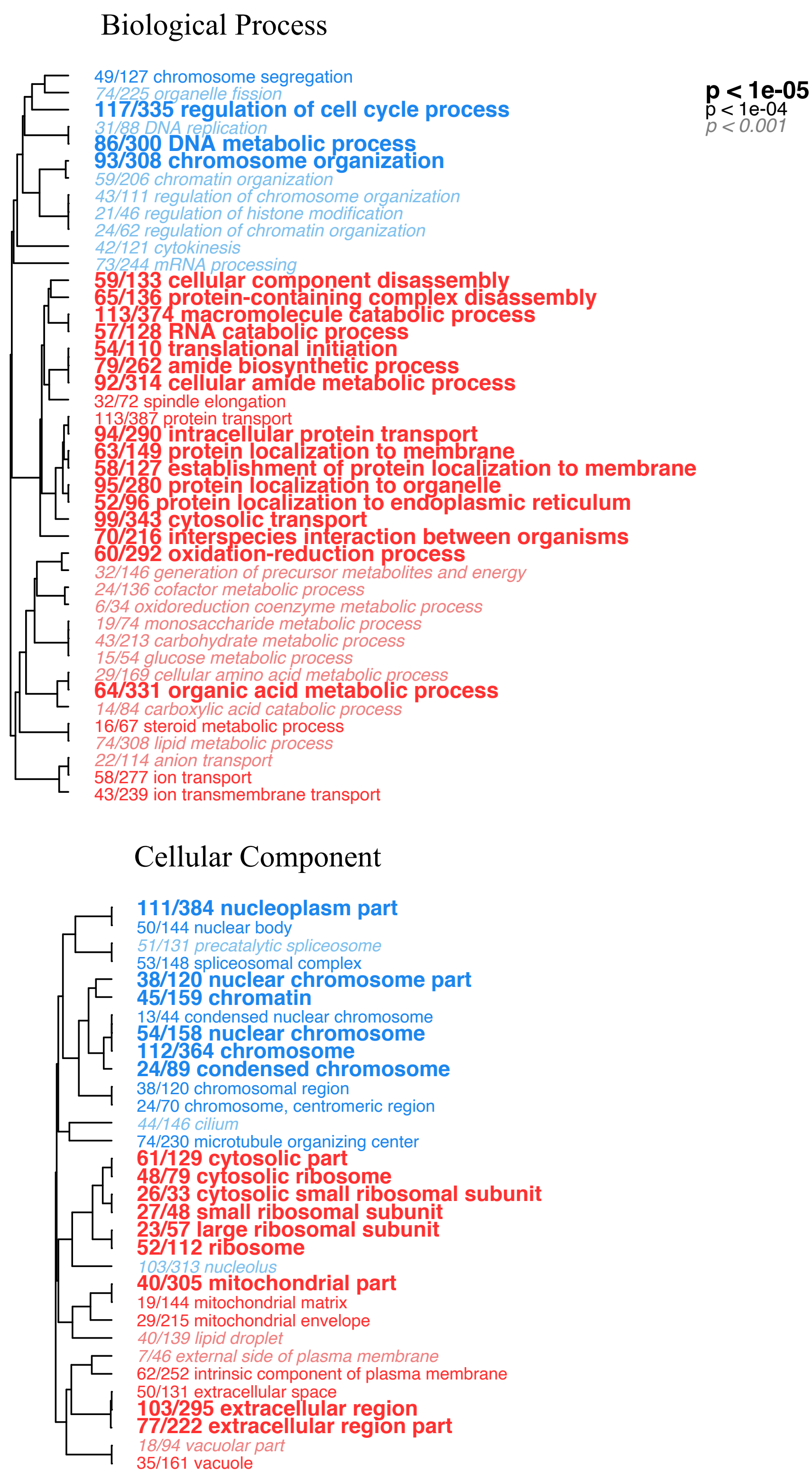
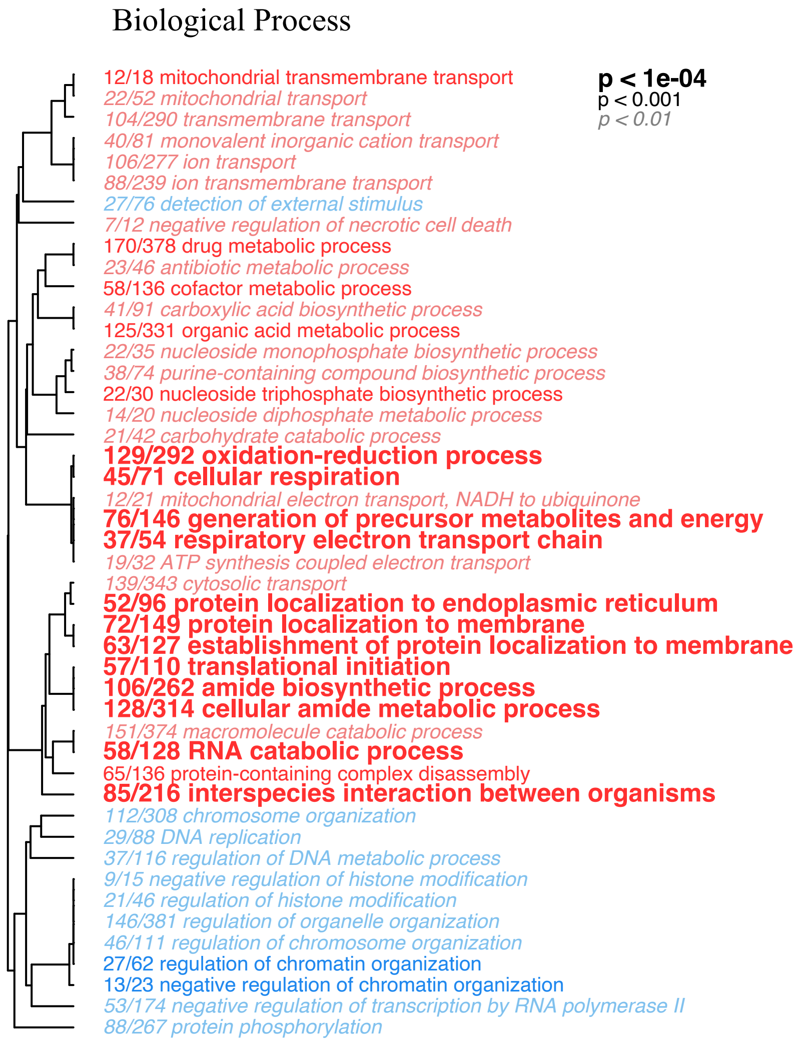


Figure 18: GO enrichment analysis between South Carolina and North Carolina at 30°C. Terms in red are enriched in South Carolina while those in blue are enriched in North Carolina.



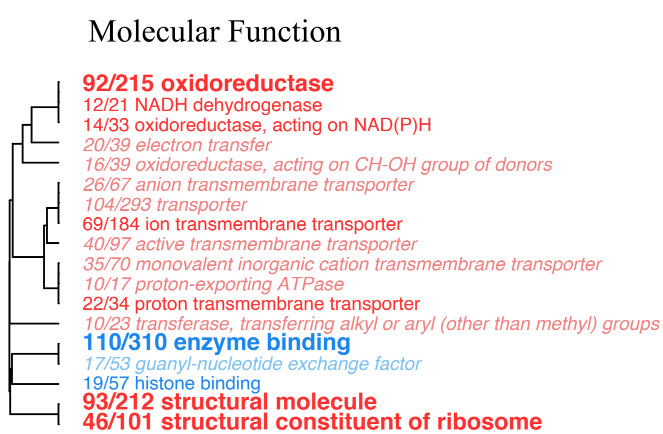


Figure 19: GO enrichment analysis between South Carolina and Nova Scotia at 30°C. Terms in red are enriched in South Carolina while those in blue are enriched in Nova Scotia.

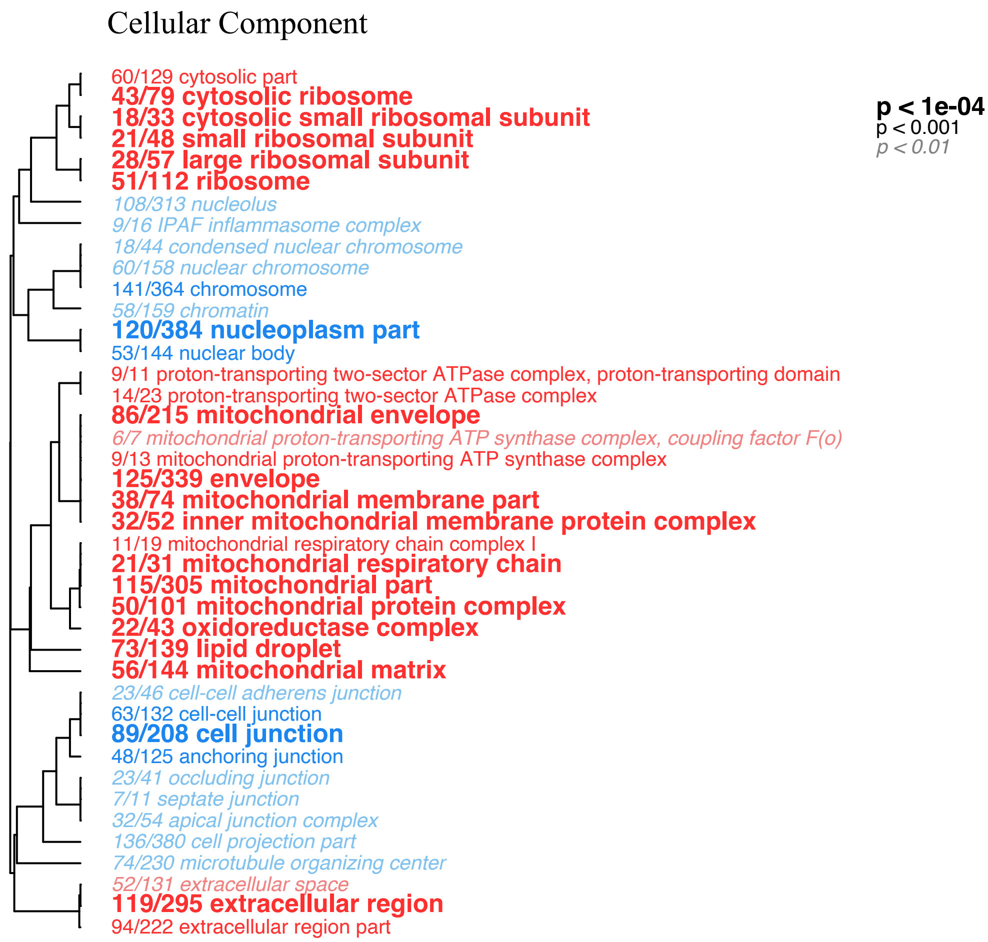


Figure 19 (continued): GO enrichment analysis between South Carolina and Nova Scotia at 30°C. Terms in red are enriched in South Carolina while those in blue are enriched in Nova Scotia.

Finally, GO enrichment analysis was performed on the remaining location-based average high temperatures. At 28°C, Nova Scotia and Maine only had GO terms enriched under cellular components which included two (p-value < 0.01) 'motile cilium' and 'ciliary plasm' indicating a possible role in fluid regulation within cells (Figure 20). No other GO terms were enriched under biological processes or molecular function at 28°C. At the lower latitude locations (North Carolina and South Carolina) 32°C was the average high temperature tested. Several GO terms were seen to be enriched across this comparison (Figure 21). Some of these terms enriched at North Carolina include 'chromosome segregation' (BP), 'tubulin binding' (MF), and 'condensed chromosome' (CC), while terms enriched at South Carolina included 'RNA catabolic process' (BP), 'structural molecule' and 'ribosome' (CC). No general pattern was observed across GO enrichment analyses at either 28°C or 32°C.

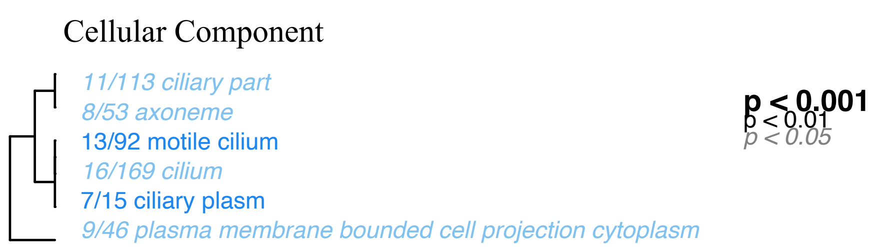


Figure 20: GO enrichment analysis between Nova Scotia and Maine at 28°C. Enrichment was observed only in the cellular component category. Terms in blue are enriched in Maine.

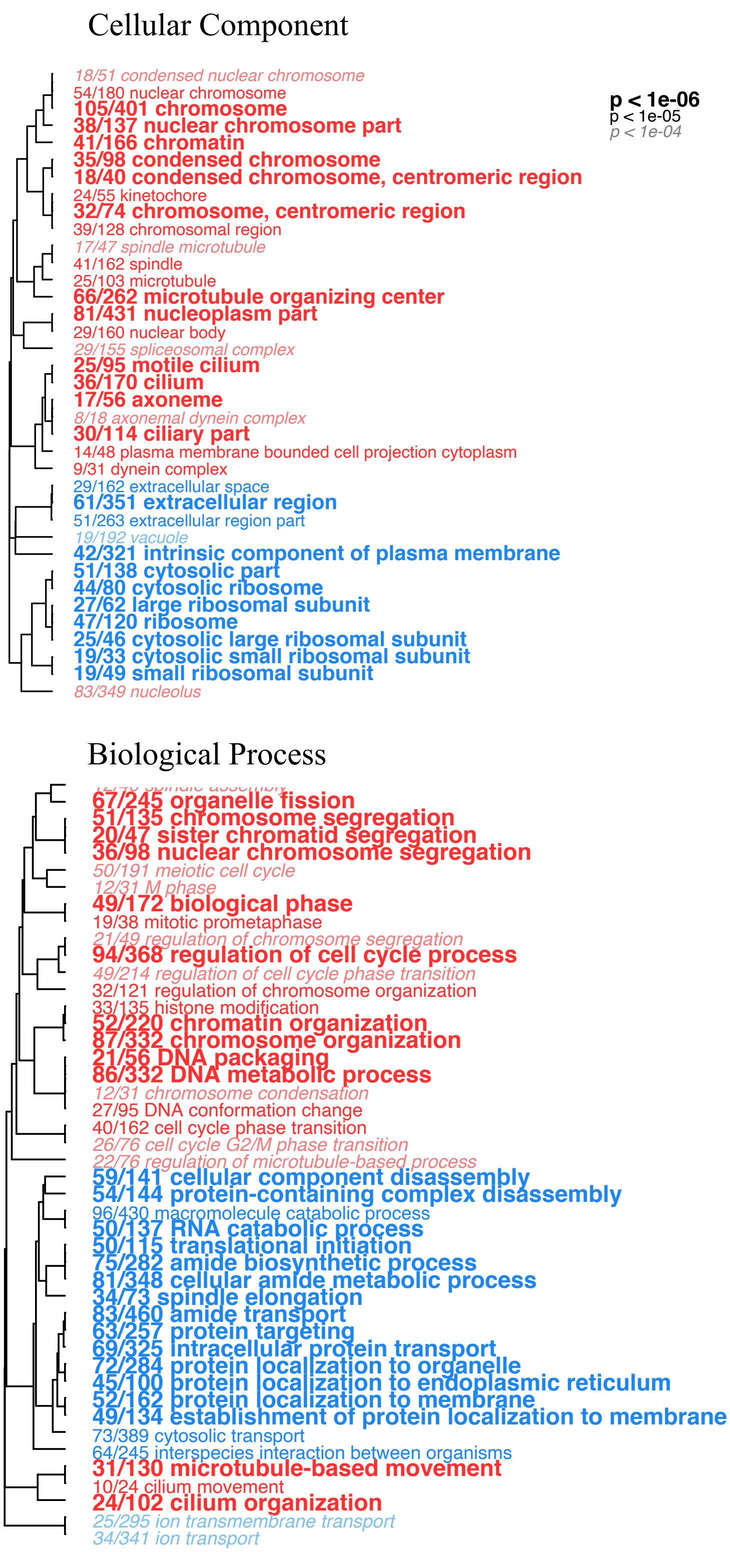


Figure 21: GO enrichment analysis between North Carolina and South Carolina at 32°C. Terms in red are enriched in North Carolina while those in blue are enriched in South Carolina.

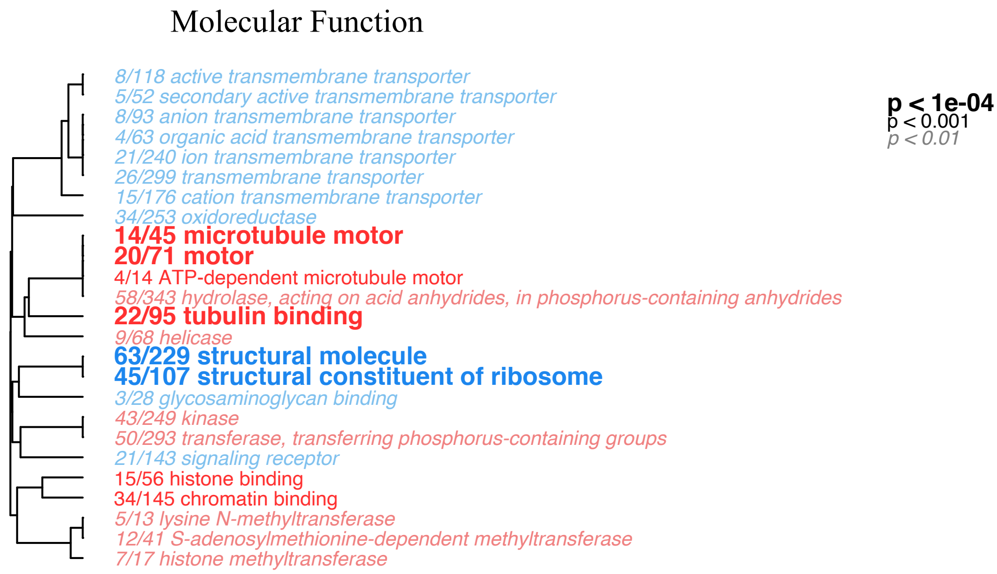


Figure 21 (continued): GO enrichment analysis between North Carolina and South Carolina at 32°C. Terms in red are enriched in North Carolina while those in blue are enriched in South Carolina.

3.2.2. Shared and Unique DEGs: Temperature within location

Previous research in *N. vectensis* and many other species has shown temperature significantly influences the expression of the transcriptome (see Introduction). Here, I compare the transcriptome-wide approach of long-term temperature differences for the five locations of *Nematostella* by comparing DEGs between each temperature treatment within location.

3.2.2.1 DEGs at different temperatures within locations

Similar to the comparisons of DEGs between locations at common temperatures, there were also hundreds of genes with significant differences in expression within a location at different temperatures.

For Nova Scotia, there were 1082 DEGs identified in the three pairwise comparisons. When compared over all comparisons, there were 492 unique genes. Twentyfour genes were DE in all comparisons, where one gene was upregulated in the higher temperature (chitin binding). Although 37% of genes (283/775) were DE in more than one comparison, a majority of genes (63%, 492/775) were unique to a particular temperature comparison (Suppl. Table 5). When I separated genes up- and down-expressed in the lower temperature compared to the high temperature, the expression was slightly biased towards more upregulation of DEGs at the higher temperature (i.e., comparison of 20°C vs 28°C: 179 genes upregulated at 28°C and 175 downregulated; 20°C vs 30°C: 129 genes upregulated at 30°C and 104 downregulated; 28°C vs 30°C: 105 genes upregulated at 30°C and 95 downregulated) (Figure 22). Comparisons of shared vs. unique DEGs in these groups of genes were consistent with the overall trends of many DEGs specific to each treatment (Suppl. Table 6). The largest similarity was between comparisons of 20°C vs 28°C and 20°C vs. 30°C, where 93 and 50 genes were up- and downregulated, respectively, in both comparisons. Some genes showed more complex expression across the treatments with opposing expression depending on the temperature comparison. For example, 21 genes were expressed at lower levels in the higher temperature for 20°C vs 28°C and 20°C vs. 30°C but had higher expression in the 30°C when compared with 28°C. Similarly, I observed that the 28°C could be a peak or a trough of expression for particular genes when compared to 20°C and 30°C. Fifty-two genes showed significantly lower expression at 28°C compared to the other two temperatures. Conversely, 46 genes had significantly higher expression at 28°C compared to the other two temperatures.

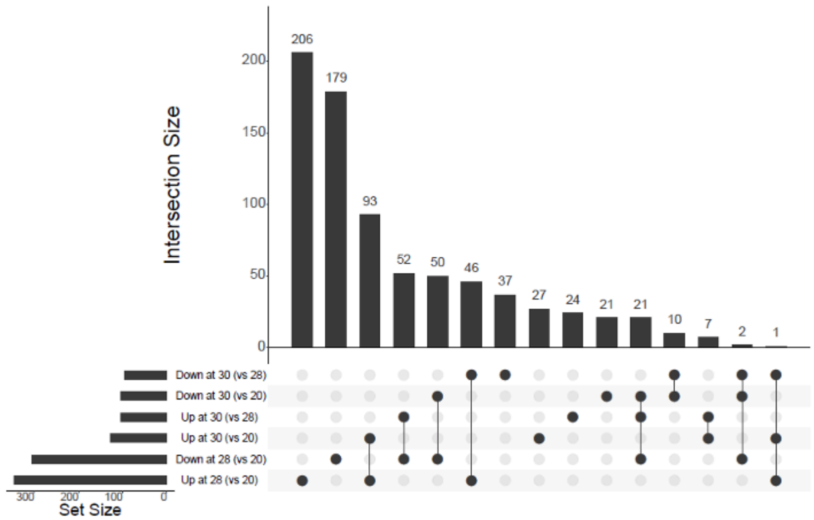


Figure 22: DEGs in Nova Scotia across temperatures.

For Maine, there were 118 DEGs identified in the three pairwise comparisons. When compared over all comparisons, there were 101 unique genes. There were no DEGs in the comparison of 28°C and 30°C. Nineteen genes were DE in each comparison, where 7 were up-regulated in the higher temperature (Figure 23). These genes included theromacin, myophilin, chitin binding, DNA-binding nuclear phosphoprotein p8, cathepsin, collagen), and vitellogenin precursor. From the 10 genes down-regulated at lower temperatures, 5 were microtubule related and the remaining were uncharacterized. Comparisons of shared vs. unique DEGs in these groups of genes were consistent with the overall trends of many DEGs specific to each treatment. However, there were 7 shared upregulated and 10 shared downregulated genes in the comparisons of 20°C vs 28°C and 20°C vs 30°C. No genes showed opposing expression across the treatments depending on the temperature comparison (i.e., expression differences were consistent across comparisons of 20°C and the higher temperatures).

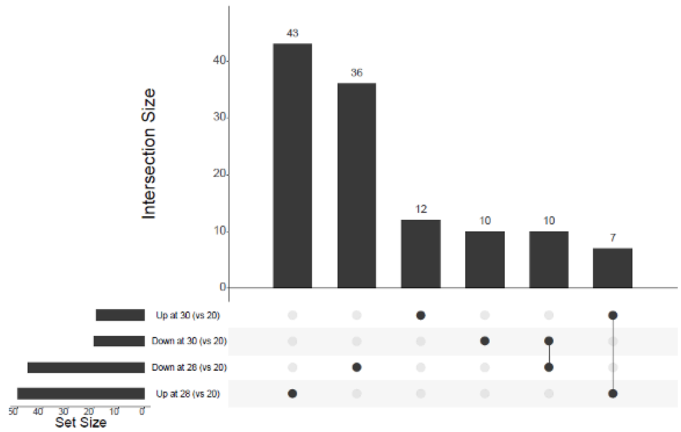


Figure 23: DEGs in Maine across temperatures.

For Massachusetts, there was only a single comparison: 20°C vs 30°C. For these individuals, higher temperature resulted in almost twice as many downregulated genes (n=96) than upregulated (n=51) (Figure 24). Interestingly, only one ('low density lipoprotein receptor-related protein') of these upregulated DEGs was shared with genes unregulated in higher temperatures in the Maine animals (nearest geographic location) (Suppl. Table 9). Similarly, of the 96 genes with decreased expression at high temperature in Massachusetts, none of these were shared with the downregulated genes in Maine. However, I did observe genes with opposite expression between locations at the same temperature comparisons (see more detail in 3.2.4).

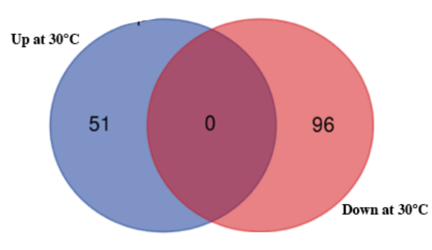


Figure 24: DEGs in Massachusetts across temperatures.

For North Carolina, there were 295 DEGs identified in the three pairwise comparisons. When compared over all comparisons, there were 254 unique genes (Suppl. Table 10). There were no DEGs in the comparison of 30°C and 32°C. Forty-one genes were DE in the two remaining comparisons: 20°C vs 30°C and 20°C vs 32°C. For these genes, 9 were upregulated at the higher temperature (Suppl. Table 11). These genes included G2 and S phase-expressed protein 1 (*GTSE1),* Cyclin-dependent kinase 1 (*CDK),* separin (ESPL*1),* reticulocalbin 1 *(rcn1*), NADPH-dependent FMN reductase *(FMN_Rdtase-like),* cell cycle regulated microtubule associated protein, thymosin beta 4, and E3 ubiquitin-protein ligase. A majority of the DEGs were unique to the temperature comparisons (total: 213 of 254, Figure 25). There were more than 4-times (238 vs. 57) the DEGs in the comparison of the more extreme temperature difference (*i.e*., 20°C vs 32°C) compared with the less extreme (*i.e*., 20°C vs 30°C). When I separated genes up- and down-expressed in the higher temperature compared to the lower temperature, the expression was biased with more genes that had downregulation at the higher temperature (*i.e.,* comparison of 20°C vs 30°C: 6 genes upregulated at 30°C and 12 downregulated; 20°C vs 32°C: 70 genes upregulated at 32°C and 129 downregulated). Comparisons of shared vs. unique DEGs in these groups of genes were consistent with the overall trends of many DEGs specific to each treatment. I observed only 8 shared upregulated genes between the comparisons and 31 shared downregulated genes. Unlike Nova Scotia, none of the DEGs switched expression pattern from down- to upregulated between temperature comparisons.

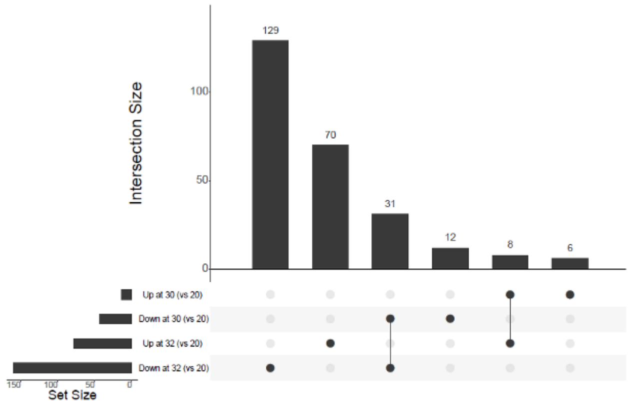


Figure 25: DEGs in North Carolina across temperatures.

For South Carolina, here were 641 DEGs identified in the three pairwise comparisons. When compared over all comparisons, there were 427 unique genes (Suppl. Table 12). Only one gene (transcriptional adapter 2-beta; *TADA2B*) was DE in all comparisons. 201 common genes were DE in the two remaining comparisons: 20°C vs 30°C and 20°C vs 32°C. Unlike in comparisons for other locations, South Carolina animals had nearly half of the genes (213 of 427) with significant differences in expression shared between comparisons (Suppl. Table 13). A vast majority (n=201) were DEG in 20°C vs 30°C and 20°C vs 32°C (Figure 26). This, in part, results from generally similar number of DEGs in each comparison, where 20°C vs 30°C and 20°C vs 32°C had 100s of DEGs (327 and 282, respectively) whereas 30°C vs 32°C had only 32 DEGs. When I separated genes up- and down-expressed in the higher temperature compared to the lower temperature, the expression was fairly similar for up- and downregulated gene expression in the more extreme temperature comparisons (i.e., comparison of 20°C vs 30°C: 153 genes upregulated at 30°C and 175 downregulated; 20°C vs 32°C: 145 genes upregulated at 32°C and 137 downregulated), but were biased to more downregulation in the comparison of

30°C vs 32°C (7 upregulated, 25 downregulated at the higher temperature) (Figure 26). Comparisons of shared vs. unique DEGs in these groups of genes were consistent with the overall trends of many DEGs specific to each treatment. I observed 97 shared upregulated genes between the broader temperature comparisons (i.e., 20°C vs 30°C and 20°C vs 32°C) and 104 shared downregulated genes for these same two comparisons. Only 1 and no DEGs were shared in up- and downregulated genes, respectively, for all three comparisons. Like Nova Scotia but unlike Maine or North Carolina, I observed DEGs that switched expression pattern from down- to upregulated between temperature comparisons. Four genes had higher expression in 30°C when compared with 20°C, but then decreased expression from 30°C to 32°C.

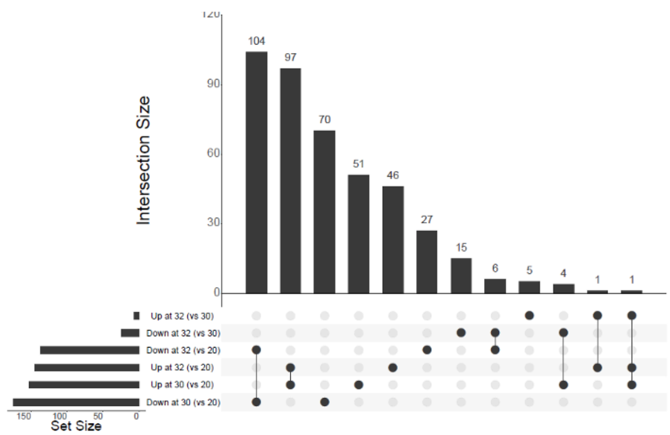


Figure 26: DEGs in South Carolina across temperatures.

3.2.2.2 Functional Enrichment of Gene Ontologies from DEGs

Interestingly, Nova Scotia at 20°C and 30°C did not have any enriched terms across any of the gene ontology categories. However, it did contain several enrichment scores 20°C vs. 28°C as well as 28°C vs. 30°C. Rather than listing terms that are seen enriched in either the higher or lower temperatures, Nova Scotia followed a patterns where similar GO terms were found enriched relative to 28°C. For example, under biological process 'cellular respiration' 'oxidation-reduction process' and respiratory electron transport chain' were all enriched at 28°C (Figure 27, Figure 28). This same pattern continued for molecular function ('oxidoreductase') and cellular component ('mitochondrial protein complex').

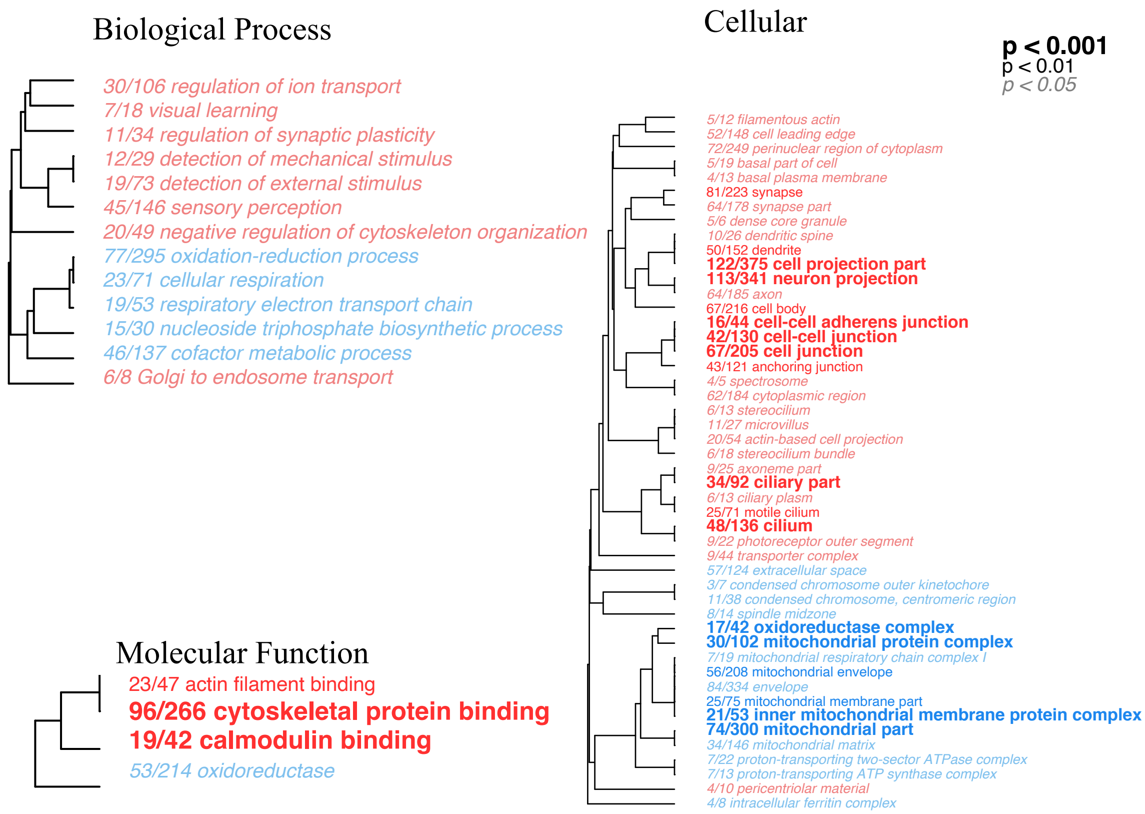


Figure 27: Enriched GO terms for Nova Scotia at 20°C vs. 28°C. Terms in red are enriched at 20°C while those in blue are enriched at 28°C.

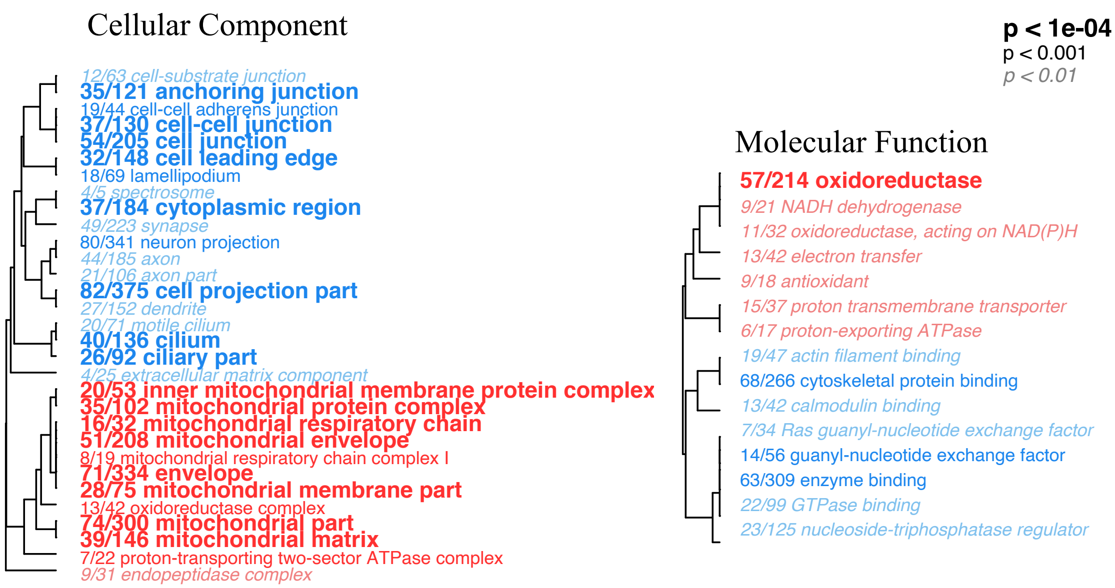


Figure 28: Enriched GO terms for Nova Scotia at 28°C vs. 30°C. Terms in red are enriched at 28°C while those in blue are enriched at 20°C.

In Maine, terms enriched at the higher temperatures (28°C and 30°C relative to 20°) included cellular respiration, electron transport chain (BP), proton transmembrane transporter, proton-exporting ATPase (MF), mitochondrial part, and mitochondrial protein complex (CC) (Figure 29, Figure 30). These could indicate a possible increased function in metabolism and energy production. When examining terms enriched at 20°C, the biological processes category contains numerous terms involved in regulation of various processes such as cell development, system processes, and locomotion. Under molecular function, four terms were enriched at lower temperatures (cytoskeletal binding, actin filament binding, calmodulin binding, and calcium ion binding). And finally, for cellular component terms such as 'neuron projection' and 'dendrite' were enriched at lower temperatures indicating a possible connection with nervous system function.

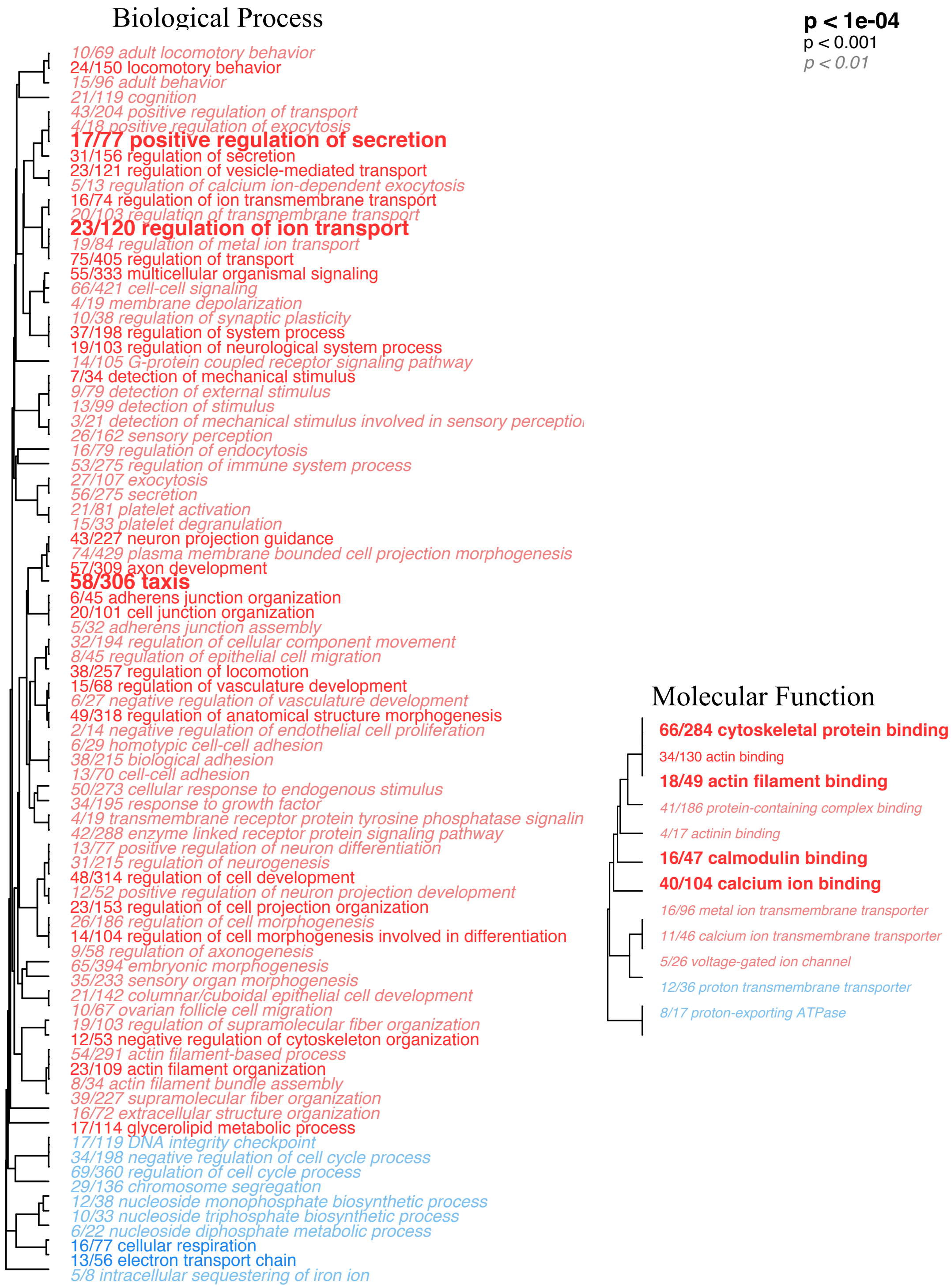


Figure 29: Enriched GO terms for Maine at 20°C vs. 30°C. Terms in red are enriched at 20°C while those in blue are enriched at 30°C.

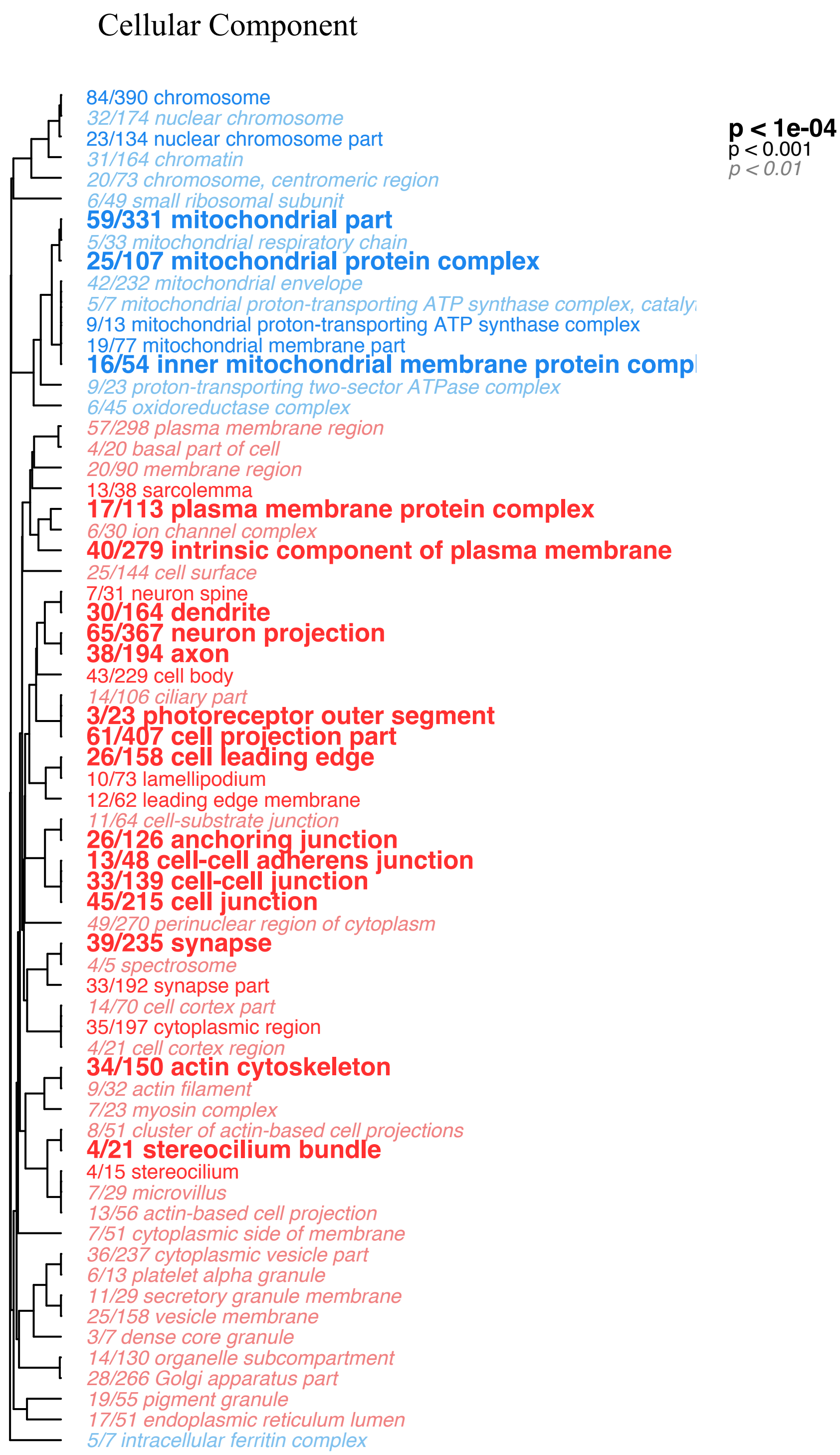


Figure 29 (continued): Enriched GO terms for Maine at 20°C vs. 30°C. Terms in red are enriched at 20°C while those in blue are enriched at 30°C.

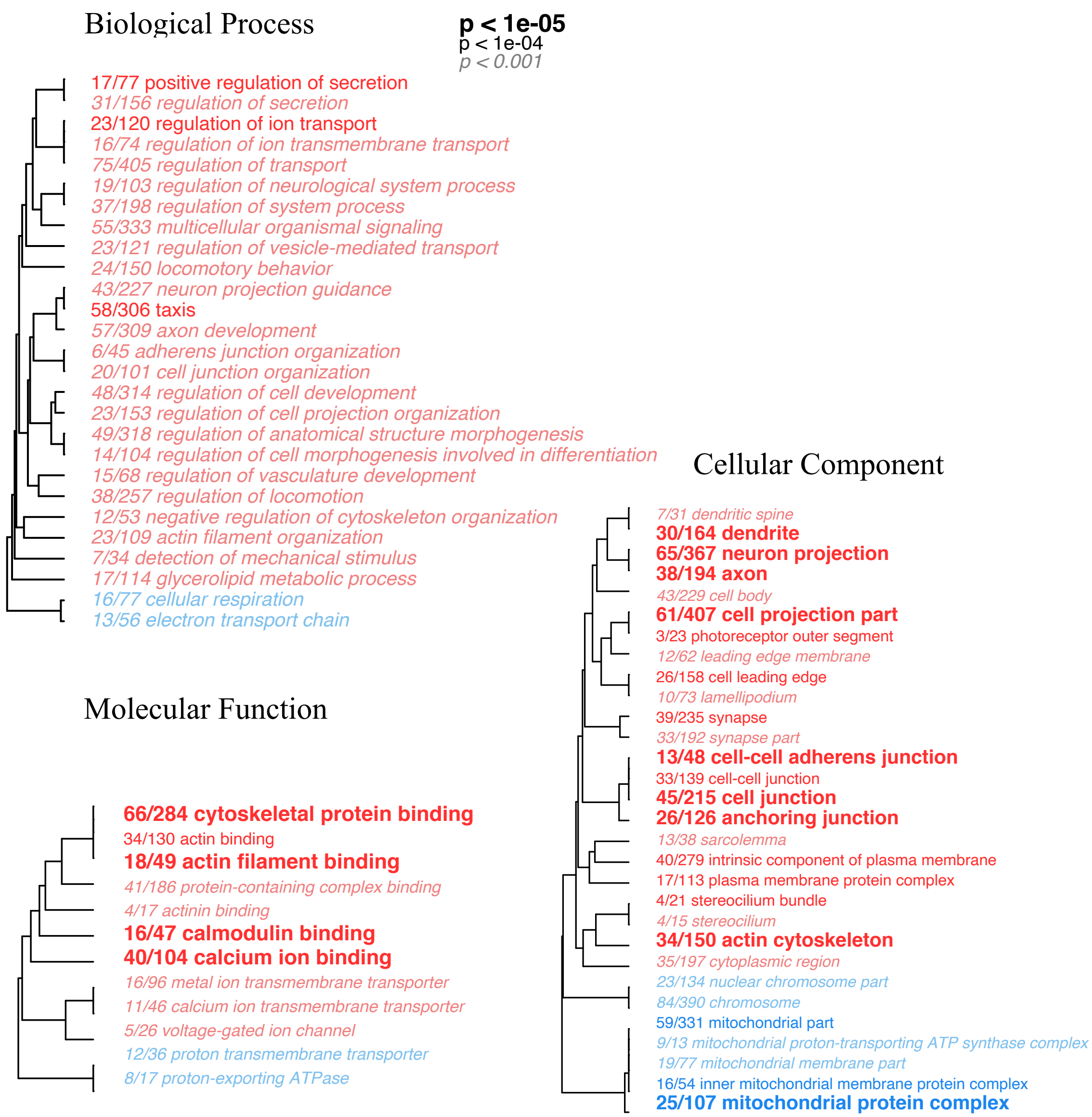


Figure 30: Enriched GO terms for Maine at 20°C vs. 28°C. Terms in red are enriched at 20°C while those in blue are enriched at 28°C.

Massachusetts contained only significant DEG when 20°C was compared against 30°C (Figure 31). These gene lists were then analyzed using a GO enrichment analysis approach. Several GO terms were seen to be enriched with a majority of them enriched at the lower temperature which has not been the case in previous comparisons. Notable gene ontologies seen enriched at 20°C include ‘regulation of system process’ (BP), ‘calmodulin binding’, ‘actin filament binding’ (MF), ‘cell projection part’, ‘neuron projection’, and ‘synapse’ (CC). Terms enriched at 30°C included ‘chromosome organization’ (BP) and ‘chromosome’ (CC) and were often related to chromosomal structure and segregation.

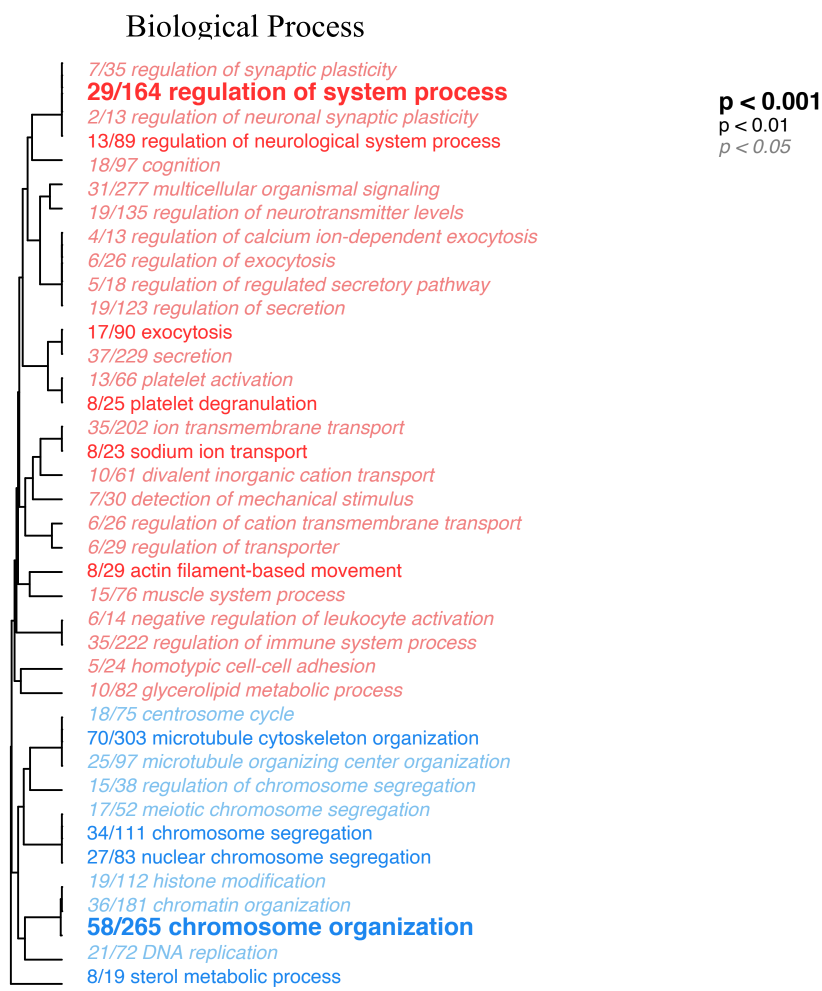


Figure 31: Enriched GO terms for Massachusetts at 20°C vs. 30°C. Terms in red are enriched at 20°C while those in blue are enriched at 30°C.

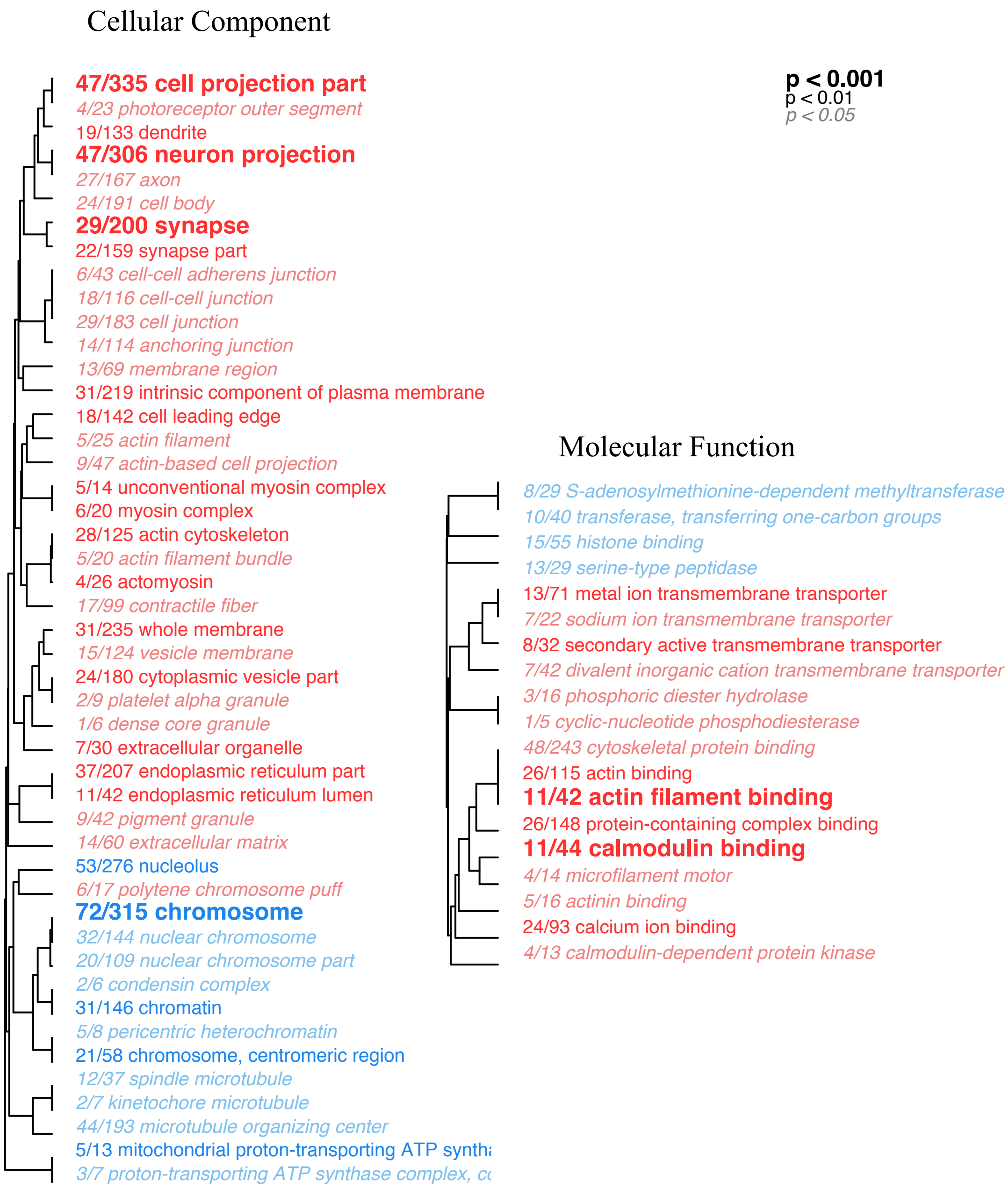


Figure 31 (continued): Enriched GO terms for Massachusetts at 20°C vs. 30°C. Terms in red are enriched at 20°C while those in blue are enriched at 30°C.

In North Carolina, very few terms were enriched at higher temperatures relative to those found at 20°C. This goes against what was previously found but may indicate that the DEG found at lower temperatures are categorized in a wider range of assigned gene ontology terms. No DEG were found when 30°C was compared against 32°C and so no resulting GO terms were enriched. For the other temperature comparisons (20°C vs 30° and 20°C vs 32°C) several terms were enriched at lower temperature while only a few were found to be enriched at the higher temperatures (Figure 32, Figure 33). Shared terms seen at higher temperatures included ‘chromosome segregation’(BP), ‘mitochondrial protein complex’, and ‘oxidoreductase complex’ (CC). On the other end, shared terms enriched at the lower temperature consisted of many under biological process (‘RNA catabolic process’ ‘taxis’ ‘regulation of locomotion’ ‘regulation of ion transport’) and cellular component (‘cytosolic ribosome’, ‘cytosolic part’, ‘ribosome’, ‘neuron projection’, ‘anchoring junction’, and ‘synapse’), while no terms overlapped for molecular function.

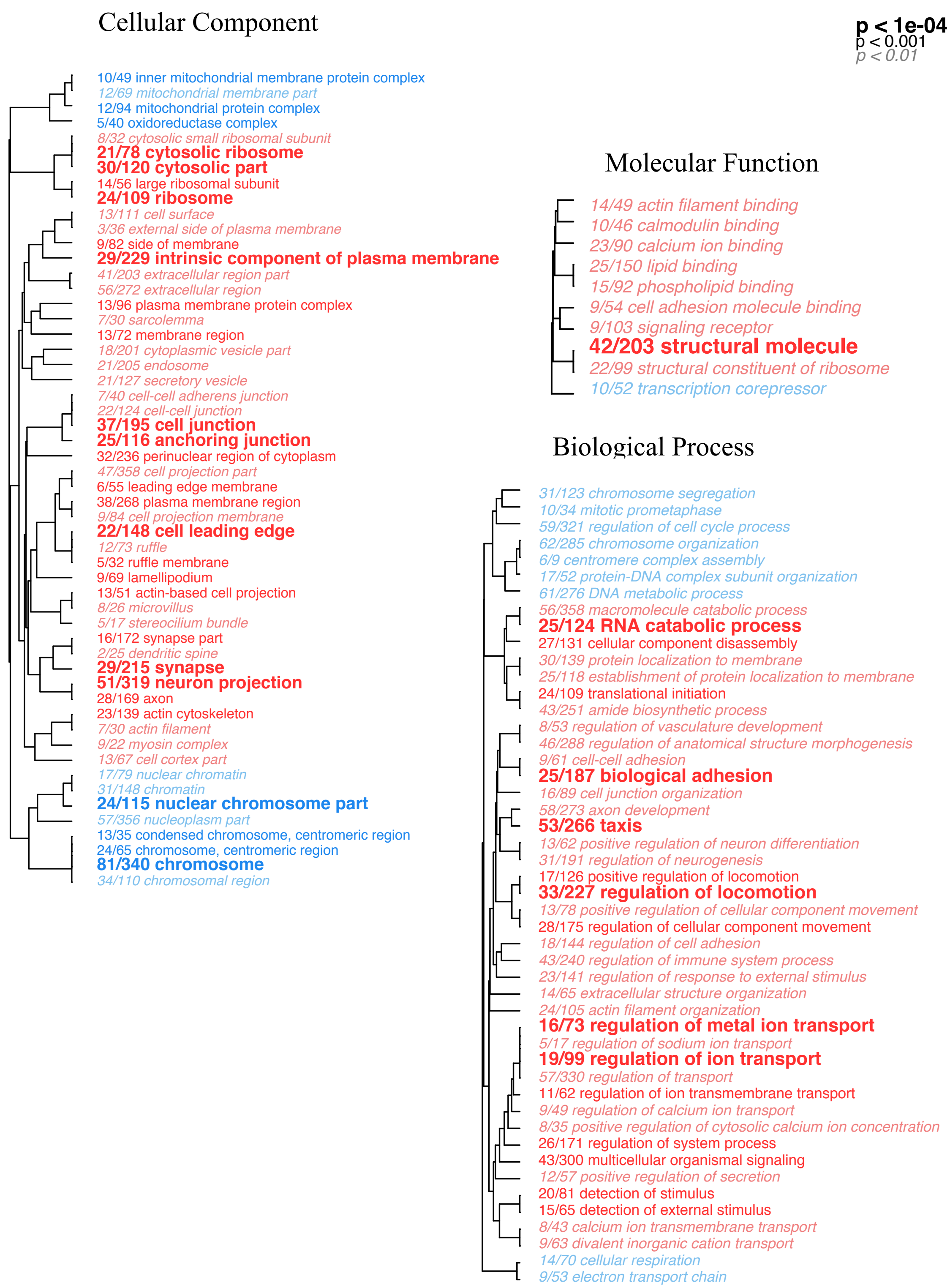


Figure 32: Enriched GO terms for North Carolina at 20°C vs. 30°C. Terms in red are enriched at 20°C while those in blue are enriched at 30°C.

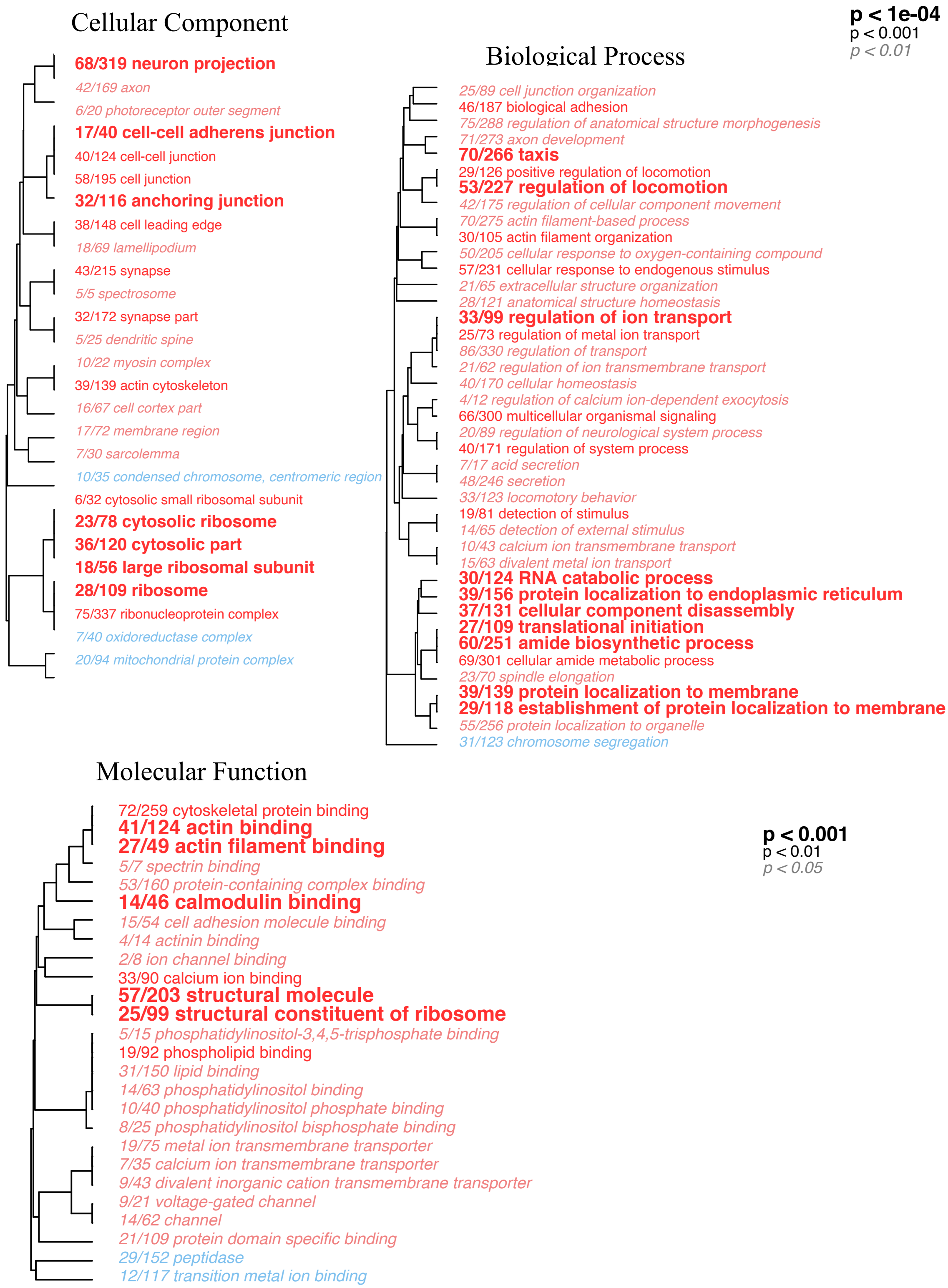


Figure 33: Enriched GO terms for North Carolina at 20°C vs. 32°C. Terms in red are enriched at 20°C while those in blue are enriched at 32°C.

In South Carolina three temperatures were compared, 20°C, 30°C, and 32°C, but more similarities were seen in functional enrichments of 20°C vs. 30°C (Figure 34) and 20°C vs. 32°C (Figure 35) than between 30°C and 32°C (Figure 36). When also comparing 20°C vs 32°C no terms were enriched under molecular function and only a few for cellular component and biological processes. One term was enriched across all three locations which was 'oxidation-reduction process' found under molecular function. When the other temperatures are compared, without 32°C, the only distinct enrichment terms observed fall under cellular components and are related to mitochondria structure.

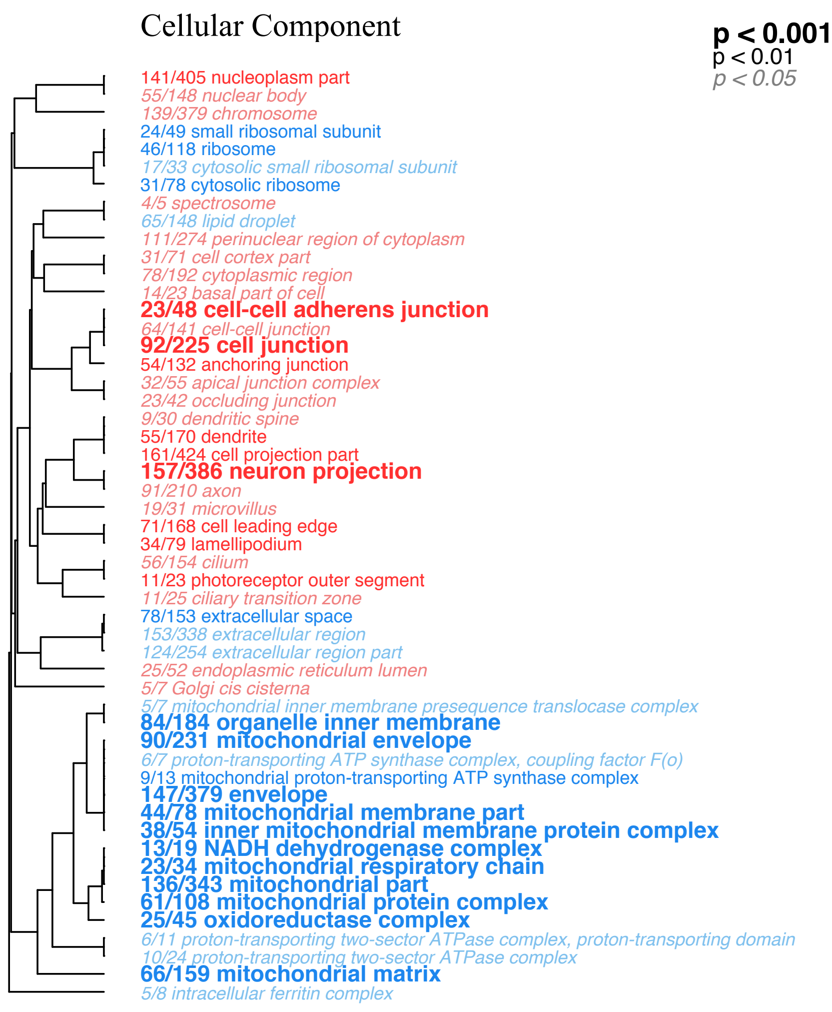


Figure 34: Enriched GO terms for South Carolina at 20°C vs. 30°C. Terms in red are enriched at 20°C while those in blue are enriched at 30°C.

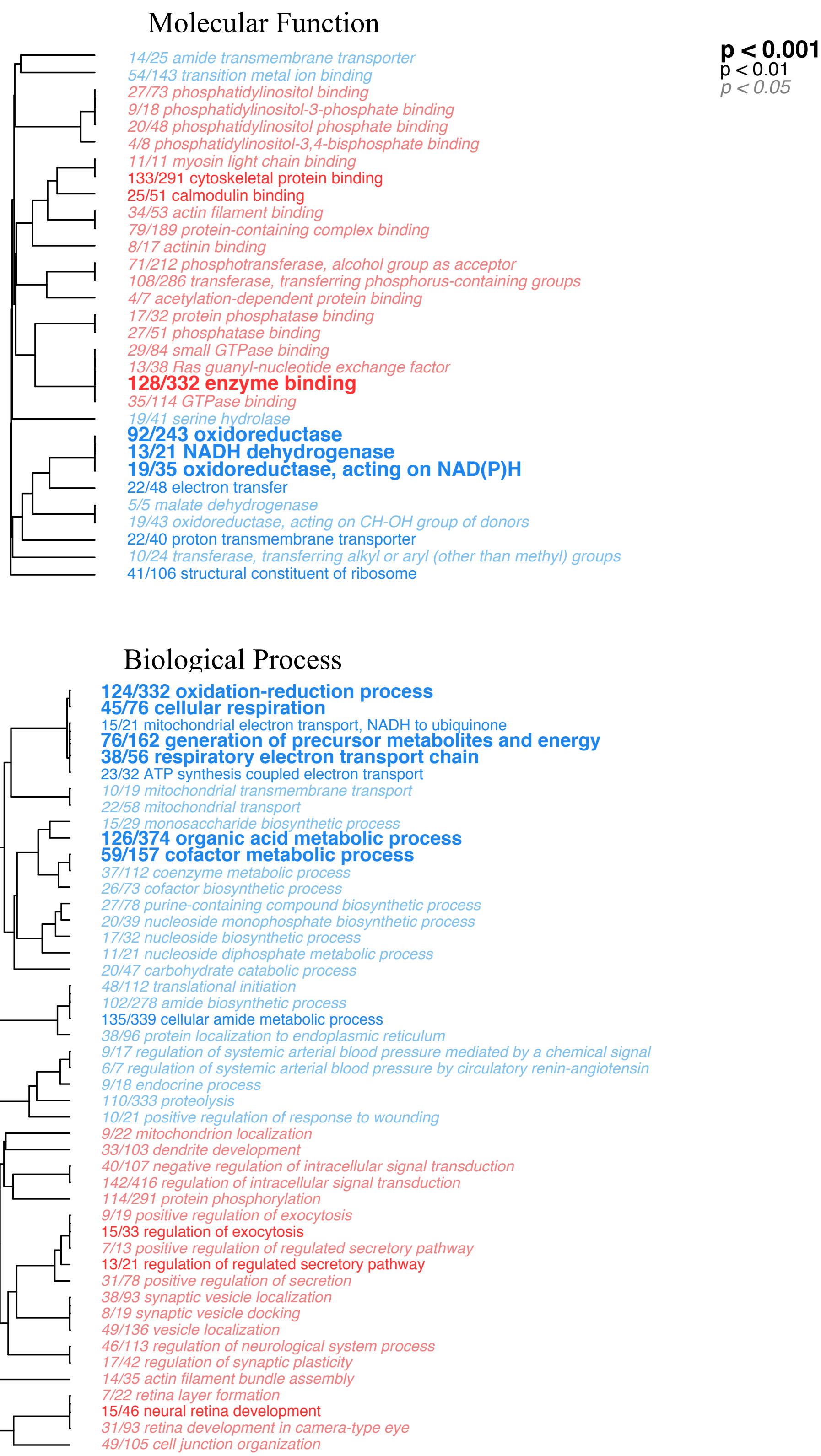


Figure 34 (continued): Enriched GO terms for South Carolina at 20°C vs. 30°C. Terms in red are enriched at 20°C while those in blue are enriched at 30°C.

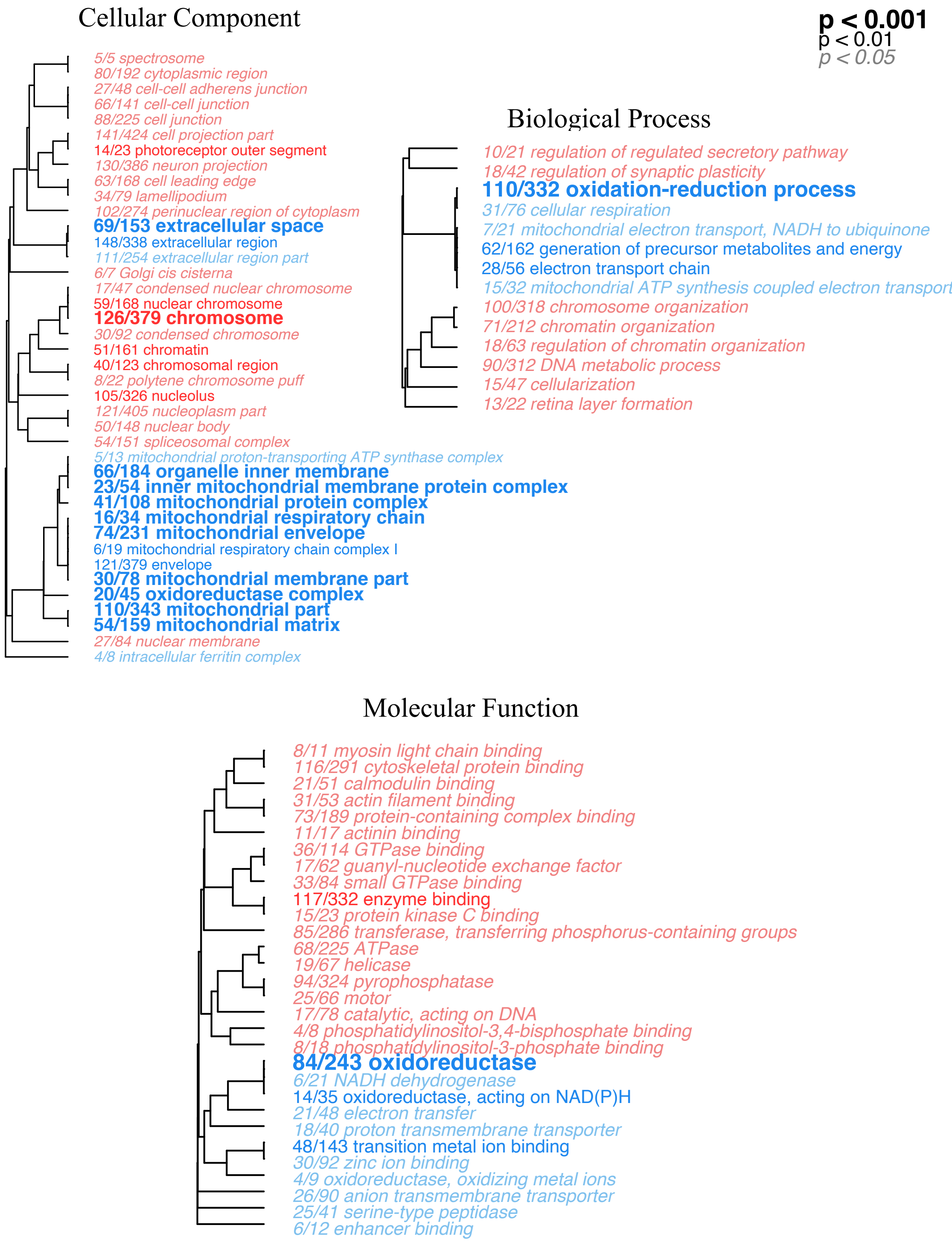


Figure 35: Enriched GO terms for South Carolina at 20°C vs. 32°C. Terms in red are enriched at 20°C while those in blue are enriched at 32°C.

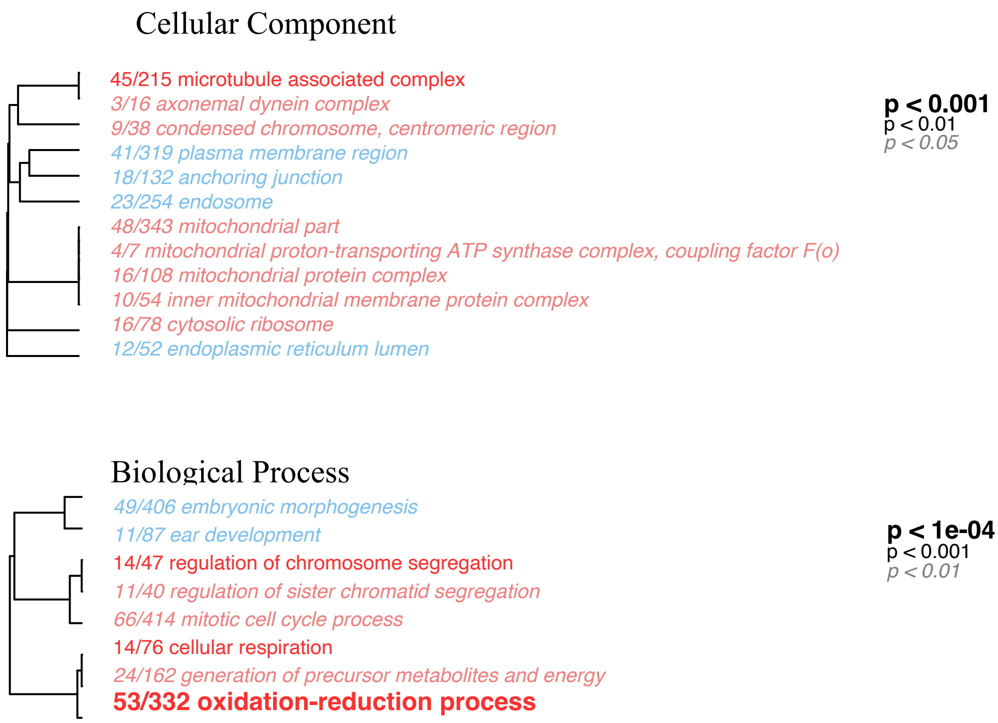


Figure 36: Enriched GO terms for South Carolina at 30°C vs. 32°C. Terms in red are enriched at 30°C while those in blue are enriched at 32°C.

3.2.3 DEGSs due to interaction of location and temperature

The multivariate model indicated the interaction of location-by-temperature was a significant factor in the differential gene expression in my data set. These interactive effects indicate that transcription of particular genes was dependent on how expression was modulated (magnitude and/or direction) in temperature contrasts between individuals from particular locations. Had different DEGs between locations in same temperature comparison: 20. Here, I compare how DEGs in the common temperature comparison for all locations (20°C vs 30°C) differ by gene ID and direction of expression, particularly focusing on genes with shifts in the direction of expression.

I first compared the DEGs in the 20°C vs 30°C contrast for all locations. Overall, there was no clear pattern for how the number of DEGs related to latitude of the population (Figure 37). The number of DEGs was largest in the most distant locations: Nova Scotia (232 DEGs) and South Carolina (327 DEGs). The locations in between these had many fewer DEGs: Maine (39 DEGs), Massachusetts (147 DEGs) and North Carolina (57 DEGs). The percentage of unique DEGs to a particular location varied considerably from 28% for Maine (11 of 39), to nearly half of the DEGs for Nova Scotia (113 of 232) to more than 75% of the DEGs (248 of 327) were only DE in South Carolina. Again, this measure of unique transcriptomic expression in response to the temperature comparisons showed no clear relationship to latitude of origin. Despite the relative uniqueness of the transcriptional response of South Carolina, it shared the most DEGs in common with Nova Scotia (n=52, 35 uniquely).

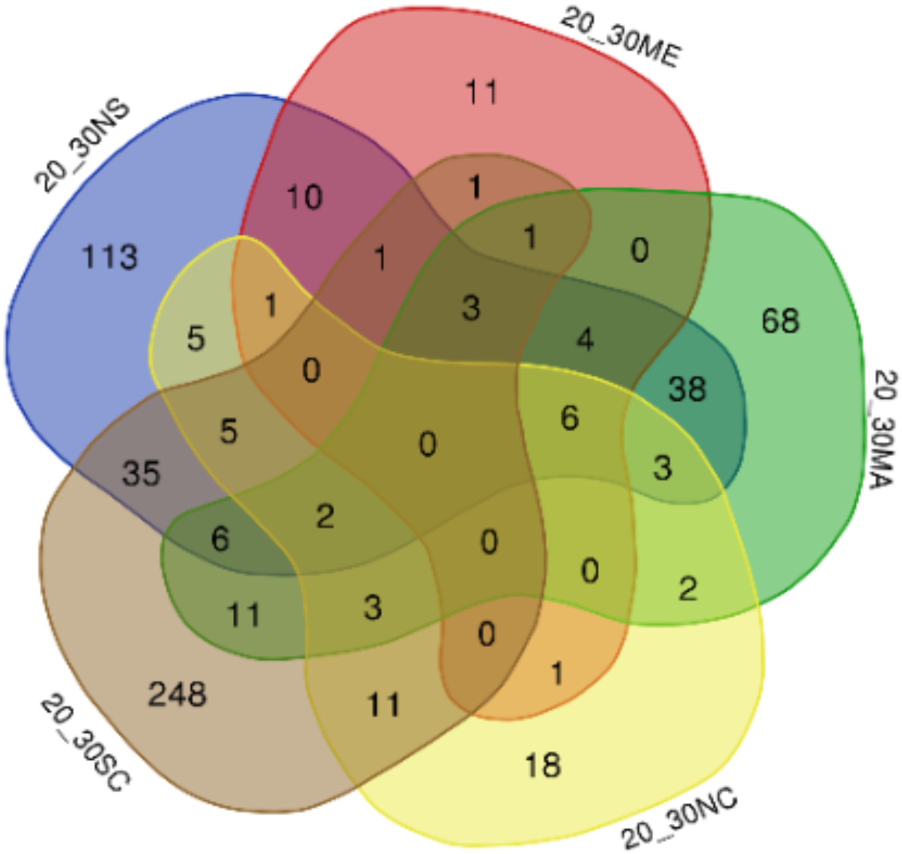


Figure 37: Venn diagram of shared and unique DEGs between 20°C and 30°C for each location.

Next, I compared only the up- and downregulated genes. For Nova Scotia, Maine, and South Carolina, there was essentially an equal proportion of up- and downregulated genes (% with higher expression at 30°C: Nova Scotia: 55%, Maine: 495, South Carolina: 47%). For Massachusetts and North Carolina, there were proportionally more downregulated genes at the higher temperature. 63% and 75%, respectively. Similar to the comparisons for overall DEGs, most of the upregulated DEGs at higher temperature were unique to a particular location. Again, Nova Scotia and South Carolina shared the most DEGs (15 genes), but Nova Scotia and Maine had a similar number (13 genes). Two of these genes were shared between these three locations. A similar pattern of unique DEGs was observed in the genes with lower expression at 30°C with a majority being specific to a location. The highest number of shared downregulated DEGs was again between Nova Scotia and South Carolina (36 genes).

Last, I compared to identify any DEGs with opposite expression patterns between locations. While comparisons between all sites were performed, I focus here on three specific comparisons: Nova Scotia and South Carolina, Maine and Massachusetts, and North Carolina and South Carolina. For the comparison of Nova Scotia and South Carolina, all but one DEG were expressed in the same direction (*i.e,* if upregulated in Nova Scotia at 30°C were also upregulated in South Carolina). Only one gene (NVE15529: unannotated) was expressed in opposing directions, where it had higher expression at 30°C in South Carolina and lower in Nova Scotia. This was surprising given the different locations and temperatures these populations experience. Comparison of one pair of nearest neighbor populations (Maine and Massachusetts) revealed genes with opposing expression patterns to the same temperature difference. There were 16 genes that were downregulated at 30°C compared to 20°C in Massachusetts that had higher expression in 30°C compared to 20°C in Maine. Conversely, there were 11 genes that were upregulated at 30°C compared to 20°C in Massachusetts that had lower expression in 30°C compared to 20°C in Maine. These opposite expression patterns are good indications of unique location-by-temperature interactions. Third, comparison of the other nearest neighbor populations (North Carolina and South Carolina) showed generally parallel expression responses for shared DEGs. Similar to the comparison of South Carolina and Nova Scotia, only one DEG (NVE7851) had opposing expression patterns between North Carolina and South Carolina. The other 20 shared DEGs had expression in the same direction for each location.

3.3 WGCNA

After identifying DEGs between experimental conditions (i.e. location and temperature) a weighted gene co-expression network analysis (WGCNA) was conducted to distinguish groups of genes (modules) exhibiting correlated expression patterns across samples. When examining location, modules were significantly and uniquely correlated to either one or multiple locations (Figure 38). Only three locations had a single unique module: Maine (darkred -Pearson's R2=-0.53, p-value= 6e-05) Massachusetts (royalblue - Pearson's R2=0.69, p-value= 2e-08), and North Carolina (cyan -Pearson's R2=-0.53, p-value=8e-05). The dark red module (37 genes) was seen to be significantly downregulated in Maine relative to all other locations, but no enriched GO terms were identified. The royal blue module (59 genes) was upregulated in Massachusetts while no other significant difference was observed in any other location or temperature condition. GO enrichment analysis for the royal blue module showed enrichment in 'innate immune response' and 'regulation of DNA-binding transcription factor' from the biological process category. The cyan module (227 genes) was downregulated in North Carolina while not other significant difference was found across experimental conditions. The GO enrichment for this module included terms from the biological processes and cellular component categories and is centered around "antigen processing and presentation' and 'proteasome core complex.' One additional location, South Carolina, had two modules that showed significant and unique co-expression of genes within the pink (Pearson's R2= -0.58, p-value=8e-06, 235 genes) and salmon (Pearson's R2=-0.73, p-value=2e-09, 142 genes) modules. The pink module contained GO enrichment of 'spliceosomal complex' and 'precatalytic spliceosome' from the cellular component category. While the salmon module revolved around RNA such as 'RNA binding' (molecular function), 'RNA biosynthesis process' (biological process), and 'ribosome' (cellular component). Beyond singular comparisons, one co-expression trend was observed between Nova Scotia and South Carolina which shared similar co-expression patterns within two modules (purple: SC-Pearson's R2= 0.78, p-value=3e-11; NS-Pearson's R2=0.28, p-value=0.05; and green: SC-Pearson's R2=-0.73, p-value=2e-09; NS-Pearson's R2=-0.27, p-value= 0.05). The purple module (1,112genes) was not enriched for any GO terms, but the green module (377 genes) was enriched for terms associated with 'positive regulation of proteolysis' (Suppl. Figure 4). Across experimental temperature conditions one module was significantly and uniquely different at 32°C (salmon module) and another at 30°C (royal blue). As previously mentioned, the salmon module was enriched in RNA binding and related GO terms while royal blue was seen to be enriched in immune response. It should also be noted that between experimental replicates no significant differences were seen across any of the 16 modules determined from the WGCNA.

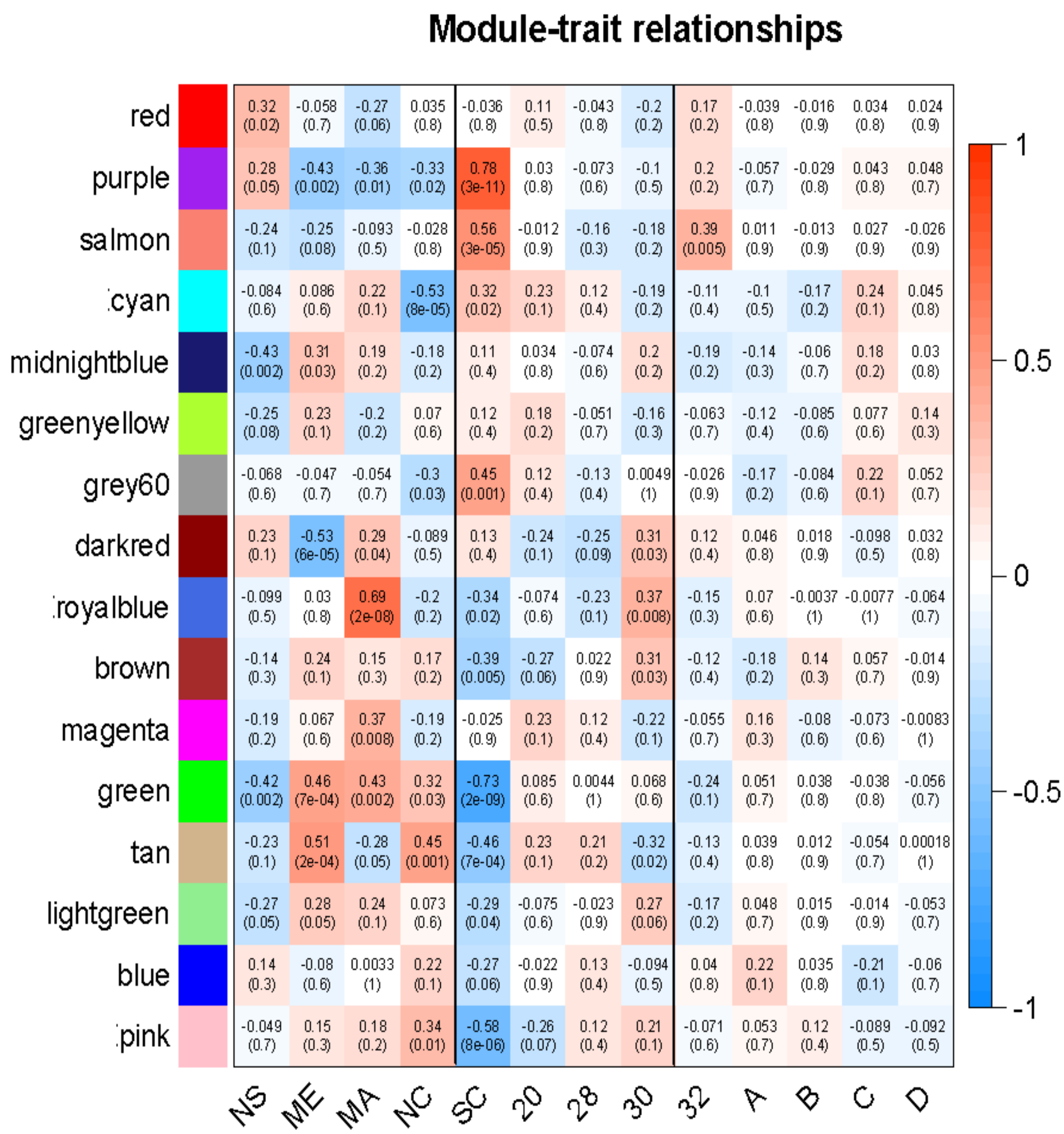


Figure 38: Weighted gene co-expression network analysis (WGCNA) illustrating correlations between location, temperature, and experimental replicate and modules (groups of genes with common expression patterns.) Modules are indicated by arbitrary colors on the left. Within the heatmap, the strength of the correlations between traits are gene expression is indicated by intensity of the colored block with red indicating a positive correlation and blue a negative correlation. Pearson's correlation value is the first number listed in each color block followed by its associated p-value.

CHAPTER 4: DISCUSSION

4.1 Gene Expression Influenced by Temperature, Location and their Interaction

Multivariate analysis of *N. vectensis* gene expression demonstrated that both location of origin, temperature exposure, and their interaction have a strong and significant impact on transcriptome-wide gene expression. This is further validated from the PCA plots in Figure 2, where locations are grouped based on proximity to each other. The three northern locations (NS, ME, and MA) were seen to be grouped together along PC2, while variance seen between locations was spread along PC1. The variance seen in North Carolina and South Carolina was also distributed along PC1 with similarities seen closer along PC2. Within each location, temperatures were seen to group together specifically within 20°C where variance was seen along PC2. I predicted that the variance seen across populations of *N. vectensis* along this thermal gradient would be grouped by temperature exposure and indicate a possible thermal stress response seen at elevated temperatures. This, however, was not the case as *N. vectensis* populations in this study were shown to be strongly affected by both temperature and location as well as their interaction. Because no obvious pattern was observed gene expression analysis was conducted on all locations at single temperatures as well as all temperatures at each location.

4.2 Evidence of Thermal Response Across Locations at 20°C and 30°C

I then compared northern populations against southern populations at both 20°C and 30°C. Northern populations consisted of Nova Scotia, Maine, and Massachusetts while southern populations included North Carolina and South Carolina (Table 4). At 20°C, 24 DEGs were established with 11 upregulated in the north and 13 in the south. Genes shown to be upregulated in northern populations include a PLAC8 gene, a tissue factor pathway inhibitor (*TFPI*), a sphinogosine-1-phosphate (*SIP*), and a gene related to ribosome biogenesis. The first three genes listed are all related to differentiation, proliferation, and apoptosis, while the ribosome biogenesis indicates a possible role in increased protein translation and growth rates. On the other hand, the genes seen to be upregulated in the southern populations include eukaryotic translation initiation factor and a fibroblast growth factor which functions by increasing protein production and cell growth and proliferation, respectively. In both conditions, genes with known functions in cellular cascades are observed indicating a larger role in overall functionality within these genes. From the gene enrichment analysis, significantly more terms were enriched in southern locations than the northern locations. There was very few overlapping GO terms enriched across multiple conditions. Three conserved GO terms were seen enriched in North Carolina and South Carolina when compared with Maine: 'ribosome,' 'large ribosome,' and 'structural constituent of ribosome' under the cellular component and molecular function categories. This is possibly indicative of an increase in ribosome and protein production and higher growth rates but contradicts what was found during differential gene expression analysis.

Table 4: List of DEG between Northern (NS, ME, MA) and Southern (NC, SC) Locations at 20°C		
Gene	Upregulated	Description
NVE14009	Southern	Component of eukaryotic translation initiation factor 3
NVE23069	Southern	Cathrin, light chain
NVE10140	Southern	Uncharacterized
NVE12877	Southern	Uncharacterized
NVE819	Southern	Ribosome biogenesis protein
NVE11103	Southern	Uncharacterized
NVE3141	Southern	Uncharacterized
NVE20016	Southern	Uncharacterized
NVE23695	Southern	Uncharacterized
NVE25877	Southern	Fibroblast growth factor receptor
NVE19184	Southern	Gelsolin
NVE20024	Southern	Collagen triple helix repeat
NVE12992	Southern	Titin
NVE20134	Northern	Coiled-coil domain containing
NVE6091	Northern	Uncharacterized
NVE20308	Northern	PLAC8 family
NVE3396	Northern	Uncharacterized
NVE17074	Northern	Sphingosine-1-phosphate phosphatase
NVE21760	Northern	Tissue factor pathway inhibitor
NVE4686	Northern	Uncharacterized
NVE18393	Northern	Uncharacterized
NVE11007	Northern	Uncharacterized

Along with 20°C, all locations were exposed to 30°C which is a known thermally stressful temperature for *N. vectensis* over a span of time. In this instance when northern populations were compared to southern populations only 8 genes were upregulated in the north and 22 in the south (Table 5). Three genes were DE in the northern populations including cytochrome B which functions in mitochondria on oxidative phosphorylation and glutamyl-prolyl tRNA synthetase that works on attaching amino acids to growing polypeptide chains. Interestingly, a gene associated with eukaryotic translation initiation was found upregulated in NS, ME, and MA. A gene with the same related function was found upregulated in southern populations at 20°C which could indicate an increased basal level of gene expression related to increased translation and protein production. The observed genes upregulated in southern locations contained mostly either poorly characterized annotations or genes related to microtubule structure. One gene of note is *TSP1* (thrombospondin type 1 domain) that functions in cell adhesion, motility, and growth. From the gene ontology enrichment results significantly more terms were enriched in South Carolina when compared against any of the three northern populations. A potential conserved thermal response is seen in North Carolina and South Carolina when compared to all northern locations at 30°C with enriched GO terms including 'cellular respiration' (BP), 'generation of precursor metabolites and energy' (BP), 'oxidoreductase' (MF), and several terms related to mitochondrial function. These terms indicate a possible increase in energy production within *N. vectensis* populations from both North and South Carolina. No terms were enriched in Maine when compared against North Carolina making it difficult to generalize this finding between northern and southern populations. This result is consistent with my original hypothesis where I anticipated thermal stress response signature would have been observed in northern populations when exposed to increased temperatures. Southern populations are predicted to be better adept at dealing with thermally stressful conditions, but further work is needed as no terms were enriched between Maine and North Carolina at 30°C. In addition, further identification and characterization is needed across *N. vectensis* transcriptome to characterize "unannotated genes."

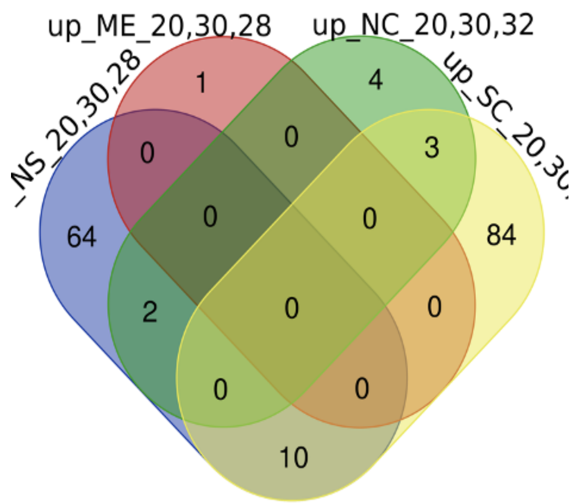
Table 5: List of DEG between Northern (NS, ME, MA) and Southern (NC, SC) Locations at 30°C

Gene	Upregulated	Description
NVE2217	Southern	Thrombospondin type 1 domain
NVE21950	Southern	Inherit from KOG: contactin associated protein-like
NVE23069	Southern	clathrin, light chain
NVE2064	Southern	reticulocalbin 1, EF-hand calcium binding domain
NVE21077	Southern	skeletal aspartic acid-rich protein 2
NVE11274	Southern	Tubulin tyrosine ligase-like family, member
NVE5432	Southern	uncharacterized protein
NVE24882	Southern	LRR domain-containing protein
NVE17049	Southern	Calponin
NVE7822	Southern	muscle lim protein
NVE24906	Southern	Pyridoxal-dependent decarboxylase conserved domain
NVE23695	Southern	uncharacterized protein
NVE2017	Southern	four-disulfide core
NVE26034	Southern	Tropomyosin
NVE7220	Southern	Selenoprotein W, 1
NVE19352	Southern	dysferlin, limb girdle muscular dystrophy 2B
NVE7820	Southern	muscle lim protein
NVE15529	Southern	uncharacterized
NVE4315	Southern	tropomyosin
NVE7494	Southern	myophilin
NVE674	Southern	Uncharacterized
NVE10548	Southern	Uncharacterized
NVE8800	Northern	Cytochrome b
NVE18925	Northern	glutamyl-prolyl-tRNA synthetase
NVE21562	Northern	Uncharacterized
NVE15163	Northern	zinc finger SWIM-type containing 8
NVE4768	Northern	Component of eukaryotic translation initiation factor 3
NVE4686	Northern	Uncharacterized
NVE18393	Northern	Uncharacterized
NVE14660	Northern	Uncharacterized

4.3 Unique Gene Expression across Temperatures

In Nova Scotia and Maine, no genes were upregulated consistently when exposed to higher temperatures indicating a lack of a conserved thermal stress response. I was not able to categorize these findings across the northern populations as Massachusetts was only exposed to 20°C and 30°C. No consistent stress response was seen in North Carolina as well. Only one gene was found upregulated consistently across all temperature comparisons in South Carolina, *TADA2B* (NVE7369) (20°C vs 30°C, 20°C vs. 32°C, and 30°C vs 32°C). This gene encodes for the transcriptional adaptor 2B which functions in promoting transcription through use of histone acetyltransferase activity. Throughout this study, South Carolina has had significantly more DEG across experimental conditions resulting in a high number of uniquely expressed genes. It is possible that an increased number of DEGs found in South Carolina was contributed to by the increased expression of this gene.

Very few genes were found across all locations when 30°C was compared to the location based average high temperature (28°C in NS & ME and 32°C in NC & SC). Because of this, I focused on DEGs upregulated at higher temperatures in each location when 20°C was compared against 30°C and the location-based average high. Consistent with the results, Nova Scotia and South Carolina had the highest number of shared DEGs with 76 and 97 genes, respectively, while Maine and North Carolina both had 9. When these lists of genes were compared 2 genes were shared between North Carolina and Nova Scotia, 10 genes shared between Nova Scotia and South Carolina, and 3 genes shared between North and South Carolina (Figure 39). The two genes upregulated at higher temperatures in Nova Scotia and North Carolina both have putative roles in cell cycle regulation (cyclin dependent kinase 1, cell cycle regulated microtubule associated protein). Increased production of new cells is indicative of a stress response, but it was surprising to find it shared between two locations that are not close in proximity. Out of the three DEGs upregulated in North Carolina and South Carolina was an NADPH-dependent FMN reductase and BIVM (basic, immunoglobulin-like variable motif containing). And finally, the ten genes seen upregulated in both Nova Scotia and South Carolina contained five that were uncharacterized, three related to microtubule structure, *SNX11* (sorting nexin 11), and cyclin I. *SNX11* is involved in protein sorting and membrane trafficking in endosomes while cyclin I functions in cell cycle regulation.



Nova Scotia & North Carolina

1. Cell cycle regulated microtubule associated protein
2. Cyclin dependent kinase 1

North Carolina & South Carolina

1. NADPH-dependent FMN reductase
2. basic, immunoglobulin-like variable motif containing
3. Uncharacterized

Nova Scotia & South Carolina

1. Myophilin
2. Sorting nexin 11
3. Calponin family repeat
4. Cyclin I
5. Tubulin
6. 5 uncharacterized

Figure 39: Venn diagram of overlapping upregulated DEGs between the two most northern locations against the two most southern locations at higher temperatures.

No evidence was found to support the notion that a conserved stress response exists across *N. vectensis* populations along the latitudinal thermal gradient found along the Atlantic coast of North America. The thermal responses observed occurred on a location-by-location basis with only slight similarities seen between higher and lower latitudes. Surprisingly, Nova Scotia showed stress responses similar to North and South Carolina despite it being the furthest away in proximity. This could indicate a possible evolutionary relationship or potential anthropogenic assisted gene flow between the locations.

4.4 Future Directions

For this study, I used the publicly available reference transcriptome for *Nematostella vectensis*. This transcriptome was generated more than ten years ago and lacks annotations for nearly 36% of the genes listed. Because of this I was not able to categorize the extent of the thermal stress response observed within *N. vectensis* populations when exposed to increasing temperatures long-term. In all aspects of my results, I discussed several genes with no known functions making it very difficult to draw any concrete conclusions. This could be the reason why no conserved thermal response was observed. A simpler explanation would be that thermal stress responses are unique across locations. This would indicate that there is limited gene flow between populations requiring each to adapt and acclimate to their own specific environments.

With a better annotated transcriptome, I plan to establish lists of genes that have known functions in stress response (*i.e.* related to higher growth rate, increased cell division, transcription factors, heat shock proteins) and perform a gene set enrichment analysis (GSEA) to determine if groups of genes are enriched in particular locations or temperatures. My aim is to determine if the mechanism of constitutive frontloading plays any role in *N. vectensis* thermal response. Evidence of constitutive frontloading was observed in *Acropora hyacinthus* (Barshis et al. 2013) where genes related to heath shock response, cell death signaling, and immunity were seen upregulated under basal conditions. This is predicted to be the case in organisms who often experience a range of thermal stressors. By having higher expression levels of these genes all the time, the organism's molecular mechanisms are quicker to respond and acclimate to increasingly stressful conditions.

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APPENDIX: SUPPLEMENTAL FILES

Supplemental Table 1: DEGs across all locations at 20°C

Names	Total	Genes
MA_NC20 MA_NS20 MA_SC20 ME_MA20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	3	NVE22573 NVE17707 NVE21340
MA_NC20 MA_NS20 MA_SC20 ME_NC20 ME_NS20 ME_SC20 NS_NC20 SC_NC20	1	NVE18269
MA_NC20 MA_NS20 ME_MA20 ME_NC20 ME_NS20 ME_SC20 SC_NC20 SC_NS20	1	NVE14792
MA_NC20 MA_NS20 MA_SC20 ME_MA20 ME_NC20 ME_SC20 NS_NC20	1	NVE491
MA_NC20 MA_NS20 MA_SC20 ME_MA20 ME_NC20 NS_NC20 SC_NC20	1	NVE8783
MA_NC20 MA_NS20 MA_SC20 ME_NC20 ME_NS20 ME_SC20 SC_NS20	1	NVE272
MA_NC20 MA_NS20 ME_MA20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	1	NVE12655
MA_NC20 MA_SC20 ME_NC20 ME_SC20 NS_NC20 SC_NC20 SC_NS20	1	NVE14660
MA_NS20 MA_SC20 ME_NC20 ME_NS20 ME_SC20 NS_NC20 SC_NC20	1	NVE18268
MA_NS20 ME_MA20 ME_NC20 ME_NS20 ME_SC20 NS_NC20 SC_NS20	1	NVE6609
MA_NC20 MA_NS20 MA_SC20 ME_NC20 ME_NS20 ME_SC20	4	NVE14133 NVE21538 NVE24376 NVE16653
MA_NC20 MA_NS20 ME_MA20 ME_NC20 ME_SC20 SC_NS20	1	NVE11138
MA_NC20 MA_NS20 ME_MA20 ME_SC20 SC_NC20 SC_NS20	1	NVE3331
MA_NC20 MA_NS20 ME_NC20 ME_NS20 SC_NC20 SC_NS20	1	NVE4465
MA_NC20 MA_NS20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	1	NVE4315
MA_NC20 MA_NS20 ME_NC20 NS_NC20 SC_NC20 SC_NS20	1	NVE18617
MA_NC20 MA_SC20 ME_MA20 ME_SC20 NS_NC20 SC_NS20	1	NVE1185

MA_NC20 MA_SC20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	24	NVE21760 NVE20024 NVE23069 NVE3141 NVE17074 NVE19184 NVE20134 NVE6091 NVE20308 NVE25877 NVE11007 NVE18393 NVE4686 NVE12992 NVE23695 NVE20016 NVE24510 NVE14009 NVE11103 NVE819 NVE3396 NVE23049 NVE10140 NVE12877
MA_NC20 MA_SC20 ME_NS20 ME_SC20 NS_NC20 SC_NS20	1	NVE1750
MA_NC20 MA_SC20 ME_SC20 NS_NC20 SC_NC20 SC_NS20	1	NVE25572
MA_NC20 ME_MA20 ME_NC20 ME_NS20 ME_SC20 NS_NC20	2	NVE17831 NVE15160
MA_NC20 ME_MA20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	1	NVE14362
MA_NC20 ME_MA20 ME_NS20 ME_SC20 NS_NC20 SC_NC20	1	NVE6446
MA_NS20 MA_SC20 ME_MA20 ME_NC20 ME_NS20 ME_SC20	1	NVE18600
MA_NS20 MA_SC20 ME_NS20 ME_SC20 NS_NC20 SC_NC20	1	NVE10958
MA_NS20 ME_MA20 ME_NC20 ME_NS20 NS_NC20 SC_NS20	1	NVE4647
MA_NS20 ME_MA20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	7	NVE25579 NVE20768 NVE12231 NVE20452 NVE11504 NVE12991 NVE17828
MA_NS20 ME_NC20 ME_NS20 ME_SC20 NS_NC20 SC_NS20	1	NVE7186
MA_SC20 ME_NC20 ME_NS20 ME_SC20 SC_NC20 SC_NS20	1	NVE14789
MA_SC20 ME_NC20 ME_SC20 NS_NC20 SC_NC20 SC_NS20	1	NVE11006
MA_NC20 MA_NS20 MA_SC20 ME_MA20 ME_NC20	1	NVE10179
MA_NC20 MA_NS20 MA_SC20 ME_NC20 ME_NS20	1	NVE7457
MA_NC20 MA_NS20 MA_SC20 ME_NC20 ME_SC20	1	NVE25333
MA_NC20 MA_NS20 ME_MA20 ME_NC20 SC_NC20	1	NVE1302
MA_NC20 MA_NS20 ME_NC20 ME_NS20 ME_SC20	3	NVE7823 NVE4464 NVE20421
MA_NC20 MA_NS20 ME_NC20 ME_NS20 SC_NS20	1	NVE22589
MA_NC20 MA_SC20 ME_MA20 NS_NC20 SC_NS20	1	NVE1602
MA_NC20 MA_SC20 ME_NC20 ME_NS20 ME_SC20	4	NVE15135 NVE15136 NVE17110 NVE589
MA_NC20 MA_SC20 ME_NC20 ME_SC20 NS_NC20	3	NVE11274 NVE15529 NVE9714

MA_NC20 MA_SC20 ME_NC20 ME_SC20 SC_NS20	5	NVE13761 NVE25341 NVE4768 NVE3540 NVE4720
MA_NC20 MA_SC20 ME_SC20 SC_NC20 SC_NS20	1	NVE12092
MA_NC20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	9	NVE19183 NVE12857 NVE9155 NVE7522 NVE17049 NVE2814 NVE25933 NVE7820 NVE5575
MA_NC20 ME_NC20 NS_NC20 SC_NC20 SC_NS20	1	NVE7822
MA_NS20 MA_SC20 ME_MA20 ME_NC20 NS_NC20	1	NVE13842
MA_NS20 ME_MA20 ME_NC20 ME_NS20 ME_SC20	1	NVE16065
MA_NS20 ME_MA20 ME_NC20 ME_SC20 SC_NS20	1	NVE19463
MA_NS20 ME_NC20 ME_NS20 NS_NC20 SC_NS20	2	NVE20160 NVE1757
MA_NS20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	2	NVE14552 NVE16356
MA_SC20 ME_NC20 ME_NS20 ME_SC20 SC_NC20	1	NVE23947
MA_SC20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	1	NVE164
MA_SC20 ME_NC20 ME_SC20 SC_NC20 SC_NS20	1	NVE12007
MA_SC20 ME_NS20 ME_SC20 SC_NC20 SC_NS20	2	NVE7408 NVE538
ME_MA20 ME_NC20 ME_NS20 ME_SC20 NS_NC20	1	NVE17336
ME_MA20 ME_NC20 ME_NS20 SC_NC20 SC_NS20	1	NVE17843
ME_MA20 ME_NC20 ME_SC20 NS_NC20 SC_NS20	1	NVE13757
MA_NC20 MA_NS20 MA_SC20 ME_MA20	5	NVE24378 NVE14622 NVE8461 NVE23717 NVE8782
MA_NC20 MA_NS20 MA_SC20 ME_NC20	1	NVE10227
MA_NC20 MA_NS20 MA_SC20 ME_NS20	1	NVE25234
MA_NC20 MA_NS20 MA_SC20 ME_SC20	1	NVE16978
MA_NC20 MA_NS20 ME_NC20 ME_NS20	1	NVE2668
MA_NC20 MA_NS20 ME_NS20 SC_NS20	1	NVE16674
MA_NC20 MA_SC20 ME_MA20 NS_NC20	1	NVE23461
MA_NC20 MA_SC20 ME_NC20 ME_SC20	7	NVE6010 NVE21815 NVE24407 NVE15726 NVE3726 NVE6269 NVE15236
MA_NC20 MA_SC20 ME_NC20 NS_NC20	2	NVE10181 NVE7220
MA_NC20 MA_SC20 NS_NC20 SC_NS20	1	NVE18144

MA_NC20 ME_NC20 ME_NS20 ME_SC20	1	NVE23810
MA_NC20 ME_NC20 ME_SC20 NS_NC20	2	NVE3113 NVE12983
MA_NC20 ME_NC20 NS_NC20 SC_NC20	11	NVE7851 NVE24906 NVE953 NVE21289 NVE6194 NVE181 NVE23724 NVE24000 NVE23460 NVE11740 NVE6850
MA_NS20 MA_SC20 ME_NS20 ME_SC20	1	NVE5879
MA_NS20 ME_MA20 ME_NC20 NS_NC20	1	NVE12867
MA_NS20 ME_MA20 ME_SC20 SC_NS20	2	NVE1560 NVE2618
MA_NS20 ME_NC20 ME_NS20 ME_SC20	1	NVE1021
MA_NS20 ME_NC20 NS_NC20 SC_NS20	3	NVE8689 NVE11431 NVE5054
MA_NS20 ME_NS20 NS_NC20 SC_NS20	9	NVE3474 NVE14814 NVE7593 NVE593 NVE23057 NVE14791 NVE14458 NVE13161 NVE20507
MA_NS20 ME_NS20 SC_NC20 SC_NS20	1	NVE7414
MA_NS20 ME_SC20 NS_NC20 SC_NS20	1	NVE26007
MA_SC20 ME_NC20 ME_NS20 ME_SC20	1	NVE16547
MA_SC20 ME_NC20 ME_SC20 SC_NS20	2	NVE1901 NVE10021
MA_SC20 ME_NS20 SC_NC20 SC_NS20	1	NVE17765
MA_SC20 ME_SC20 NS_NC20 SC_NC20	1	NVE4727
MA_SC20 ME_SC20 SC_NC20 SC_NS20	7	NVE7022 NVE10854 NVE17046 NVE23326 NVE4770 NVE7149 NVE891
ME_MA20 ME_NC20 ME_NS20 ME_SC20	18	NVE10584 NVE25866 NVE7526 NVE9598 NVE21670 NVE11140 NVE6785 NVE4603 NVE11693 NVE17845 NVE4604 NVE17335 NVE17830 NVE23781 NVE1244 NVE5522 NVE15132 NVE1825
ME_MA20 ME_NC20 ME_SC20 NS_NC20	3	NVE23474 NVE17823 NVE23473
ME_MA20 ME_NC20 ME_SC20 SC_NS20	2	NVE10278 NVE15943
ME_MA20 ME_NS20 ME_SC20 NS_NC20	1	NVE2767
ME_MA20 ME_SC20 SC_NC20 SC_NS20	1	NVE15760
ME_NC20 ME_SC20 NS_NC20 SC_NS20	9	NVE15137 NVE7520 NVE925 NVE11208 NVE25676 NVE2428 NVE6402 NVE17829 NVE6666
MA_NC20 MA_NS20 ME_MA20	2	NVE13779 NVE20744
MA_NC20 MA_SC20 ME_MA20	3	NVE22370 NVE18523 NVE14556
MA_NC20 MA_SC20 ME_NC20	1	NVE15163
MA_NC20 MA_SC20 SC_NS20	2	NVE16798 NVE2197
MA_NC20 ME_NC20 ME_NS20	1	NVE20814
MA_NC20 ME_NC20 ME_SC20	6	NVE14006 NVE10633 NVE13045 NVE2670 NVE17825 NVE16906
MA_NC20 ME_NC20 NS_NC20	12	NVE5832 NVE20077 NVE21105 NVE14719 NVE9541 NVE21627 NVE15238 NVE10757 NVE21107 NVE17222 NVE24499 NVE19791
MA_NC20 ME_NC20 SC_NC20	2	NVE305 NVE5412
MA_NS20 MA_SC20 ME_MA20	3	NVE25600 NVE4847 NVE18608
MA_NS20 MA_SC20 ME_SC20	1	NVE295
MA_NS20 MA_SC20 NS_NC20	1	NVE10067

MA_NS20 ME_NC20 ME_NS20	1	NVE14796
MA_NS20 ME_NS20 ME_SC20	1	NVE14423
MA_NS20 ME_NS20 NS_NC20	2	NVE309 NVE22528
MA_NS20 ME_NS20 SC_NS20	4	NVE21119 NVE24174 NVE17842 NVE9916
MA_NS20 ME_SC20 SC_NS20	1	NVE25706
MA_NS20 NS_NC20 SC_NC20	1	NVE7359
MA_NS20 NS_NC20 SC_NS20	5	NVE11897 NVE4232 NVE13041 NVE9889 NVE25659
MA_SC20 ME_NC20 ME_SC20	4	NVE25162 NVE4769 NVE2383 NVE9629
MA_SC20 ME_NC20 SC_NC20	1	NVE18925
MA_SC20 ME_SC20 SC_NC20	2	NVE22208 NVE13579
MA_SC20 ME_SC20 SC_NS20	12	NVE21562 NVE1541 NVE12780 NVE1688 NVE13487 NVE21563 NVE19871 NVE3142 NVE8917 NVE19403 NVE17170 NVE8125
MA_SC20 NS_NC20 SC_NS20	1	NVE21187
ME_MA20 ME_NC20 ME_NS20	2	NVE22146 NVE11593
ME_MA20 ME_NC20 ME_SC20	9	NVE18805 NVE1869 NVE9847 NVE5877 NVE18491 NVE5622 NVE11944 NVE17827 NVE18650
ME_NC20 ME_NS20 ME_SC20	20	NVE25226 NVE22158 NVE1903 NVE21802 NVE418 NVE1276 NVE8749 NVE21084 NVE5857 NVE13758 NVE9622 NVE25662 NVE25406 NVE13106 NVE4927 NVE2183 NVE11497 NVE12279 NVE21570 NVE9125
ME_NC20 ME_SC20 NS_NC20	4	NVE8075 NVE20303 NVE23472 NVE150
ME_NC20 ME_SC20 SC_NS20	2	NVE8332 NVE9056
ME_NC20 NS_NC20 SC_NC20	4	NVE23993 NVE6148 NVE22624 NVE7744
ME_NC20 NS_NC20 SC_NS20	8	NVE7184 NVE26034 NVE20941 NVE478 NVE1808 NVE3912 NVE20895 NVE5432
ME_NS20 ME_SC20 NS_NC20	2	NVE14177 NVE6295
ME_NS20 NS_NC20 SC_NS20	3	NVE17043 NVE23587 NVE1932
ME_SC20 NS_NC20 SC_NS20	2	NVE6997 NVE8124
ME_SC20 SC_NC20 SC_NS20	3	NVE22599 NVE22558 NVE9780
MA_NC20 MA_NS20	1	NVE17685
MA_NC20 MA_SC20	7	NVE401 NVE24882 NVE7833 NVE7760 NVE10393 NVE11912 NVE14663
MA_NC20 ME_MA20	2	NVE12250 NVE9257
MA_NC20 ME_NC20	18	NVE4340 NVE11837 NVE21782 NVE19993 NVE11519 NVE10372 NVE10147 NVE12218 NVE22194 NVE22092 NVE2017 NVE21943 NVE14253 NVE24758 NVE1100 NVE4741 NVE19282 NVE10438
MA_NC20 NS_NC20	1	NVE20913
MA_NC20 SC_NC20	2	NVE22805 NVE1868
MA_NS20 MA_SC20	2	NVE25334 NVE18777
MA_NS20 ME_MA20	4	NVE11319 NVE12211 NVE25651 NVE12463
MA_NS20 ME_NS20	3	NVE25059 NVE16027 NVE5855
MA_NS20 NS_NC20	7	NVE13176 NVE24889 NVE24747 NVE14528 NVE2938 NVE2542 NVE13778
MA_NS20 SC_NS20	9	NVE2876 NVE10051 NVE13257 NVE16052 NVE3432 NVE22257 NVE19039 NVE21711 NVE5239
MA_SC20 ME_MA20	3	NVE4448 NVE25599 NVE21887
MA_SC20 ME_SC20	14	NVE7854 NVE13823 NVE26011 NVE1815 NVE18385 NVE21421 NVE5391 NVE20703 NVE9601 NVE17679 NVE25777 NVE11584 NVE7733 NVE19229
MA_SC20 SC_NC20	2	NVE25571 NVE17751
MA_SC20 SC_NS20	5	NVE17316 NVE15563 NVE10180 NVE12994 NVE14392
ME_MA20 ME_NC20	8	NVE19657 NVE6316 NVE13396 NVE2702 NVE8020 NVE3472 NVE2016 NVE23113

ME_MA20 ME_NS20	4	NVE15403 NVE25060 NVE17844 NVE14983
ME_MA20 ME_SC20	4	NVE8197 NVE11126 NVE13149 NVE10550
ME_NC20 ME_NS20	10	NVE6671 NVE2190 NVE9103 NVE16289 NVE1179 NVE14847 NVE18688 NVE13841 NVE19696 NVE6964
ME_NC20 ME_SC20	36	NVE6694 NVE14201 NVE16190 NVE7411 NVE23274 NVE22591 NVE11282 NVE358 NVE5875 NVE417 NVE10523 NVE7821 NVE16306 NVE5978 NVE17570 NVE5142 NVE6588 NVE19352 NVE13592 NVE15350 NVE21527 NVE23392 NVE10836 NVE17246 NVE6502 NVE20412 NVE2217 NVE24408 NVE12754 NVE23457 NVE20235 NVE17166 NVE4159 NVE22269 NVE12631 NVE21393
ME_NC20 NS_NC20	26	NVE14934 NVE7494 NVE23439 NVE16002 NVE20536 NVE381 NVE14289 NVE17154 NVE13315 NVE12768 NVE18456 NVE24165 NVE22954 NVE24872 NVE20881 NVE24899 NVE4324 NVE4987 NVE12912 NVE10940 NVE12215 NVE8330 NVE7138 NVE6998 NVE4170 NVE10753
ME_NC20 SC_NC20	1	NVE22285
ME_NS20 ME_SC20	6	NVE6821 NVE13434 NVE25820 NVE5154 NVE12366 NVE23953
ME_NS20 NS_NC20	4	NVE4745 NVE17724 NVE19300 NVE16580
ME_NS20 SC_NS20	2	NVE3845 NVE7487
ME_SC20 SC_NC20	9	NVE1122 NVE6366 NVE2482 NVE23344 NVE19285 NVE4662 NVE21209 NVE7291 NVE25224
ME_SC20 SC_NS20	15	NVE10760 NVE1046 NVE3816 NVE193 NVE11971 NVE13857 NVE4492 NVE3705 NVE6636 NVE10879 NVE2930 NVE14725 NVE2310 NVE1614 NVE47
NS_NC20 SC_NS20	15	NVE10977 NVE1725 NVE1625 NVE24288 NVE10224 NVE4901 NVE5521 NVE15740 NVE10262 NVE10763 NVE14880 NVE23813 NVE13490 NVE20880 NVE21846
SC_NC20 SC_NS20	2	NVE12939 NVE7446
MA_NC20	23	NVE6181 NVE19289 NVE2455 NVE20704 NVE12386 NVE14168 NVE13799 NVE18975 NVE4825 NVE5400 NVE3286 NVE1143 NVE1502 NVE16246 NVE842 NVE10817 NVE25207 NVE20094 NVE26192 NVE24191 NVE19829 NVE23561 NVE12138
MA_NS20	16	NVE13162 NVE6401 NVE15894 NVE14670 NVE4672 NVE23277 NVE12374 NVE1253 NVE6753 NVE12899 NVE12148 NVE1246 NVE24199 NVE14569 NVE910 NVE9794
MA_SC20	22	NVE15383 NVE25578 NVE25782 NVE9170 NVE11435 NVE5570 NVE19335 NVE16219 NVE23560 NVE22820 NVE25792 NVE14332 NVE17173 NVE18536 NVE7136 NVE16565 NVE15806 NVE5753 NVE19085 NVE25581 NVE23087 NVE19109
ME_MA20	17	NVE17615 NVE10575 NVE10586 NVE13575 NVE13026 NVE25687 NVE23425 NVE6614 NVE20648 NVE18546 NVE3126 NVE18649 NVE13626 NVE17082 NVE21624 NVE1567 NVE25672
ME_NC20	86	NVE24810 NVE10573 NVE20133 NVE7392 NVE11974 NVE5375 NVE22060 NVE332 NVE23040 NVE796 NVE2508 NVE17634 NVE10837 NVE14254 NVE13226 NVE10444 NVE15830 NVE17848 NVE17090 NVE7211 NVE17329 NVE20338 NVE6160 NVE13968 NVE6376 NVE13565 NVE24732 NVE4823 NVE22359 NVE4760 NVE4653 NVE8884 NVE13456 NVE42 NVE8190 NVE17343 NVE12840 NVE16084 NVE24081 NVE10571 NVE25265 NVE22483 NVE13204 NVE6697 NVE24066 NVE25082 NVE17500 NVE4323 NVE13065 NVE1102 NVE17129 NVE16296 NVE1389 NVE2015 NVE18863 NVE2216 NVE15510 NVE17896 NVE26042 NVE16807 NVE25965 NVE5282 NVE3795 NVE13564 NVE24900 NVE22614 NVE25725 NVE18224 NVE15707 NVE11689 NVE16872 NVE2951 NVE21665 NVE12106 NVE1561 NVE5347 NVE19863 NVE19792 NVE444 NVE25762 NVE4716 NVE4826 NVE6020 NVE16938 NVE3475 NVE16461
ME_NS20	12	NVE9447 NVE26003 NVE25365 NVE3959 NVE3211 NVE16116 NVE18973 NVE15608 NVE13449 NVE18382 NVE14456 NVE7504
ME_SC20	67	NVE542 NVE10419 NVE26015 NVE2088 NVE10359 NVE15272 NVE13843 NVE11413 NVE21167 NVE12620 NVE25966 NVE19272 NVE18510 NVE18038 NVE17839 NVE23609 NVE10791 NVE9925 NVE17138 NVE1971 NVE21600 NVE9203 NVE3206 NVE9318 NVE25729 NVE22893 NVE5410 NVE13150 NVE13346 NVE12352 NVE13703 NVE15970 NVE22506 NVE22604 NVE6803 NVE10594 NVE3616 NVE16671 NVE7775 NVE18002 NVE22302 NVE16405 NVE5876 NVE6973 NVE8184 NVE4349 NVE12843 NVE13131 NVE15803 NVE7113 NVE19134 NVE16715 NVE9193 NVE9234 NVE22073 NVE11272 NVE13571 NVE4966 NVE20605 NVE20630 NVE15134 NVE24551 NVE4052 NVE11482 NVE4810 NVE23147 NVE8547
NS_NC20	31	NVE5695 NVE5382 NVE7892 NVE17083 NVE13457 NVE16005 NVE1350 NVE857 NVE4759 NVE14802 NVE4380 NVE24988 NVE23818 NVE5662 NVE5331 NVE2733 NVE4510 NVE24705 NVE1280 NVE15833 NVE14258 NVE22804 NVE20959 NVE18976 NVE13292 NVE3978 NVE18145 NVE22299 NVE6085 NVE18293 NVE18453
SC_NC20	1	NVE19208
SC_NS20	44	NVE1748 NVE14217 NVE14406 NVE18926 NVE16731 NVE12183 NVE9208 NVE21065 NVE21950 NVE25650 NVE8462 NVE14564 NVE6777 NVE17104 NVE25470 NVE21696 NVE14124 NVE21566 NVE2223 NVE15150 NVE18958 NVE4248 NVE25393 NVE5785 NVE13233 NVE6336 NVE12085 NVE12427 NVE4249 NVE16024 NVE15518 NVE24164 NVE22590 NVE12091 NVE25062 NVE1532 NVE19258 NVE4602 NVE4905 NVE21064 NVE22906 NVE7131 NVE1957 NVE8861

Supplemental Table 2: DEGs across all locations at 30°C		
Names	**Total**	**Genes**
MAvNC30 MAvSC30 MAvsNS30 MEvNC30 MEvNS30 MEvSC30 NSvNC30 SCvNS30	2	NVE24510 NVE1757
MAvNC30 MAvSC30 MEvMA30 MEvNC30 MEvNS30 MEvSC30 NSvNC30 SCvNS30	3	NVE17831 NVE15134 NVE589
MAvNC30 MAvSC30 MEvMA30 MEvNS30 MEvSC30 NSvNC30 SCvNC30 SCvNS30	1	NVE25572
MAvSC30 MAvsNS30 MEvMA30 MEvNC30 MEvNS30 MEvSC30 SCvNC30 SCvNS30	1	NVE12007
MAvNC30 MAvSC30 MAvsNS30 MEvMA30 MEvNC30 NSvNC30 SCvNS30	1	NVE1252
MAvNC30 MAvSC30 MAvsNS30 MEvMA30 MEvSC30 SCvNC30 SCvNS30	1	NVE8461
MAvNC30 MAvSC30 MAvsNS30 MEvNC30 MEvNS30 MEvSC30 NSvNC30	1	NVE18269
MAvNC30 MAvSC30 MAvsNS30 MEvNC30 MEvNS30 MEvSC30 SCvNS30	1	NVE272
MAvNC30 MAvSC30 MAvsNS30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	2	NVE8330 NVE14934
MAvNC30 MAvsNS30 MEvMA30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	3	NVE17707 NVE17828 NVE22573
MAvNC30 MAvSC30 MEvMA30 MEvNC30 MEvNS30 MEvSC30 NSvNC30	1	NVE15135
MAvNC30 MAvSC30 MEvNC30 MEvSC30 NSvNC30 SCvNC30 SCvNS30	1	NVE11007
MAvsNS30 MEvMA30 MEvNC30 MEvNS30 MEvSC30 NSvNC30 SCvNS30	3	NVE1021 NVE6609 NVE21340
MAvNC30 MAvSC30 MAvsNS30 MEvMA30 MEvNS30 MEvSC30	1	NVE25581
MAvNC30 MAvSC30 MAvsNS30 MEvMA30 NSvNC30 SCvNS30	1	NVE491
MAvNC30 MAvSC30 MAvsNS30 MEvNC30 MEvNS30 MEvSC30	5	NVE18268 NVE7457 NVE10958 NVE910 NVE22589
MAvNC30 MAvSC30 MAvsNS30 MEvSC30 NSvNC30 SCvNS30	1	NVE1750
MAvNC30 MAvsNS30 MEvMA30 MEvSC30 SCvNC30 SCvNS30	1	NVE1868
MAvNC30 MAvsNS30 MEvNC30 MEvNS30 SCvNC30 SCvNS30	5	NVE7526 NVE4464 NVE16216 NVE14796 NVE4465

MAvNC30 MAvSC30 MEvMA30 MEvSC30 NSvNC30 SCvNS30	1	NVE1185
MAvNC30 MAvSC30 MEvNC30 MEvSC30 NSvNC30 SCvNC30	1	NVE6194
MAvNC30 MAvSC30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	30	NVE7494 NVE21562 NVE23069 NVE2064 NVE21950 NVE7822 NVE18925 NVE18393 NVE15163 NVE4686 NVE2017 NVE23695 NVE674 NVE15529 NVE10548 NVE2217 NVE7820 NVE26034 NVE4768 NVE14660 NVE24882 NVE24906 NVE21077 NVE19352 NVE17049 NVE11274 NVE5432 NVE8800 NVE7220 NVE4315
MAvNC30 MAvSC30 MEvNC30 MEvSC30 SCvNC30 SCvNS30	1	NVE12092
MAvNC30 MEvMA30 MEvNS30 MEvSC30 NSvNC30 SCvNC30	1	NVE6446
MAvSC30 MAvsNS30 MEvMA30 MEvNC30 MEvNS30 MEvSC30	1	NVE7823
MAvSC30 MAvsNS30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	2	NVE478 NVE16796
MAvSC30 MAvsNS30 MEvNS30 MEvSC30 NSvNC30 SCvNC30	1	NVE5239
MAvSC30 MAvsNS30 MEvNS30 MEvSC30 NSvNC30 SCvNS30	1	NVE7186
MAvSC30 MAvsNS30 MEvNS30 MEvSC30 SCvNC30 SCvNS30	1	NVE21084
MAvSC30 MAvsNS30 MEvSC30 NSvNC30 SCvNC30 SCvNS30	1	NVE7149
MAvsNS30 MEvMA30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	2	NVE17001 NVE9155
MAvSC30 MEvNC30 MEvSC30 NSvNC30 SCvNC30 SCvNS30	1	NVE7520
MAvNC30 MAvSC30 MAvsNS30 MEvMA30 NSvNC30	1	NVE10179
MAvNC30 MAvSC30 MAvsNS30 MEvMA30 SCvNS30	1	NVE14622
MAvNC30 MAvSC30 MAvsNS30 MEvNC30 MEvNS30	1	NVE7620
MAvNC30 MAvSC30 MAvsNS30 MEvNS30 MEvSC30	1	NVE21133
MAvNC30 MAvSC30 MAvsNS30 MEvNS30 SCvNS30	1	NVE16548
MAvNC30 MAvsNS30 MEvMA30 MEvSC30 SCvNS30	1	NVE1253
MAvNC30 MAvsNS30 MEvMA30 SCvNC30 SCvNS30	1	NVE17896
MAvNC30 MAvsNS30 MEvSC30 SCvNC30 SCvNS30	1	NVE4653
MAvNC30 MAvSC30 MEvMA30 MEvSC30 SCvNS30	1	NVE323

MAvNC30 MAvSC30 MEvMA30 NSvNC30 SCvNS30	2	NVE16681 NVE10140
MAvNC30 MAvSC30 MEvNC30 MEvSC30 NSvNC30	2	NVE19993 NVE15136
MAvNC30 MAvSC30 MEvNC30 MEvSC30 SCvNS30	1	NVE16547
MAvNC30 MAvSC30 MEvNC30 NSvNC30 SCvNC30	1	NVE16286
MAvNC30 MAvSC30 MEvSC30 NSvNC30 SCvNS30	7	NVE5716 NVE19019 NVE18144 NVE5400 NVE4324 NVE14462 NVE8417
MAvNC30 MEvNC30 MEvNS30 NSvNC30 SCvNC30	1	NVE23460
MAvNC30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	1	NVE8332
MAvNC30 MEvNC30 NSvNC30 SCvNC30 SCvNS30	1	NVE21760
MAvSC30 MAvsNS30 MEvMA30 NSvNC30 SCvNC30	1	NVE4746
MAvSC30 MAvsNS30 MEvNS30 NSvNC30 SCvNS30	2	NVE23786 NVE14814
MAvSC30 MAvsNS30 MEvSC30 NSvNC30 SCvNC30	1	NVE10067
MAvSC30 MAvsNS30 MEvSC30 NSvNC30 SCvNS30	1	NVE23818
MAvSC30 MAvsNS30 MEvSC30 SCvNC30 SCvNS30	1	NVE17046
MAvsNS30 MEvMA30 MEvNC30 MEvSC30 SCvNS30	1	NVE11584
MAvsNS30 MEvMA30 MEvSC30 NSvNC30 SCvNS30	1	NVE12211
MAvsNS30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	2	NVE7522 NVE2814
MAvSC30 MEvMA30 MEvNC30 MEvNS30 MEvSC30	1	NVE18600
MAvSC30 MEvMA30 MEvSC30 SCvNC30 SCvNS30	1	NVE8075
MAvSC30 MEvNC30 MEvSC30 NSvNC30 SCvNC30	1	NVE16807
MAvSC30 MEvNC30 MEvSC30 NSvNC30 SCvNS30	4	NVE24937 NVE3427 NVE15137 NVE17827
MAvSC30 MEvSC30 NSvNC30 SCvNC30 SCvNS30	2	NVE19791 NVE16356
MEvMA30 MEvNC30 MEvNS30 MEvSC30 SCvNS30	1	NVE2633
MAvNC30 MAvSC30 MAvsNS30 MEvMA30	10	NVE25599 NVE8782 NVE13778 NVE8783 NVE17775 NVE4779 NVE1302 NVE13626 NVE17082 NVE24191
MAvNC30 MAvSC30 MAvsNS30 MEvNC30	5	NVE21538 NVE24758 NVE15236 NVE14423 NVE2452
MAvNC30 MAvSC30 MAvsNS30 MEvSC30	1	NVE1815
MAvNC30 MAvsNS30 SCvNC30 SCvNS30	2	NVE20133 NVE20814
MAvNC30 MAvSC30 MEvMA30 MEvNS30	1	NVE23725
MAvNC30 MAvSC30 MEvMA30 MEvSC30	1	NVE6912

MAvNC30 MAvSC30 MEvNC30 MEvSC30	3	NVE8579 NVE24408 NVE25333
MAvNC30 MAvSC30 MEvNC30 NSvNC30	1	NVE2197
MAvNC30 MAvSC30 MEvSC30 SCvNS30	2	NVE15469 NVE2216
MAvNC30 MAvSC30 NSvNC30 SCvNS30	8	NVE22370 NVE18971 NVE15615 NVE17861 NVE24544 NVE14234 NVE4323 NVE21549
MAvNC30 MEvNC30 MEvSC30 NSvNC30	4	NVE17823 NVE15160 NVE17222 NVE16906
MAvNC30 MEvNC30 NSvNC30 SCvNC30	8	NVE23810 NVE18733 NVE10181 NVE19975 NVE6850 NVE6148 NVE18617 NVE181
MAvNC30 MEvSC30 NSvNC30 SCvNC30	1	NVE24070
MAvNC30 MEvSC30 SCvNC30 SCvNS30	1	NVE3785
MAvSC30 MAvsNS30 MEvMA30 SCvNC30	1	NVE4847
MAvSC30 MAvsNS30 MEvNS30 MEvSC30	2	NVE3980 NVE18382
MAvSC30 MAvsNS30 MEvSC30 SCvNC30	1	NVE20913
MAvsNS30 MEvMA30 MEvNC30 NSvNC30	1	NVE7852
MAvsNS30 MEvMA30 NSvNC30 SCvNS30	1	NVE25138
MAvsNS30 MEvNC30 NSvNC30 SCvNS30	2	NVE12231 NVE21846
MAvsNS30 MEvNS30 NSvNC30 SCvNS30	15	NVE4745 NVE7593 NVE10952 NVE17765 NVE7359 NVE14528 NVE20880 NVE7289 NVE2998 NVE3432 NVE23587 NVE5879 NVE24174 NVE14458 NVE14725
MAvsNS30 MEvSC30 NSvNC30 SCvNS30	2	NVE21242 NVE4647
MAvsNS30 MEvSC30 SCvNC30 SCvNS30	1	NVE758
MAvSC30 MEvMA30 MEvNS30 SCvNS30	2	NVE14556 NVE24607
MAvSC30 MEvMA30 SCvNC30 SCvNS30	1	NVE9622
MAvSC30 MEvNC30 MEvSC30 SCvNS30	7	NVE6269 NVE9714 NVE6666 NVE3816 NVE10147 NVE17829 NVE4159
MAvSC30 MEvNC30 NSvNC30 SCvNC30	1	NVE25651
MAvSC30 MEvNC30 SCvNC30 SCvNS30	2	NVE21665 NVE25452
MAvSC30 MEvNS30 NSvNC30 SCvNS30	1	NVE21187
MAvSC30 MEvSC30 NSvNC30 SCvNS30	16	NVE20941 NVE12771 NVE24899 NVE15532 NVE18976 NVE24161 NVE19183 NVE21105 NVE21563 NVE11431 NVE5598 NVE14362 NVE842 NVE4987 NVE14253 NVE3101
MAvSC30 MEvSC30 SCvNC30 SCvNS30	46	NVE26015 NVE7851 NVE796 NVE16799 NVE20160 NVE23458 NVE19184 NVE16635 NVE22208 NVE6408 NVE20525 NVE24637 NVE10879 NVE24705 NVE9104 NVE4703 NVE18989 NVE23457 NVE7446 NVE19403 NVE17170 NVE444 NVE5329 NVE21570 NVE10760 NVE6366 NVE11006 NVE25536 NVE17084 NVE23425 NVE14874 NVE18931 NVE19630 NVE5410 NVE4476 NVE18920 NVE4658 NVE24081 NVE3912 NVE17751 NVE7408 NVE7733 NVE2822 NVE2600 NVE24162 NVE18453
MEvMA30 MEvNC30 MEvNS30 MEvSC30	6	NVE1122 NVE14006 NVE7369 NVE14847 NVE18782 NVE5522
MEvMA30 MEvNC30 MEvSC30 SCvNS30	2	NVE20452 NVE342
MEvMA30 MEvNC30 NSvNC30 SCvNC30	1	NVE1246
MEvNC30 MEvSC30 NSvNC30 SCvNS30	3	NVE20768 NVE17246 NVE4232
MEvNS30 MEvSC30 NSvNC30 SCvNC30	2	NVE4727 NVE14177

MAvNC30 MAvSC30 MAvsNS30	10	NVE16219 NVE7760 NVE12374 NVE23717 NVE12138 NVE25580 NVE11837 NVE23680 NVE25777 NVE17825
MAvNC30 MAvsNS30 SCvNS30	3	NVE9201 NVE11519 NVE18002
MAvNC30 MAvSC30 MEvMA30	2	NVE6106 NVE12767
MAvNC30 MAvSC30 MEvNC30	1	NVE8689
MAvNC30 MAvSC30 MEvSC30	2	NVE21477 NVE26011
MAvNC30 MAvSC30 NSvNC30	3	NVE24064 NVE2343 NVE18145
MAvNC30 MAvSC30 SCvNS30	4	NVE18805 NVE4433 NVE2856 NVE24065
MAvNC30 MEvMA30 MEvSC30	1	NVE21670
MAvNC30 MEvNC30 MEvSC30	1	NVE24866
MAvNC30 MEvNC30 NSvNC30	3	NVE13564 NVE7184 NVE21627
MAvNC30 MEvNC30 SCvNC30	2	NVE24376 NVE8917
MAvNC30 NSvNC30 SCvNC30	1	NVE10069
MAvNC30 NSvNC30 SCvNS30	4	NVE14450 NVE8270 NVE22171 NVE3220
MAvNC30 SCvNC30 SCvNS30	2	NVE6685 NVE1179
MAvSC30 MAvsNS30 MEvMA30	7	NVE25883 NVE10550 NVE11138 NVE19279 NVE9257 NVE18608 NVE25672
MAvSC30 MAvsNS30 MEvSC30	1	NVE25059
MAvsNS30 MEvMA30 SCvNS30	1	NVE3331
MAvsNS30 MEvNS30 NSvNC30	1	NVE20507
MAvsNS30 MEvNS30 SCvNS30	3	NVE13041 NVE9592 NVE18711
MAvsNS30 MEvSC30 SCvNS30	2	NVE1980 NVE3672
MAvsNS30 NSvNC30 SCvNS30	9	NVE13106 NVE23700 NVE18284 NVE17842 NVE3474 NVE17721 NVE17845 NVE4901 NVE16674
MAvsNS30 SCvNC30 SCvNS30	1	NVE7910
MAvSC30 MEvMA30 MEvNC30	1	NVE17335
MAvSC30 MEvNC30 MEvSC30	1	NVE7984
MAvSC30 MEvNS30 MEvSC30	1	NVE19696
MAvSC30 MEvNS30 SCvNS30	2	NVE23726 NVE9156
MAvSC30 MEvSC30 SCvNC30	2	NVE9447 NVE19366
MAvSC30 MEvSC30 SCvNS30	155	NVE23240 NVE13843 NVE24097 NVE19503 NVE13557 NVE1541 NVE1725 NVE16707 NVE15287 NVE15247 NVE14200 NVE16731 NVE12442 NVE20609 NVE21815 NVE9354 NVE11271 NVE17328 NVE2492 NVE17630 NVE17430 NVE24509 NVE3406 NVE19871 NVE12510 NVE17074 NVE7288 NVE12918 NVE20512 NVE5570 NVE18909 NVE19215 NVE22493 NVE14008 NVE21834 NVE15238 NVE22737 NVE4213 NVE14093 NVE273 NVE8626 NVE25802 NVE19957 NVE14186 NVE24795 NVE19755 NVE5521 NVE9824 NVE4314 NVE19775 NVE12643 NVE984 NVE20729 NVE3929 NVE18331 NVE10075 NVE12427 NVE15776 NVE23801 NVE10393 NVE10878 NVE1826 NVE22656 NVE19984 NVE12039 NVE24074 NVE24825 NVE20233 NVE10888 NVE16433 NVE12983 NVE23049 NVE3819 NVE15539 NVE13415 NVE6579 NVE13955 NVE8083 NVE16646 NVE4052 NVE25540 NVE11124 NVE8125 NVE1674 NVE25579 NVE536 NVE48 NVE76 NVE18163 NVE23807 NVE1901 NVE9596 NVE857 NVE14667 NVE2455 NVE24828 NVE16644 NVE14254 NVE4004

		NVE14800 NVE7850 NVE12857 NVE19102 NVE9650 NVE16965 NVE18385 NVE22658 NVE16413 NVE1236 NVE16136 NVE8462 NVE17329 NVE20867 NVE23694 NVE5399 NVE3286 NVE10911 NVE25569 NVE477 NVE4141 NVE14552 NVE15860 NVE14940 NVE8034 NVE16592 NVE15259 NVE11125 NVE21943 NVE12337 NVE16678 NVE3404 NVE7481 NVE23792 NVE13583 NVE10325 NVE9423 NVE2996 NVE24945 NVE672 NVE14231 NVE25813 NVE6956 NVE13203 NVE11272 NVE13571 NVE24347 NVE15088 NVE818 NVE12556 NVE7523 NVE8113 NVE1157 NVE14563 NVE23664 NVE14789
MAvSC30 NSvNC30 SCvNS30	7	NVE14982 NVE14216 NVE15563 NVE14201 NVE10071 NVE3540 NVE2938
MAvSC30 SCvNC30 SCvNS30	16	NVE2692 NVE3113 NVE14426 NVE13758 NVE21517 NVE1795 NVE11497 NVE1903 NVE11388 NVE21802 NVE8271 NVE12121 NVE1505 NVE19863 NVE10786 NVE16112
MEvNC30 MEvNS30 MEvSC30	1	NVE1560
MEvNC30 MEvNS30 SCvNS30	1	NVE11853
MEvNC30 MEvSC30 NSvNC30	1	NVE17908
MEvNC30 NSvNC30 SCvNC30	2	NVE13226 NVE9964
MEvNC30 NSvNC30 SCvNS30	2	NVE11852 NVE14719
MEvNS30 NSvNC30 SCvNS30	4	NVE14217 NVE570 NVE953 NVE10342
MEvSC30 NSvNC30 SCvNC30	1	NVE22623
MEvSC30 NSvNC30 SCvNS30	1	NVE10189
MEvSC30 SCvNC30 SCvNS30	4	NVE2426 NVE18522 NVE22411 NVE8465
MAvNC30 MAvsNS30	3	NVE4778 NVE8266 NVE1216
MAvNC30 MAvSC30	17	NVE12210 NVE9180 NVE16306 NVE22893 NVE1143 NVE2323 NVE1244 NVE18114 NVE11370 NVE2431 NVE849 NVE7831 NVE628 NVE10814 NVE18929 NVE23847 NVE1602
MAvNC30 MEvNC30	8	NVE11282 NVE18456 NVE19335 NVE13853 NVE23947 NVE13045 NVE22571 NVE16938
MAvNC30 NSvNC30	6	NVE19772 NVE23259 NVE10817 NVE16024 NVE3978 NVE20844
MAvNC30 SCvNC30	1	NVE23153
MAvNC30 SCvNS30	1	NVE20303
MAvSC30 MAvsNS30	8	NVE25578 NVE14569 NVE22099 NVE22977 NVE7833 NVE18777 NVE8644 NVE5054
MAvsNS30 MEvMA30	2	NVE8329 NVE20813
MAvsNS30 NSvNC30	2	NVE6401 NVE23699
MAvsNS30 SCvNS30	12	NVE2701 NVE16867 NVE10188 NVE21584 NVE17844 NVE25659 NVE5857 NVE16604 NVE1679 NVE15608 NVE23371 NVE4826
MAvSC30 MEvMA30	7	NVE16503 NVE19703 NVE10684 NVE9886 NVE21825 NVE23781 NVE23787
MAvSC30 MEvSC30	30	NVE542 NVE25332 NVE23477 NVE24407 NVE11008 NVE17909 NVE20185 NVE25334 NVE7496 NVE22548 NVE15029 NVE16425 NVE23575 NVE21706 NVE11447 NVE16671 NVE24497 NVE16142 NVE24900 NVE9234 NVE295 NVE23285 NVE529 NVE11533 NVE15383 NVE3397 NVE14619 NVE339 NVE11770 NVE13773
MAvSC30 NSvNC30	1	NVE129
MAvSC30 SCvNC30	3	NVE5479 NVE13823 NVE13065
MAvSC30 SCvNS30	230	NVE10089 NVE17114 NVE23181 NVE14737 NVE3139 NVE16002 NVE5375 NVE15195 NVE23867 NVE18119 NVE12769 NVE10278 NVE3904 NVE2588 NVE8469 NVE8627 NVE2195 NVE4109 NVE21334 NVE6531 NVE7873 NVE10553 NVE3078 NVE17350 NVE22837 NVE21058 NVE24911 NVE13582 NVE15792 NVE22134 NVE19676 NVE21048 NVE16226 NVE23894 NVE18234 NVE7832 NVE17043 NVE14670 NVE24009 NVE1879 NVE18538 NVE15470 NVE17337 NVE6332 NVE17146 NVE2554 NVE942 NVE11023 NVE128 NVE25083 NVE23052 NVE14094 NVE21893 NVE22659 NVE24172 NVE25681 NVE5490 NVE15604 NVE21051 NVE15798 NVE2999 NVE16284 NVE6477 NVE14194 NVE11342 NVE12008 NVE25474 NVE483 NVE12255 NVE3571 NVE3857 NVE11488 NVE2159 NVE5594 NVE25689 NVE13084 NVE17024 NVE22273 NVE6335 NVE11383 NVE13747 NVE701 NVE18699 NVE11684 NVE11583 NVE242 NVE14280 NVE23710 NVE25836 NVE553 NVE17285 NVE25254 NVE15833 NVE22414 NVE22129 NVE4927 NVE10354 NVE26105 NVE13931 NVE14009 NVE13591 NVE21151 NVE4567 NVE23473 NVE21887 NVE2977 NVE1938 NVE11862 NVE7290 NVE18903 NVE8026 NVE9870 NVE3712 NVE19683 NVE12967 NVE14731 NVE2854 NVE13749 NVE1737

		NVE3708 NVE14853 NVE211 NVE12366 NVE16837 NVE12279 NVE13802 NVE9032 NVE1495 NVE6520 NVE24137 NVE26185 NVE9223 NVE20392 NVE16891 NVE6248 NVE1390 NVE24820 NVE22695 NVE23474 NVE21580 NVE17113 NVE23006 NVE15214 NVE26003 NVE21729 NVE8223 NVE7964 NVE10220 NVE13834 NVE25687 NVE13662 NVE18455 NVE21927 NVE16798 NVE26075 NVE6915 NVE3887 NVE6612 NVE3206 NVE9450 NVE15822 NVE2340 NVE11611 NVE10953 NVE15734 NVE2314 NVE1001 NVE16502 NVE3472 NVE15422 NVE7749 NVE21541 NVE10119 NVE17280 NVE23817 NVE42 NVE11278 NVE12511 NVE23916 NVE22313 NVE2049 NVE19208 NVE22955 NVE11665 NVE23290 NVE25265 NVE4371 NVE13123 NVE14907 NVE9150 NVE16558 NVE15451 NVE6758 NVE26190 NVE25393 NVE25565 NVE19427 NVE18460 NVE23472 NVE18548 NVE9089 NVE22533 NVE10173 NVE10082 NVE15119 NVE16063 NVE4702 NVE2183 NVE4820 NVE19079 NVE23679 NVE15832 NVE19903 NVE21596 NVE20476 NVE2337 NVE19829 NVE2538 NVE22522 NVE18267 NVE23087 NVE22112 NVE11482 NVE13620 NVE4944 NVE6274 NVE20421 NVE10414 NVE22374 NVE21559
MEvMA30 MEvNC30	1	NVE19463
MEvMA30 MEvNS30	1	NVE4355
MEvMA30 MEvSC30	2	NVE1869 NVE22146
MEvNC30 MEvNS30	1	NVE14133
MEvNC30 MEvSC30	10	NVE15226 NVE10584 NVE10837 NVE26086 NVE8482 NVE10836 NVE13632 NVE20308 NVE17110 NVE16065
MEvNC30 NSvNC30	4	NVE20024 NVE6997 NVE13575 NVE6091
MEvNC30 SCvNC30	4	NVE6210 NVE12655 NVE18688 NVE25933
MEvNC30 SCvNS30	2	NVE21561 NVE13313
MEvNS30 MEvSC30	3	NVE25866 NVE9598 NVE4626
MEvNS30 NSvNC30	1	NVE6311
MEvNS30 SCvNS30	7	NVE4486 NVE19831 NVE18649 NVE18413 NVE19523 NVE8481 NVE15132
MEvSC30 NSvNC30	1	NVE15350
MEvSC30 SCvNS30	68	NVE4748 NVE2161 NVE19155 NVE25071 NVE5001 NVE778 NVE11856 NVE7035 NVE305 NVE19532 NVE23835 NVE12192 NVE8909 NVE16712 NVE19700 NVE14291 NVE6777 NVE4662 NVE14939 NVE12912 NVE12559 NVE7744 NVE5785 NVE18815 NVE25974 NVE844 NVE11103 NVE3726 NVE7357 NVE10103 NVE2583 NVE23813 NVE6169 NVE3976 NVE13573 NVE24377 NVE11407 NVE4320 NVE10633 NVE16211 NVE7493 NVE9787 NVE22722 NVE15168 NVE9221 NVE4672 NVE4492 NVE6824 NVE10634 NVE23675 NVE9487 NVE22604 NVE21289 NVE15276 NVE2428 NVE1642 NVE10245 NVE1450 NVE12953 NVE24871 NVE16820 NVE1939 NVE13854 NVE37 NVE26074 NVE5740 NVE10970 NVE12406
NSvNC30 SCvNC30	2	NVE4899 NVE8287
NSvNC30 SCvNS30	42	NVE18915 NVE24103 NVE17634 NVE1688 NVE4448 NVE6145 NVE17722 NVE8309 NVE18461 NVE24288 NVE6794 NVE1808 NVE12125 NVE25676 NVE8902 NVE17724 NVE24289 NVE6736 NVE10262 NVE12215 NVE16064 NVE10190 NVE5764 NVE6580 NVE12877 NVE790 NVE13127 NVE14955 NVE20418 NVE18387 NVE25366 NVE12545 NVE21525 NVE21566 NVE20016 NVE17325 NVE12091 NVE23373 NVE819 NVE3316 NVE10818 NVE13091
SCvNC30 SCvNS30	7	NVE4083 NVE5142 NVE18687 NVE3505 NVE4251 NVE3068 NVE891
MAvNC30	30	NVE11578 NVE6960 NVE3141 NVE10218 NVE8502 NVE25662 NVE4604 NVE18990 NVE22805 NVE23612 NVE13022 NVE4720 NVE4068 NVE9071 NVE23805 NVE11693 NVE2752 NVE13578 NVE8333 NVE5331 NVE25792 NVE3437 NVE9702 NVE5412 NVE12148 NVE24155 NVE13667 NVE16404 NVE15730 NVE2965
MAvsNS30	9	NVE17841 NVE16246 NVE6970 NVE10940 NVE18201 NVE6156 NVE18469 NVE7138 NVE16856

MAvSC30	283	NVE15828 NVE8081 NVE13761 NVE3363 NVE10359 NVE1875 NVE24539 NVE4685 NVE17070 NVE23281 NVE8978 NVE3371 NVE7022 NVE4279 NVE13079 NVE11067 NVE11721 NVE24609 NVE13015 NVE20674 NVE24183 NVE22768 NVE16978 NVE1342 NVE10097 NVE8084 NVE5749 NVE15911 NVE8009 NVE18005 NVE21412 NVE8453 NVE9847 NVE13000 NVE10459 NVE1568 NVE8551 NVE10458 NVE1706 NVE10291 NVE3211 NVE16808 NVE12823 NVE761 NVE21972 NVE11817 NVE24168 NVE9664 NVE1954 NVE18254 NVE4103 NVE8286 NVE288 NVE20135 NVE22359 NVE140 NVE12890 NVE3463 NVE15384 NVE17412 NVE743 NVE6632 NVE1273 NVE10862 NVE9058 NVE17812 NVE6447 NVE9538 NVE9112 NVE641 NVE10468 NVE23708 NVE2509 NVE20113 NVE15531 NVE9732 NVE20598 NVE2670 NVE3868 NVE10844 NVE21000 NVE13842 NVE19383 NVE17523 NVE17679 NVE7489 NVE13181 NVE8463 NVE8051 NVE12457 NVE24997 NVE9648 NVE4981 NVE23711 NVE19115 NVE14284 NVE13427 NVE18536 NVE2092 NVE18086 NVE15999 NVE24958 NVE2296 NVE6473 NVE18520 NVE879 NVE15677 NVE2383 NVE16149 NVE24381 NVE7297 NVE9635 NVE3038 NVE20076 NVE13984 NVE20235 NVE11261 NVE4317 NVE3291 NVE15905 NVE25730 NVE3596 NVE5374 NVE12991 NVE16424 NVE12859 NVE20003 NVE721 NVE5931 NVE3883 NVE20413 NVE14769 NVE17053 NVE25371 NVE9464 NVE19543 NVE5323 NVE14663 NVE3475 NVE3848 NVE9629 NVE8679 NVE5695 NVE19553 NVE6375 NVE20078 NVE22476 NVE13323 NVE17688 NVE24474 NVE23581 NVE13759 NVE24914 NVE24785 NVE7644 NVE14406 NVE4157 NVE21391 NVE18960 NVE20752 NVE18287 NVE25305 NVE13717 NVE10586 NVE24280 NVE16634 NVE9850 NVE15162 NVE18111 NVE9954 NVE13315 NVE7662 NVE13122 NVE7793 NVE4740 NVE1730 NVE14303 NVE2259 NVE9417 NVE4367 NVE14208 NVE5784 NVE13325 NVE2321 NVE2520 NVE11688 NVE25600 NVE9575 NVE10461 NVE13257 NVE18491 NVE10874 NVE6121 NVE20134 NVE632 NVE25080 NVE20487 NVE18260 NVE19285 NVE13593 NVE22144 NVE13565 NVE20339 NVE3366 NVE442 NVE1494 NVE12218 NVE3610 NVE13159 NVE6587 NVE13724 NVE13365 NVE25060 NVE22553 NVE25369 NVE4520 NVE13515 NVE12356 NVE9896 NVE14946 NVE25570 NVE22820 NVE9105 NVE16630 NVE101 NVE11113 NVE8178 NVE230 NVE13656 NVE12225 NVE11324 NVE8184 NVE18784 NVE4015 NVE13459 NVE23759 NVE19667 NVE12994 NVE17173 NVE13604 NVE22376 NVE8516 NVE3942 NVE7136 NVE22173 NVE16565 NVE3716 NVE14919 NVE4982 NVE9542 NVE8638 NVE6006 NVE9959 NVE25910 NVE20068 NVE3199 NVE3203 NVE6398 NVE17166 NVE22120 NVE20182 NVE2951 NVE10227 NVE20806 NVE24200 NVE9927 NVE15129 NVE24731 NVE13995 NVE5 NVE21332 NVE2042 NVE13490 NVE17947 NVE18721 NVE14647 NVE23054 NVE24992 NVE17898 NVE18365 NVE10187 NVE20470 NVE3509
MEvMA30	2	NVE16905 NVE11126
MEvNC30	10	NVE2320 NVE15152 NVE21132 NVE24499 NVE4966 NVE6616 NVE20692 NVE20687 NVE14792 NVE2673
MEvNS30	9	NVE6821 NVE11593 NVE18788 NVE2767 NVE5337 NVE22158 NVE18442 NVE13434 NVE1825
MEvSC30	52	NVE6310 NVE2088 NVE20539 NVE5523 NVE18871 NVE17262 NVE9203 NVE19107 NVE5978 NVE12257 NVE14264 NVE12352 NVE1863 NVE19460 NVE24650 NVE8996 NVE6644 NVE9443 NVE20217 NVE21426 NVE13844 NVE15724 NVE6250 NVE15707 NVE710 NVE23766 NVE9331 NVE4582 NVE3266 NVE20878 NVE25966 NVE371 NVE19272 NVE3517 NVE16289 NVE18996 NVE22048 NVE2534 NVE6086 NVE25406 NVE20811 NVE21032 NVE21423 NVE6519 NVE3274 NVE21189 NVE8268 NVE20412 NVE14604 NVE15943 NVE6230 NVE20728
NSvNC30	29	NVE9404 NVE20536 NVE20827 NVE19848 NVE15855 NVE9167 NVE481 NVE15740 NVE23459 NVE11224 NVE283 NVE538 NVE2310 NVE14422 NVE6555 NVE20622 NVE10753 NVE7892 NVE8092 NVE9601 NVE10139 NVE15687 NVE1649 NVE16809 NVE16115 NVE11504 NVE23559 NVE11422 NVE8861
SCvNC30	9	NVE7996 NVE7626 NVE16086 NVE15571 NVE15569 NVE10203 NVE9780 NVE12887 NVE10150
SCvNS30	347	NVE10060 NVE419 NVE15514 NVE22617 NVE24011 NVE11974 NVE25233 NVE5774 NVE3355 NVE20522 NVE18023 NVE5856 NVE3103 NVE2619 NVE2550 NVE16391 NVE2120 NVE4771 NVE17883 NVE24728 NVE8847 NVE20370 NVE14289 NVE16097 NVE12141 NVE15893 NVE3460 NVE17714 NVE20254 NVE19965 NVE17843 NVE2315 NVE8079 NVE16562 NVE16422 NVE10441 NVE4713 NVE17919 NVE11548 NVE6785 NVE16676 NVE5588 NVE7414 NVE1775 NVE259 NVE5080 NVE23812 NVE11109 NVE18553 NVE11603 NVE12110 NVE25650 NVE18622 NVE22605 NVE2366 NVE10155

		NVE6078 NVE7539 NVE24495 NVE24203 NVE25471 NVE17135 NVE16813 NVE9477 NVE14564 NVE12986 NVE2367 NVE535 NVE2218 NVE19872 NVE16195 NVE697 NVE17576 NVE3012 NVE11208 NVE10095 NVE24732 NVE15535 NVE25698 NVE19483 NVE17944 NVE6120 NVE20686 NVE24521 NVE23674 NVE6993 NVE8820 NVE2781 NVE23021 NVE11467 NVE8179 NVE23844 NVE17343 NVE5125 NVE24305 NVE18914 NVE480 NVE18196 NVE24745 NVE14416 NVE13874 NVE7351 NVE19375 NVE25639 NVE12904 NVE3911 NVE15150 NVE9904 NVE2252 NVE24413 NVE16466 NVE25241 NVE782 NVE21111 NVE23701 NVE6973 NVE1264 NVE16617 NVE25490 NVE17322 NVE1017 NVE11452 NVE22602 NVE2668 NVE21982 NVE22721 NVE11644 NVE8199 NVE3649 NVE17282 NVE6287 NVE20568 NVE23989 NVE24466 NVE19588 NVE25543 NVE16817 NVE10077 NVE7862 NVE23962 NVE25706 NVE15133 NVE12557 NVE19594 NVE18540 NVE8143 NVE25062 NVE215 NVE24104 NVE13292 NVE5154 NVE385 NVE25725 NVE14880 NVE17609 NVE8236 NVE10286 NVE16428 NVE1532 NVE19804 NVE3262 NVE25289 NVE17875 NVE13473 NVE935 NVE7638 NVE12284 NVE14456 NVE10626 NVE12207 NVE9822 NVE19013 NVE10438 NVE9437 NVE2629 NVE25290 NVE18376 NVE127 NVE8163 NVE8357 NVE3085 NVE14059 NVE7269 NVE7371 NVE15856 NVE19194 NVE10722 NVE24970 NVE16531 NVE25496 NVE18195 NVE13904 NVE5153 NVE11040 NVE13457 NVE10854 NVE22650 NVE23023 NVE17511 NVE19760 NVE17892 NVE1774 NVE1154 NVE8832 NVE23796 NVE9828 NVE6646 NVE11865 NVE22395 NVE21544 NVE8815 NVE12183 NVE21797 NVE14047 NVE25317 NVE22984 NVE6358 NVE5628 NVE2472 NVE16207 NVE17066 NVE18566 NVE2326 NVE10252 NVE360 NVE11843 NVE4825 NVE6251 NVE4430 NVE7689 NVE25854 NVE11297 NVE10243 NVE8429 NVE14028 NVE6249 NVE7441 NVE1816 NVE16036 NVE20161 NVE13982 NVE24912 NVE26032 NVE10096 NVE10373 NVE12434 NVE3140 NVE17949 NVE6802 NVE9994 NVE10510 NVE2275 NVE38 NVE7538 NVE4091 NVE19751 NVE7135 NVE19082 NVE6383 NVE16672 NVE24096 NVE15471 NVE15323 NVE7967 NVE21696 NVE23811 NVE9857 NVE2219 NVE20404 NVE9998 NVE14374 NVE13748 NVE19732 NVE4124 NVE25541 NVE20089 NVE15621 NVE3117 NVE3071 NVE17436 NVE3281 NVE15941 NVE23123 NVE2935 NVE2733 NVE15710 NVE4687 NVE19457 NVE10948 NVE16953 NVE15748 NVE22425 NVE15870 NVE21177 NVE20999 NVE7117 NVE24639 NVE13163 NVE13685 NVE14891 NVE4021 NVE4349 NVE25136 NVE17194 NVE15773 NVE22740 NVE23029 NVE10887 NVE1932 NVE13233 NVE17290 NVE14561 NVE10424 NVE1738 NVE10763 NVE6502 NVE2945 NVE15644 NVE17116 NVE22614 NVE873 NVE9802 NVE309 NVE13149 NVE1271 NVE17450 NVE9769 NVE20445 NVE16257 NVE2258 NVE25309 NVE10336 NVE6295 NVE459 NVE15093 NVE16580 NVE9141 NVE8086 NVE16181 NVE21284 NVE12752 NVE17901 NVE1957 NVE9329 NVE9125 NVE24779 NVE19355

Supplemental table 3: Unique DEG up- or downregulated at 28°C

DEG upregulated in Maine	**DEG upregulated in Nova Scotia**
NVE16906, NVE18608, NVE7526, NVE589, NVE15760, NVE10051, NVE10584, NVE14217, NVE6616, NVE14133, NVE5879, NVE3474, NVE24607, NVE11853, NVE4727, NVE7359, NVE10139, NVE910, NVE15134, NVE22589, NVE14423, NVE6612, NVE6235, NVE16065, NVE14006, NVE15132, NVE5522, NVE22370, NVE18782, NVE15136, NVE15707, NVE7457, NVE12202, NVE15135, NVE12631, NVE10067, NVE14177, NVE9447, NVE14814, NVE23785, NVE24274, NVE8481, NVE18915, NVE13322, NVE7544, NVE23587, NVE2701, NVE5068, NVE8410, NVE17265, NVE7620, NVE22158, NVE25513, NVE5911, NVE6446, NVE10262, NVE14955, NVE15606, NVE14880, NVE4464, NVE2998, NVE14458, NVE6271, NVE6609, NVE2999, NVE18268, NVE7593, NVE1021, NVE12125, NVE18600, NVE1757, NVE4465, NVE10958, NVE18269	NVE16064, NVE19696, NVE2767, NVE21133, NVE8075, NVE23510, NVE21761, NVE1122, NVE11627, NVE9385, NVE4745, NVE17766, NVE18777, NVE22146, NVE14556, NVE13796, NVE20507, NVE24866, NVE13844, NVE12006, NVE14425, NVE25059, NVE8861, NVE24510, NVE7823, NVE1356, NVE17625, NVE21670, NVE8461, NVE3432, NVE6821, NVE11837, NVE19300, NVE2190, NVE7760, NVE24407, NVE17831, NVE24408, NVE2188, NVE17845, NVE2189, NVE25581, NVE2187, NVE18436, NVE20439, NVE16593, NVE7289, NVE6982, NVE8482, NVE2555, NVE17830, NVE9534, NVE25060, NVE18417, NVE1825, NVE14725, NVE272, NVE14630, NVE12007, NVE17765, NVE16216, NVE1091, NVE14796, NVE15403, NVE6736, NVE25062, NVE23036, NVE1750, NVE15726, NVE417, NVE17843, NVE2263, NVE13659, NVE16024, NVE7357, NVE14528, NVE1560, NVE14660, NVE12092

Supplemental table 4: Unique DEG up- or downregulated at 32°C	
Genes upregulated in North Carolina	**Genes upregulated in South Carolina**
NVE4981, NVE3980, NVE3917, NVE17714, NVE23510, NVE9538, NVE10753, NVE10754, NVE73, NVE7512, NVE17812, NVE24607, NVE7408, NVE1747, NVE12113, NVE15350, NVE21584, NVE25572, NVE24070, NVE11856, NVE10067, NVE3831, NVE6983, NVE6850, NVE4083, NVE25892, NVE2188, NVE15999, NVE16807, NVE14670, NVE15471, NVE4727, NVE14789, NVE2535, NVE14792, NVE14714, NVE12352, NVE6148, NVE9203, NVE16085, NVE22208, NVE13397, NVE12092, NVE5893, NVE11109, NVE20949, NVE6446, NVE6634, NVE19262, NVE14177, NVE12007	NVE5385, NVE22750, NVE10879, NVE11627, NVE15287, NVE11948, NVE672, NVE23792, NVE18777, NVE22411, NVE8462, NVE6447, NVE19388, NVE15822, NVE3634, NVE15088, NVE6194, NVE23087, NVE23153, NVE4760, NVE24162, NVE16820, NVE891, NVE14622, NVE16433, NVE18733, NVE24347, NVE10854, NVE4742, NVE5410, NVE25581, NVE6248, NVE16630, NVE18931, NVE9533, NVE2619, NVE7457, NVE16785, NVE9534, NVE23835, NVE1901, NVE25579, NVE23458, NVE15247, NVE12272, NVE2554, NVE10760, NVE2553, NVE16112

Supplemental Table 5: All DEG across temperature comparisons in Nova Scotia

Names	total	elements
20_28NS 20_30NS 28_30NS	24	NVE1903 NVE21802 NVE14406 NVE5875 NVE17336 NVE9847 NVE1706 NVE13758 NVE9622 NVE17104 NVE9964 NVE17335 NVE12840 NVE2670 NVE2668 NVE4927 NVE2183 NVE11497 NVE20235 NVE17166 NVE12279 NVE20421 NVE21570 NVE9629
20_28NS 20_30NS	143	NVE17218 NVE10573 NVE5802 NVE7494 NVE14926 NVE23810 NVE10359 NVE18993 NVE12898 NVE20499 NVE253 NVE9353 NVE18479 NVE2876 NVE7022 NVE25562 NVE13904 NVE15845 NVE19366 NVE418 NVE7996 NVE25580 NVE8055 NVE9886 NVE18231 NVE16799 NVE15674 NVE1276 NVE16377 NVE6585 NVE26011 NVE7848 NVE13575 NVE521 NVE25782 NVE15431 NVE5749 NVE25687 NVE16780 NVE6614 NVE23699 NVE16798 NVE15059 NVE5877 NVE24211 NVE18461 NVE17817 NVE3845 NVE17855 NVE10086 NVE19046 NVE18268 NVE19335 NVE1143 NVE7520 NVE16796 NVE16116 NVE12688 NVE5564 NVE2535 NVE23824 NVE19262 NVE6120 NVE1494 NVE7823 NVE339 NVE18973 NVE22385 NVE13177 NVE21051 NVE16797 NVE18546 NVE8179 NVE1391 NVE10862 NVE17812 NVE25802 NVE7724 NVE3806 NVE21102 NVE19989 NVE7777 NVE19039 NVE18958 NVE12939 NVE1939 NVE1253 NVE8280 NVE21150 NVE16086 NVE8465 NVE16429 NVE23947 NVE4301 NVE23717 NVE24199 NVE4981 NVE6336 NVE4720 NVE15296 NVE13427 NVE25820 NVE18608 NVE19845 NVE19980 NVE20304 NVE538 NVE5207 NVE4982 NVE309 NVE10021 NVE25705 NVE9320 NVE9767 NVE23373 NVE17236 NVE19902 NVE12983 NVE4655 NVE1602 NVE5239 NVE14422 NVE23460 NVE12938 NVE19040 NVE8661 NVE26027 NVE10227 NVE13161 NVE22528 NVE13440 NVE21064 NVE25581 NVE647 NVE17219 NVE6616 NVE7061 NVE25556 NVE24033 NVE8861 NVE21507 NVE1495 NVE4068
20_28NS 28_30NS	99	NVE6010 NVE14934 NVE13761 NVE24810 NVE10339 NVE7851 NVE6685 NVE17083 NVE5223 NVE23807 NVE23020 NVE10278 NVE21561 NVE796 NVE23474 NVE7526 NVE21580 NVE22591 NVE24835 NVE9201 NVE3113 NVE22099 NVE758 NVE7035 NVE14426 NVE25335 NVE4004 NVE16978 NVE16634 NVE20160 NVE9354 NVE478 NVE13582 NVE5857 NVE6785 NVE9787 NVE7822 NVE24509 NVE5400 NVE4778 NVE14216 NVE17337 NVE25569 NVE9541 NVE4847 NVE6393 NVE8461 NVE13313 NVE3432 NVE21893 NVE4823 NVE4251 NVE14362 NVE3472 NVE18688 NVE7570 NVE12992 NVE42 NVE4658 NVE22553 NVE2017 NVE25244 NVE10179 NVE25265 NVE19375 NVE9368 NVE8917 NVE13626 NVE10139 NVE3912 NVE14332 NVE14462 NVE23472 NVE13796 NVE9104 NVE17825 NVE9885 NVE20604 NVE21151 NVE23473 NVE21887 NVE1100 NVE17341 NVE16166 NVE20076 NVE12967 NVE4355 NVE23813 NVE7446 NVE20844 NVE12991 NVE444 NVE9609 NVE18847 NVE20507 NVE891 NVE14563 NVE3475 NVE23664
20_30NS 28_30NS	17	NVE1748 NVE20941 NVE11271 NVE25138 NVE2870 NVE25273 NVE8266 NVE2633 NVE20303 NVE15529 NVE10190 NVE25933 NVE23457 NVE18506 NVE7149 NVE8800 NVE25290
20_28NS	384	NVE9898 NVE15399 NVE8081 NVE5695 NVE9606 NVE22974 NVE1219 NVE48 NVE20133 NVE419 NVE3139 NVE2132 NVE10420 NVE13249 NVE18687 NVE14872 NVE13713 NVE16915 NVE1875 NVE11362 NVE4340 NVE11852 NVE14201 NVE24970 NVE8705 NVE19991 NVE9331 NVE13823 NVE9071 NVE13323 NVE21098 NVE18805 NVE1791 NVE6286 NVE22060 NVE20321 NVE11388 NVE24474 NVE11287 NVE18022 NVE13457 NVE13759 NVE19183 NVE19733 NVE23424 NVE24785 NVE20536 NVE24264 NVE23968 NVE15781 NVE2482 NVE6663 NVE23274 NVE1605 NVE20077 NVE2517 NVE1719 NVE20227 NVE25336 NVE24828 NVE26003 NVE12141 NVE25536 NVE11822 NVE13376 NVE11299 NVE11466 NVE22692 NVE10039 NVE13026 NVE12442 NVE3373 NVE7850 NVE25578 NVE13829 NVE9208 NVE1815 NVE1342 NVE12744 NVE21801 NVE15615 NVE22317 NVE8271 NVE24071 NVE716 NVE26084 NVE20814 NVE21058 NVE12768 NVE7348 NVE9139 NVE2609 NVE12386 NVE11140 NVE17919 NVE12211 NVE23425 NVE3482 NVE23982 NVE12051 NVE26039 NVE13434 NVE22977 NVE8075 NVE7063 NVE1971 NVE2492 NVE14874 NVE13277 NVE4603 NVE10252 NVE19510 NVE18553 NVE4768 NVE8502 NVE1270 NVE3887 NVE9103 NVE16136 NVE14884 NVE877 NVE15175 NVE3880 NVE20088 NVE628 NVE15242 NVE13658 NVE25771 NVE25337 NVE17074 NVE3446 NVE825 NVE13524 NVE19184 NVE18491 NVE16052 NVE21214 NVE6160 NVE2677 NVE11323 NVE5399 NVE5570 NVE24882 NVE23818 NVE19990 NVE6091 NVE22714 NVE11431 NVE17852 NVE22893 NVE11527 NVE18909 NVE4672 NVE7404 NVE247 NVE13992 NVE24070 NVE19431 NVE7510 NVE18260 NVE11023 NVE4735 NVE4141 NVE19778 NVE697 NVE1179 NVE20633 NVE5893 NVE22689 NVE6118 NVE14718 NVE25662 NVE18393 NVE15160 NVE19917 NVE4662 NVE4899 NVE6912 NVE2081 NVE8992 NVE12119 NVE22742 NVE114 NVE2702 NVE22359 NVE11872 NVE15402 NVE4604 NVE14413 NVE4779 NVE22248 NVE4476 NVE24823 NVE17818 NVE22485 NVE7094 NVE15323 NVE13945 NVE4324 NVE9857 NVE5385 NVE9487 NVE9956 NVE18954 NVE14746 NVE2219 NVE14592 NVE743 NVE9120 NVE21022 NVE17318 NVE21973 NVE11922 NVE16411 NVE18144 NVE19482 NVE25331 NVE4987 NVE5352 NVE12337 NVE1245 NVE8159 NVE19732 NVE11342 NVE24801 NVE975 NVE24915 NVE5206 NVE3426 NVE18987 NVE3749 NVE20113 NVE7481 NVE25651 NVE23123 NVE22754 NVE2733 NVE2446 NVE3445 NVE9610 NVE15649 NVE23143 NVE2252 NVE24413 NVE4951 NVE17999 NVE12315 NVE24197 NVE5594 NVE25571 NVE18002 NVE11863 NVE7512 NVE19884 NVE6615 NVE10879 NVE5876 NVE3437 NVE20702 NVE19383 NVE14260 NVE1234 NVE13065 NVE17049 NVE9702 NVE22307 NVE21971 NVE5412 NVE25686 NVE20913 NVE7715 NVE8071 NVE5785 NVE21823 NVE12658 NVE6462 NVE22972 NVE25008 NVE19667 NVE10215 NVE6402 NVE13429 NVE11743 NVE16593 NVE23324 NVE7408 NVE18012 NVE21390 NVE8330 NVE18115 NVE16216 NVE18416 NVE23801 NVE14012 NVE16251 NVE24958 NVE15518 NVE9607 NVE7862 NVE16063 NVE10878 NVE24337 NVE25706 NVE4702 NVE14796 NVE655 NVE19696 NVE15644 NVE11584 NVE4820 NVE4723 NVE23113 NVE4025 NVE9797 NVE5154

		NVE18272 NVE4666 NVE21242 NVE15925 NVE22400 NVE11770 NVE8101 NVE23847 NVE18224 NVE24381 NVE16286 NVE8537 NVE1252 NVE4746 NVE11742 NVE3199 NVE16027 NVE20445 NVE1721 NVE7910 NVE11652 NVE22028 NVE6110 NVE18113 NVE21286 NVE21821 NVE23956 NVE16872 NVE2579 NVE6295 NVE12092 NVE15129 NVE459 NVE19315 NVE19863 NVE15093 NVE14050 NVE6085 NVE16580 NVE19012 NVE9488 NVE17170 NVE4220 NVE6666 NVE20003 NVE18267 NVE6007 NVE250 NVE16837 NVE7504 NVE20126 NVE11482 NVE2555 NVE21741 NVE19207 NVE4810 NVE16447 NVE20275 NVE10187 NVE3354 NVE20439 NVE21645 NVE26059 NVE9125 NVE17867 NVE13802 NVE14789 NVE4209 NVE4315
20_30NS	48	NVE2256 NVE24137 NVE2962 NVE24301 NVE9143 NVE3427 NVE21562 NVE14622 NVE18926 NVE2508 NVE4109 NVE13176 NVE17721 NVE193 NVE21815 NVE19786 NVE13799 NVE164 NVE19676 NVE8462 NVE814 NVE15721 NVE1808 NVE11208 NVE9167 NVE8197 NVE3705 NVE7760 NVE4477 NVE21566 NVE23459 NVE23700 NVE21786 NVE7114 NVE7626 NVE12899 NVE4770 NVE19398 NVE19594 NVE1750 NVE22589 NVE4602 NVE5054 NVE17574 NVE4085 NVE8049 NVE15385 NVE20880
28_30NS	60	NVE17615 NVE14667 NVE23796 NVE17634 NVE358 NVE17843 NVE21797 NVE9650 NVE17861 NVE18456 NVE18711 NVE21119 NVE3153 NVE16219 NVE1001 NVE14008 NVE23052 NVE323 NVE12545 NVE4653 NVE15860 NVE9332 NVE8034 NVE16867 NVE23695 NVE21943 NVE6588 NVE20703 NVE2223 NVE15621 NVE24637 NVE25792 NVE570 NVE14794 NVE15748 NVE230 NVE25777 NVE1017 NVE14561 NVE17844 NVE24466 NVE1825 NVE22173 NVE17842 NVE11103 NVE987 NVE20813 NVE13844 NVE13149 NVE3401 NVE16428 NVE8668 NVE7820 NVE1737 NVE5017 NVE24065 NVE25572 NVE5329 NVE10414 NVE8163

Table 6: Genes up- or downregulated at higher temperatures in Nova Scotia

Comparison	Total	Genes
20_28NSup 20_30NSup 28_30NSdown	1	NVE9964
20_28NSdown 20_30NSdown 28_30NSup	21	NVE1903 NVE21802 NVE5875 NVE17336 NVE9847 NVE13758 NVE9622 NVE17104 NVE17335 NVE12840 NVE2670 NVE2668 NVE4927 NVE2183 NVE11497 NVE17166 NVE20235 NVE12279 NVE20421 NVE21570 NVE9629
20_28NSdown 20_30NSdown 28_30NSdown	2	NVE14406 NVE1706
20_28NSup 20_30NSup	75	NVE17218 NVE5802 NVE7494 NVE23810 NVE10359 NVE18993 NVE12898 NVE20499 NVE9353 NVE18479 NVE25562 NVE13904 NVE15845 NVE19366 NVE7996 NVE8055 NVE9886 NVE18231 NVE16377 NVE6585 NVE7848 NVE521 NVE25782 NVE6614 NVE16798 NVE15059 NVE24211 NVE18461 NVE17817 NVE17855 NVE10086 NVE19046 NVE1143 NVE7520 NVE16796 NVE12688 NVE5564 NVE2535 NVE19262 NVE6120 NVE7823 NVE21051 NVE16797 NVE18546 NVE10862 NVE25802 NVE21102 NVE7777 NVE12939 NVE1939 NVE21150 NVE16086 NVE16429 NVE23947 NVE4301 NVE15296 NVE19845 NVE538 NVE5207 NVE25705 NVE9320 NVE9767 NVE23373 NVE19902 NVE5239 NVE14422 NVE12938 NVE26027 NVE25581 NVE17219 NVE6616 NVE7061 NVE25556 NVE24033 NVE8861
20_28NSup 28_30NSdown	33	NVE6685 NVE23807 NVE7526 NVE24835 NVE14426 NVE20160 NVE9354 NVE5857 NVE6785 NVE9787 NVE7822 NVE5400 NVE25569 NVE4847 NVE6393 NVE4251 NVE14362 NVE18688 NVE4658 NVE22553 NVE9368 NVE8917 NVE3912 NVE13796 NVE9104 NVE9885 NVE21151 NVE21887 NVE20076 NVE23813 NVE7446 NVE20844 NVE891
20_30NSup 28_30NSup	7	NVE1748 NVE25138 NVE2870 NVE8266 NVE20303 NVE7149 NVE8800
20_28NSdown 28_30NSup	42	NVE13761 NVE24810 NVE10278 NVE21561 NVE796 NVE23474 NVE21580 NVE22591 NVE3113 NVE22099 NVE758 NVE25335 NVE4004 NVE16978 NVE13582 NVE24509 NVE4778 NVE17337 NVE9541 NVE13313 NVE3432 NVE4823 NVE3472 NVE12992 NVE42 NVE25244 NVE10179 NVE25265 NVE19375 NVE13626 NVE10139 NVE14332 NVE23472 NVE20604 NVE23473 NVE12967 NVE12991 NVE444 NVE9609 NVE14563 NVE3475 NVE23664
20_28NSdown 20_30NSdown	38	NVE10573 NVE14926 NVE253 NVE7022 NVE418 NVE1276 NVE5749 NVE16780 NVE5877 NVE3845 NVE18268 NVE19335 NVE23824 NVE1494 NVE339 NVE18973 NVE1391 NVE19989 NVE19039 NVE18958 NVE1253 NVE24199 NVE4720 NVE25820 NVE18608 NVE309 NVE17236 NVE12983 NVE4655 NVE19040 NVE8661 NVE10227 NVE13161 NVE22528 NVE13440 NVE21064 NVE647 NVE1495
20_30NSdown 28_30NSdown	10	NVE20941 NVE11271 NVE25273 NVE2633 NVE15529 NVE10190 NVE25933 NVE23457 NVE18506 NVE25290
20_28NSup	70	NVE15399 NVE8081 NVE22974 NVE14872 NVE9331 NVE13323 NVE24785 NVE20536 NVE13026 NVE12442 NVE25578 NVE17919 NVE23425 NVE16136 NVE19184 NVE18491 NVE5399 NVE5570 NVE23818 NVE11431 NVE22893 NVE13992 NVE697 NVE5893 NVE25662 NVE15160 NVE12119 NVE15402 NVE14413 NVE4324 NVE9857 NVE9120 NVE4987 NVE19732 NVE20113 NVE25651 NVE2446 NVE2733 NVE25571 NVE18002 NVE19884 NVE6615 NVE10879 NVE14260 NVE1234 NVE5785 NVE21823 NVE25706 NVE19696 NVE11584

		NVE4820 NVE4723 NVE21242 NVE8101 NVE23847 NVE18224 NVE16286 NVE4746 NVE12092 NVE6085 NVE16580 NVE17170 NVE6666 NVE11482 NVE21741 NVE16447 NVE3354 NVE9125 NVE4209 NVE4315
20_30NSup	45	NVE24137 NVE24301 NVE9143 NVE3427 NVE2876 NVE21562 NVE25580 NVE15674 NVE16799 NVE2508 NVE13575 NVE17721 NVE19786 NVE23699 NVE13799 NVE164 NVE19676 NVE814 NVE16116 NVE1808 NVE11208 NVE9167 NVE22385 NVE3705 NVE7760 NVE8179 NVE17812 NVE21566 NVE3806 NVE23459 NVE23700 NVE21786 NVE7626 NVE8280 NVE23717 NVE4981 NVE19594 NVE20304 NVE4982 NVE1750 NVE22589 NVE23460 NVE15385 NVE20880 NVE4068
28_30NSup	34	NVE6010 NVE10339 NVE17083 NVE14667 NVE17634 NVE358 NVE7035 NVE16634 NVE17843 NVE21119 NVE3153 NVE16219 NVE323 NVE21893 NVE12545 NVE7570 NVE21943 NVE24637 NVE25792 NVE15748 NVE17825 NVE17844 NVE24466 NVE1825 NVE22173 NVE17842 NVE1100 NVE17341 NVE20813 NVE16428 NVE1737 NVE5017 NVE24065 NVE10414
20_28NSdown	72	NVE9898 NVE14201 NVE13823 NVE22060 NVE11388 NVE13759 NVE15781 NVE1605 NVE25336 NVE11299 NVE11466 NVE716 NVE21058 NVE12768 NVE1971 NVE14874 NVE10252 NVE14884 NVE15175 NVE25337 NVE13524 NVE6160 NVE19990 NVE11527 NVE7510 NVE11023 NVE4735 NVE14718 NVE6912 NVE8992 NVE22359 NVE13945 NVE743 NVE21973 NVE18144 NVE25331 NVE12337 NVE11342 NVE5206 NVE22754 NVE3445 NVE9610 NVE2252 NVE24413 NVE4951 NVE5876 NVE21971 NVE25686 NVE10215 NVE6402 NVE7408 NVE16216 NVE18416 NVE23801 NVE24958 NVE16063 NVE15644 NVE23113 NVE11770 NVE3199 NVE16027 NVE1721 NVE22028 NVE18113 NVE6295 NVE459 NVE14050 NVE9488 NVE18267 NVE19207 NVE13802 NVE17867
20_30NSdown	33	NVE2256 NVE2962 NVE14622 NVE18926 NVE4109 NVE13176 NVE26011 NVE193 NVE21815 NVE15431 NVE25687 NVE8462 NVE15721 NVE8197 NVE13177 NVE4477 NVE7724 NVE7114 NVE12899 NVE8465 NVE4770 NVE6336 NVE13427 NVE19398 NVE19980 NVE10021 NVE1602 NVE4602 NVE5054 NVE17574 NVE4085 NVE8049 NVE21507
28_30NSdown	50	NVE14934 NVE7851 NVE5223 NVE23020 NVE17615 NVE23796 NVE9201 NVE21797 NVE9650 NVE478 NVE17861 NVE18456 NVE18711 NVE14216 NVE1001 NVE8461 NVE14008 NVE23052 NVE4653 NVE15860 NVE9332 NVE8034 NVE16867 NVE2017 NVE23695 NVE6588 NVE20703 NVE2223 NVE15621 NVE570 NVE14794 NVE230 NVE14462 NVE25777 NVE1017 NVE14561 NVE11103 NVE987 NVE13844 NVE13149 NVE16166 NVE3401 NVE4355 NVE8668 NVE7820 NVE18847 NVE20507 NVE25572 NVE5329 NVE8163

Table 7: DEG across all temperatures in Maine		
Names	**Total**	**Genes**
20_28ME 20_30ME	19	NVE12939 NVE12840 NVE18733 NVE7494 NVE9964 NVE21150 NVE21823 NVE16429 NVE647 NVE1706 NVE521 NVE23717 NVE24199 NVE6616 NVE20024 NVE4720 NVE19335 NVE2188 NVE16216
20°C vs 28°C ME	77	NVE15399 NVE25706 NVE11837 NVE8661 NVE9886 NVE23473 NVE2535 NVE7526 NVE5893 NVE24544 NVE24509 NVE23472 NVE1240 NVE1270 NVE23824 NVE17110 NVE3113 NVE17104 NVE15059 NVE22528 NVE16377 NVE19315 NVE12991 NVE5877 NVE19863 NVE5400 NVE5875 NVE2962 NVE18461 NVE1100 NVE7715 NVE3880 NVE16086 NVE628 NVE14426 NVE150 NVE6120 NVE21020 NVE1494 NVE14201 NVE3584 NVE825 NVE25578 NVE8101 NVE17818 NVE6609 NVE2189 NVE19046 NVE10278 NVE4778 NVE11742 NVE24607 NVE2252 NVE1747 NVE13183 NVE25687 NVE14216 NVE17337 NVE26012 NVE16780 NVE15296 NVE8330 NVE21051 NVE4897 NVE6614 NVE9885 NVE16796 NVE6615 NVE1560 NVE5239 NVE9629 NVE13951 NVE17336 NVE17335 NVE23474 NVE10594 NVE23956
20°C vs 30°C ME	20	NVE164 NVE22590 NVE538 NVE21527 NVE1122 NVE8465 NVE17574 NVE4769 NVE4770 NVE10021 NVE15383 NVE14564 NVE4826 NVE17845 NVE1748 NVE23459 NVE23457 NVE8266 NVE11504 NVE1808
28° vs 30°C ME	0	

Table 8: List of genes up- or downregulated across temperatures in Maine		
Names	**Total**	**Genes**
20_28MEup 20_30MEup	9	NVE12939 NVE7494 NVE9964 NVE21150 NVE21823 NVE16429 NVE521 NVE23717 NVE6616
20_28MEdown 20_30MEdown	10	NVE12840 NVE18733 NVE647 NVE1706 NVE24199 NVE20024 NVE4720 NVE19335 NVE2188 NVE16216
20_28MEup	41	NVE15399 NVE25706 NVE9886 NVE2535 NVE7526 NVE5893 NVE24544 NVE1240 NVE15059 NVE16377 NVE19863 NVE5400 NVE18461 NVE7715 NVE3880 NVE16086 NVE14426 NVE6120 NVE21020 NVE3584 NVE825 NVE25578 NVE8101 NVE17818 NVE6609 NVE19046 NVE11742 NVE24607 NVE1747 NVE13183 NVE14216 NVE15296 NVE8330 NVE21051 NVE6614 NVE9885 NVE16796 NVE6615 NVE5239 NVE13951 NVE23956
20_30MEup	10	NVE164 NVE22590 NVE538 NVE21527 NVE14564 NVE4826 NVE17845 NVE1748 NVE8266 NVE1808
20_28MEdown	36	NVE11837 NVE8661 NVE23473 NVE24509 NVE23472 NVE1270 NVE23824 NVE17110 NVE3113 NVE17104 NVE22528 NVE19315 NVE12991 NVE5877 NVE5875 NVE2962 NVE1100 NVE628 NVE150 NVE1494 NVE14201 NVE2189 NVE10278 NVE4778 NVE2252 NVE25687 NVE17337 NVE26012 NVE16780 NVE4897 NVE1560 NVE9629 NVE17336 NVE17335 NVE23474 NVE10594
20_30MEdown	10	NVE1122 NVE8465 NVE17574 NVE4769 NVE4770 NVE10021 NVE15383 NVE23459 NVE23457 NVE11504

Supplemental Table 9: Genes up- and downregulated at higher temperatures in Massachusetts	
20°C vs 30°C Down	**20°C vs 30° Up**
NVE23006, NVE17114, NVE19383, NVE5385, NVE7369, NVE6120, NVE2618, NVE17074, NVE20806, NVE6609, NVE21340, NVE4068, NVE2337, NVE8029, NVE7494, NVE17189, NVE7060, NVE16149, NVE24168, NVE8767, NVE10359, NVE8055, NVE9353, NVE8861, NVE19676, NVE7512, NVE9201, NVE5564, NVE15999, NVE7852, NVE4982, NVE20133, NVE22974, NVE8463, NVE21729, NVE8081, NVE17812, NVE2535, NVE18002, NVE9959, NVE7526, NVE4604, NVE7848, NVE21741, NVE15296, NVE5207, NVE9146, NVE21459, NVE4209, NVE7111, NVE4981, NVE1747, NVE17855, NVE12688, NVE19046, NVE5893, NVE17818, NVE8178, NVE23424, NVE23425, NVE9538, NVE15845, NVE26027, NVE25706, NVE21051, NVE17817, NVE2577, NVE10862, NVE12898, NVE18115, NVE13183, NVE23019, NVE11742, NVE11863, NVE18546, NVE25556, NVE19366, NVE6585, NVE25705, NVE25782, NVE538, NVE21398, NVE25802, NVE19279, NVE19884, NVE521, NVE2042, NVE8101, NVE17219, NVE11822, NVE4301, NVE5802, NVE17218, NVE18479, NVE20499, NVE19845	NVE9850, NVE8689, NVE14406, NVE11319, NVE253, NVE5605, NVE7833, NVE11584, NVE16216, NVE20068, NVE1706, NVE20077, NVE2343, NVE19335, NVE4720, NVE647, NVE24378, NVE7724, NVE1252, NVE12983, NVE23113, NVE4655, NVE1560, NVE12141, NVE4778, NVE4770, NVE13440, NVE24607, NVE15806, NVE10051, NVE19040, NVE4779, NVE6250, NVE23457, NVE12840, NVE8462, NVE19991, NVE13110, NVE14719, NVE24337, NVE1280, NVE17336, NVE24509, NVE21821, NVE23324, NVE10021, NVE5875, NVE17574, NVE1495, NVE23054, NVE8465

Supplemental Table 10: DEG across all temperatures in North Carolina		
Names	**Total**	**Genes**
20_30NC 20_32NC	41	NVE23473 NVE9847 NVE10573 NVE24544 NVE24509 NVE18925 NVE2183 NVE5400 NVE5207 NVE11852 NVE14201 NVE19991 NVE1706 NVE17817 NVE18805 NVE10021 NVE10278 NVE19990 NVE19335 NVE22714 NVE12983 NVE16872 NVE19040 NVE10227 NVE7626 NVE23472 NVE18733 NVE25336 NVE11740 NVE21511 NVE6912 NVE8465 NVE305 NVE6402 NVE13183 NVE4720 NVE2064 NVE8330 NVE18115 NVE21570 NVE17336
20_30NC	16	NVE7851 NVE3220 NVE7744 NVE18268 NVE19039 NVE10958 NVE8767 NVE2965 NVE26011 NVE8270 NVE12887 NVE4770 NVE24199 NVE21815 NVE1389 NVE26012
20_32NC	197	NVE6010 NVE15399 NVE25579 NVE24337 NVE14874 NVE25706 NVE13761 NVE982 NVE12840 NVE6502 NVE21943 NVE20133 NVE14737 NVE19932 NVE2088 NVE538 NVE11971 NVE7494 NVE4820 NVE14926 NVE8462 NVE7369 NVE4723 NVE23113 NVE18990 NVE10977 NVE1279 NVE414 NVE11497 NVE20698 NVE6235 NVE21761 NVE23510 NVE19375 NVE8455 NVE6525 NVE22673 NVE17707 NVE13257 NVE19903 NVE13524 NVE15925 NVE309 NVE13758 NVE253 NVE11611 NVE1903 NVE6160 NVE22060 NVE9622 NVE13626 NVE23847 NVE5399 NVE9610 NVE19989 NVE20024 NVE2670 NVE24413 NVE11629 NVE17337 NVE15350 NVE14817 NVE24408 NVE12592 NVE18707 NVE18002 NVE17236 NVE23787 NVE23459 NVE21802 NVE24235 NVE7512 NVE4672 NVE17751 NVE24070 NVE6234 NVE17166 NVE20235 NVE20536 NVE23474 NVE11023 NVE12122 NVE12643 NVE12620 NVE22571 NVE12935 NVE13065 NVE9886 NVE2535 NVE7526 NVE10585 NVE2499 NVE925 NVE13346 NVE6295 NVE18926 NVE14847 NVE3113 NVE19975 NVE25452 NVE5412 NVE25662 NVE19388 NVE12991 NVE22099 NVE17900 NVE25686 NVE5875 NVE19483 NVE13313 NVE123 NVE16943 NVE14426 NVE5017 NVE20895 NVE21064 NVE1494 NVE444 NVE3432 NVE20336 NVE12867 NVE21823 NVE21893 NVE10804 NVE22194 NVE22359 NVE2668 NVE12604 NVE25335 NVE647 NVE16978 NVE23153 NVE4604 NVE18267 NVE1280 NVE17217 NVE721 NVE22573 NVE1538 NVE7850 NVE17084 NVE25578 NVE272 NVE20881 NVE6325 NVE7094 NVE21084 NVE23858 NVE21257 NVE16502 NVE12655 NVE4927 NVE20145 NVE24899 NVE24065 NVE24000 NVE16965 NVE20941 NVE11482 NVE12378 NVE6196 NVE12279 NVE23324 NVE8084 NVE5749 NVE6336 NVE12558 NVE16780 NVE25820 NVE15608 NVE10019 NVE23425 NVE24278 NVE2015 NVE20421 NVE11519 NVE24466 NVE5857 NVE3475 NVE16216 NVE21846 NVE25341 NVE22265 NVE6746 NVE42 NVE19786 NVE8861 NVE1971 NVE3402 NVE9629 NVE13802 NVE9125 NVE17335 NVE14789 NVE21950 NVE15276 NVE1495

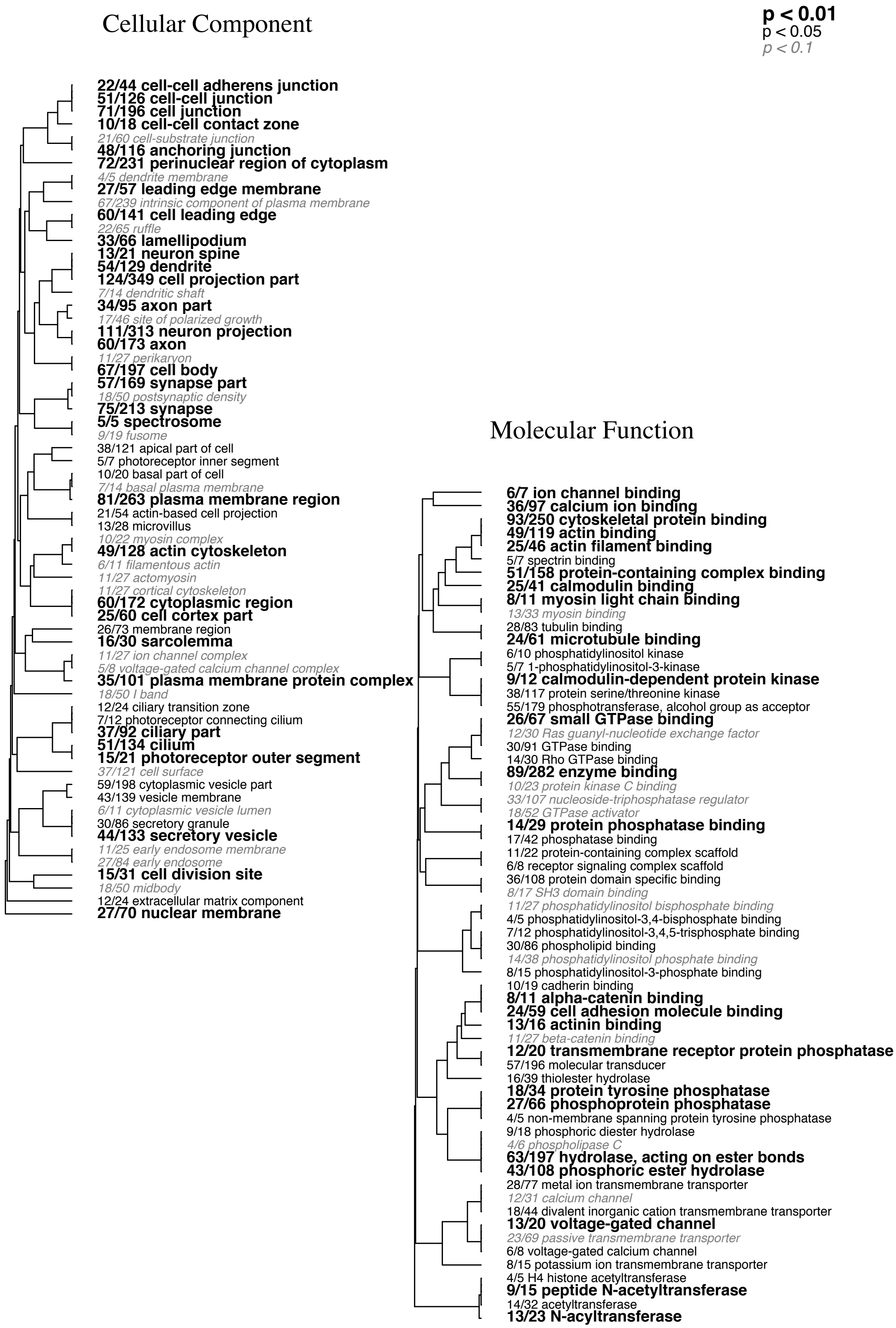
Supplemental Table 11: Genes up- or downregulated at higher temperature in North Carolina		
Names	**Total**	**Genes**
NC20vs30up NC20vs32up	9	NVE24544 NVE5400 NVE5207 NVE17817 NVE7626 NVE13183 NVE2064 NVE8330 NVE18115
NC20vs30down NC20vs32down	32	NVE23473 NVE9847 NVE10573 NVE24509 NVE18925 NVE2183 NVE11852 NVE14201 NVE19991 NVE1706 NVE18805 NVE10021 NVE10278 NVE19990 NVE19335 NVE22714 NVE12983 NVE19040 NVE16872 NVE10227 NVE23472 NVE18733 NVE11740 NVE25336 NVE21511 NVE6912 NVE8465 NVE305 NVE6402 NVE4720 NVE21570 NVE17336
NC20vs32up	69	NVE15399 NVE25706 NVE982 NVE20133 NVE14737 NVE538 NVE11971 NVE7494 NVE4820 NVE7369 NVE4723 NVE18990 NVE20698 NVE6235 NVE23510 NVE22673 NVE13257 NVE11611 NVE23847 NVE5399 NVE11629 NVE15350 NVE14817 NVE12592 NVE18002 NVE23787 NVE24235 NVE7512 NVE24070 NVE17751 NVE6234 NVE20536 NVE12643 NVE9886 NVE2535 NVE7526 NVE2499 NVE14847 NVE25662 NVE16943 NVE14426 NVE21823 NVE10804 NVE4604 NVE1538 NVE7850 NVE17084 NVE25578 NVE20881 NVE21084 NVE16502 NVE20145 NVE16965 NVE11482 NVE12378 NVE8084 NVE10019 NVE15608 NVE23425 NVE11519 NVE5857 NVE25341 NVE22265 NVE6746 NVE19786 NVE8861 NVE3402 NVE9125 NVE21950
NC20vs30up	6	NC20vs30up NVE3220 NVE8767 NVE2965 NVE8270 NVE12887
NC20vs32down	128	NVE6010 NVE25579 NVE24337 NVE14874 NVE13761 NVE12840 NVE6502 NVE21943 NVE19932 NVE2088 NVE14926 NVE8462 NVE23113 NVE10977 NVE1279 NVE414 NVE11497 NVE21761 NVE19375 NVE8455 NVE6525 NVE17707 NVE19903 NVE13524 NVE15925 NVE309 NVE13758 NVE253 NVE1903 NVE6160 NVE22060 NVE9622 NVE13626 NVE9610 NVE19989 NVE20024 NVE2670 NVE24413 NVE17337 NVE24408 NVE18707 NVE17236 NVE23459 NVE21802 NVE4672 NVE17166 NVE20235 NVE23474 NVE11023 NVE12122 NVE12620 NVE22571 NVE12935 NVE13065 NVE10585 NVE925 NVE13346 NVE6295 NVE18926 NVE3113 NVE19975 NVE25452 NVE5412 NVE19388 NVE12991 NVE22099 NVE17900 NVE25686 NVE5875 NVE19483 NVE13313 NVE123 NVE5017 NVE20895 NVE21064 NVE1494 NVE444 NVE3432 NVE20336 NVE12867 NVE21893 NVE22194 NVE22359 NVE12604 NVE2668 NVE25335 NVE647 NVE16978 NVE23153 NVE18267 NVE1280 NVE17217 NVE721 NVE22573 NVE272 NVE6325 NVE7094 NVE23858 NVE21257 NVE12655 NVE4927 NVE24899 NVE24065 NVE24000 NVE20941 NVE6196 NVE12279 NVE23324 NVE5749

		NVE6336 NVE12558 NVE16780 NVE25820 NVE24278 NVE2015 NVE20421 NVE24466 NVE3475 NVE16216 NVE21846 NVE42 NVE1971 NVE9629 NVE13802 NVE17335 NVE14789 NVE15276 NVE1495
NC20vs30down	11	NVE7851 NVE7744 NVE18268 NVE19039 NVE10958 NVE26011 NVE4770 NVE24199 NVE21815 NVE1389 NVE26012

Supplemental Table 12: List of all DEGs in South Carolina across temperatures

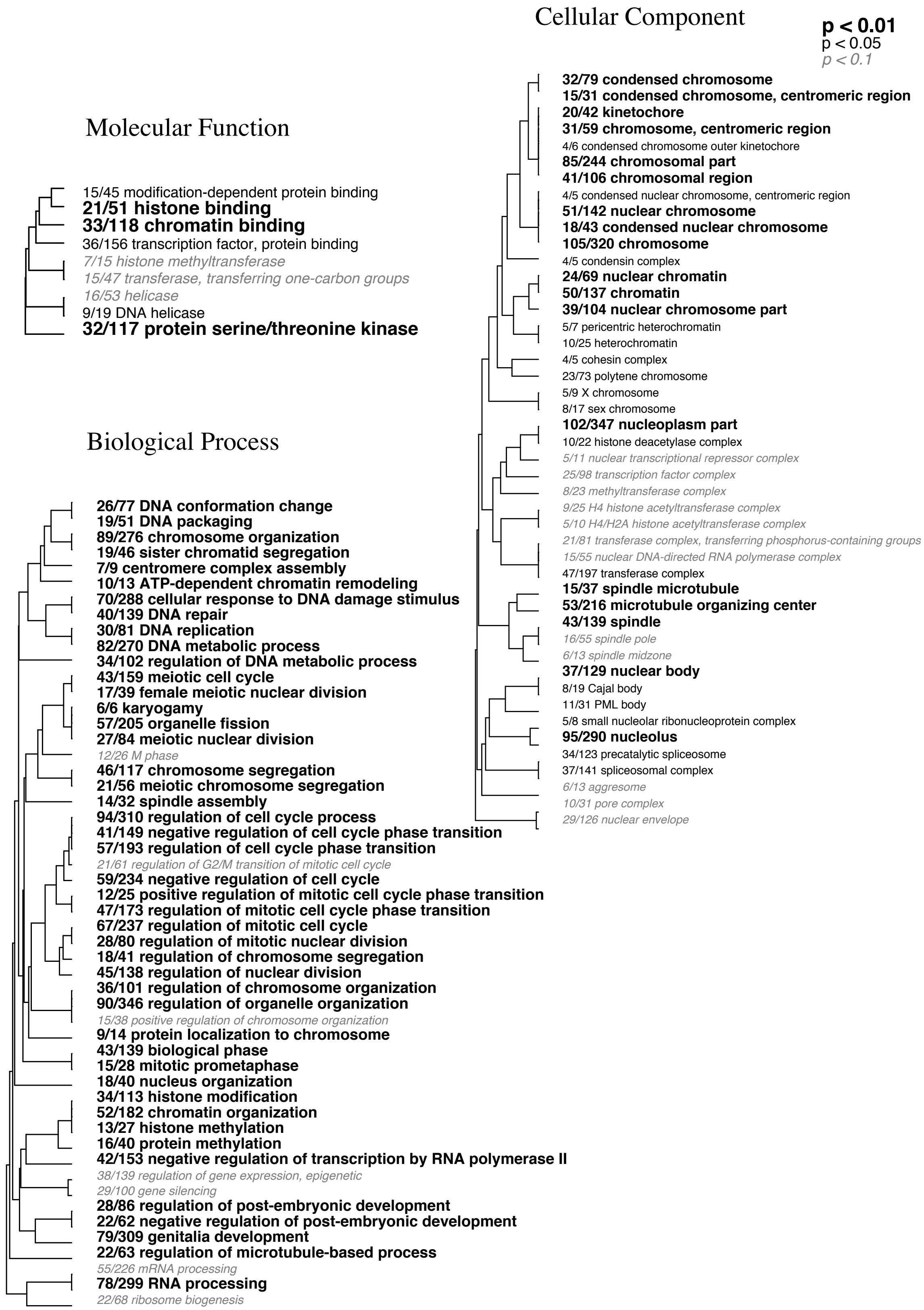
Names	Total	Genes
20_30SC 20_32SC 30_32SC	1	NVE7369
20_30SC 20_32SC	201	NVE6010 NVE6194 NVE1674 NVE14934 NVE4987 NVE13761 NVE12840 NVE5352 NVE21943 NVE12337 NVE14737 NVE5697 NVE13874 NVE23240 NVE7494 NVE2223 NVE14201 NVE19375 NVE3071 NVE13823 NVE1903 NVE18805 NVE22060 NVE10245 NVE11388 NVE13626 NVE9353 NVE18163 NVE4131 NVE23807 NVE24097 NVE7775 NVE10278 NVE20024 NVE24413 NVE2670 NVE13457 NVE13759 NVE19366 NVE18002 NVE21802 NVE418 NVE5716 NVE21561 NVE7996 NVE22695 NVE14462 NVE23474 NVE6973 NVE7526 NVE8184 NVE23472 NVE984 NVE8847 NVE3113 NVE20729 NVE21150 NVE22099 NVE25686 NVE5875 NVE12141 NVE2668 NVE4004 NVE24309 NVE16978 NVE11274 NVE7850 NVE17084 NVE12780 NVE21084 NVE20609 NVE10215 NVE4927 NVE6402 NVE9650 NVE22317 NVE16965 NVE8084 NVE478 NVE25820 NVE10019 NVE8330 NVE18608 NVE13579 NVE23425 NVE13582 NVE5857 NVE6785 NVE16216 NVE25543 NVE4740 NVE1560 NVE19786 NVE1971 NVE17336 NVE17328 NVE21151 NVE25706 NVE14874 NVE23473 NVE21105 NVE4603 NVE10252 NVE9847 NVE10147 NVE6502 NVE24544 NVE24509 NVE18925 NVE11971 NVE2183 NVE19696 NVE5400 NVE5877 NVE3206 NVE4820 NVE11862 NVE11497 NVE8429 NVE19463 NVE17707 NVE13257 NVE21242 NVE21119 NVE18931 NVE18491 NVE309 NVE13758 NVE1879 NVE11611 NVE9622 NVE5399 NVE23847 NVE8537 NVE24882 NVE23818 NVE3199 NVE14216 NVE3262 NVE17337 NVE20476 NVE1143 NVE7520 NVE12592 NVE19791 NVE12983 NVE1757 NVE20235 NVE3101 NVE17166 NVE5239 NVE24906 NVE8767 NVE7820 NVE10550 NVE2951 NVE5347 NVE128 NVE23824 NVE25662 NVE12991 NVE25698 NVE6120 NVE1494 NVE444 NVE4823 NVE13490 NVE18267 NVE4653 NVE8113 NVE20507 NVE14362 NVE16837 NVE16502 NVE11482 NVE4324 NVE3472 NVE9857 NVE15422 NVE12279 NVE9956 NVE13177 NVE18920 NVE15608 NVE14563 NVE743 NVE20421 NVE21570 NVE3475 NVE15532 NVE42 NVE8861 NVE21973 NVE17867 NVE13802 NVE9629 NVE16112 NVE17335 NVE2017 NVE4315 NVE1495
20_32SC 30_32SC	7	NVE8455 NVE8465 NVE25341 NVE1706 NVE16219 NVE13346 NVE23561
20_30SC 30_32SC	4	NVE3912 NVE17049 NVE20160 NVE7822
20_32SC	73	NVE536 NVE9930 NVE14926 NVE18990 NVE6235 NVE11852 NVE16630 NVE23792 NVE21102 NVE3273 NVE8783 NVE10180 NVE17463 NVE20702 NVE13522 NVE9886 NVE7957 NVE16799 NVE5412 NVE13685 NVE21584 NVE15287 NVE21823 NVE14034 NVE10051 NVE1280 NVE3373 NVE18111 NVE8689 NVE13315 NVE16653 NVE4720 NVE2064 NVE17138 NVE7318 NVE22379 NVE3477 NVE5576 NVE17823 NVE16400 NVE11344 NVE9193 NVE2964 NVE16052 NVE2618 NVE8852 NVE21133 NVE18224 NVE2682 NVE14220 NVE4778 NVE15088 NVE1614 NVE23120 NVE1808 NVE13301 NVE20844 NVE24003 NVE925 NVE9839 NVE13313 NVE2965 NVE4899 NVE21332 NVE10804 NVE25581 NVE18683 NVE7499 NVE18753 NVE13244 NVE529 NVE17346 NVE24278
20_30SC	121	NVE15828 NVE16284 NVE2049 NVE25244 NVE17724 NVE6310 NVE5142 NVE17114 NVE16425 NVE10573 NVE20133 NVE19732 NVE3139 NVE12356 NVE24081 NVE23810 NVE25265 NVE3616 NVE11021 NVE7851 NVE6685 NVE15117 NVE7522 NVE24795 NVE24970 NVE19991 NVE15195 NVE16891 NVE9071 NVE7481 NVE23123 NVE6609 NVE25496 NVE2252 NVE19183 NVE10731 NVE796 NVE5876 NVE857 NVE11918 NVE45 NVE13747 NVE13065 NVE21580 NVE1939 NVE1253 NVE882 NVE14289 NVE3141 NVE17900 NVE16086 NVE25536 NVE10325 NVE7035 NVE17194 NVE14800 NVE22414 NVE10173 NVE15615 NVE15529 NVE4643 NVE5749 NVE21058 NVE11140 NVE24466 NVE26007 NVE26074 NVE9787 NVE8075 NVE23801 NVE8474 NVE4436 NVE21950 NVE16063 NVE24337 NVE4702 NVE26075 NVE18553 NVE23458 NVE4723 NVE3406 NVE23726 NVE5154 NVE19184 NVE2340 NVE16286 NVE8236 NVE10911 NVE20233 NVE23813 NVE20445 NVE21972 NVE10888 NVE25569 NVE22417 NVE12771 NVE4141 NVE5351 NVE10227 NVE6295 NVE10095 NVE459 NVE19863 NVE24336 NVE21893 NVE647 NVE7823 NVE11119 NVE339 NVE12366 NVE8902 NVE12655 NVE18293 NVE24162 NVE19543 NVE15604 NVE12992 NVE23664 NVE3509 NVE9125 NVE4658
30_32SC	20	NVE19460 NVE7854 NVE4730 NVE7512 NVE11820 NVE6890 NVE23153 NVE10075 NVE4448 NVE10189 NVE22411 NVE24607 NVE2217 NVE2187 NVE4672 NVE7446 NVE22750 NVE8461 NVE24899 NVE5385

Supplemental Table 13: Genes up- or downregulated at higher temperatures in South Carolina		
Names	**Total**	**Genes**
20_30SCup 20_32SCup 30_32SCup	1	NVE7369
20_30SCdown 20_32SCdown	104	NVE6010 NVE6194 NVE1674 NVE13761 NVE12840 NVE5352 NVE21943 NVE12337 NVE5697 NVE13874 NVE14201 NVE19375 NVE13823 NVE1903 NVE18805 NVE22060 NVE11388 NVE13626 NVE7775 NVE10278 NVE20024 NVE2670 NVE24413 NVE13759 NVE21802 NVE418 NVE21561 NVE23474 NVE6973 NVE8184 NVE23472 NVE984 NVE3113 NVE20729 NVE22099 NVE5875 NVE25686 NVE12141 NVE2668 NVE4004 NVE16978 NVE12780 NVE10215 NVE4927 NVE6402 NVE25820 NVE18608 NVE13579 NVE13582 NVE16216 NVE4740 NVE1560 NVE1971 NVE17336 NVE14874 NVE23473 NVE10252 NVE9847 NVE6502 NVE24509 NVE18925 NVE2183 NVE5877 NVE11862 NVE11497 NVE19463 NVE17707 NVE21119 NVE309 NVE13758 NVE9622 NVE3199 NVE17337 NVE20476 NVE12983 NVE20235 NVE3101 NVE17166 NVE2951 NVE5347 NVE128 NVE23824 NVE12991 NVE444 NVE1494 NVE4823 NVE18267 NVE16837 NVE3472 NVE15422 NVE12279 NVE13177 NVE14563 NVE743 NVE20421 NVE21570 NVE3475 NVE42 NVE21973 NVE9629 NVE13802 NVE17867 NVE17335 NVE1495
20_32SCdown 30_32Sdown	6	NVE8455 NVE8465 NVE1706 NVE16219 NVE13346 NVE23561
20_30SCup 30_32Sdown	4	NVE3912 NVE17049 NVE20160 NVE7822
20_30SCup 20_32SCup	97	NVE14934 NVE4987 NVE14737 NVE23240 NVE7494 NVE2223 NVE3071 NVE10245 NVE9353 NVE18163 NVE4131 NVE23807 NVE24097 NVE13457 NVE19366 NVE18002 NVE5716 NVE7996 NVE22695 NVE14462 NVE7526 NVE8847 NVE21150 NVE24309 NVE11274 NVE17084 NVE7850 NVE21084 NVE20609 NVE9650 NVE22317 NVE16965 NVE8084 NVE478 NVE10019 NVE8330 NVE23425 NVE5857 NVE6785 NVE25543 NVE19786 NVE17328 NVE21151 NVE25706 NVE21105 NVE4603 NVE10147 NVE24544 NVE11971 NVE19696 NVE5400 NVE4820 NVE3206 NVE8429 NVE13257 NVE21242 NVE18931 NVE18491 NVE11611 NVE1879 NVE23847 NVE5399 NVE8537 NVE23818 NVE24882 NVE3262 NVE14216 NVE1143 NVE7520 NVE12592 NVE19791 NVE1757 NVE5239 NVE8767 NVE24906 NVE7820 NVE10550 NVE25662 NVE25698 NVE6120 NVE13490 NVE4653 NVE8113 NVE20507 NVE14362 NVE16502 NVE11482 NVE4324 NVE9857 NVE9956 NVE18920 NVE15608 NVE15532 NVE8861 NVE16112 NVE4315 NVE2017
20_32SCup 30_32SCup	1	NVE25341
20_32SCdown	27	NVE14926 NVE11852 NVE3273 NVE20702 NVE13522 NVE7957 NVE5412 NVE21584 NVE14034 NVE10051 NVE1280 NVE3373 NVE8689 NVE18111 NVE16653 NVE4720 NVE3477 NVE17823 NVE8852 NVE4778 NVE925 NVE9839 NVE13313 NVE4899 NVE7499 NVE13244 NVE24278
20_30SCdown	70	NVE15828 NVE16284 NVE25244 NVE17724 NVE5142 NVE17114 NVE16425 NVE10573 NVE3139 NVE12356 NVE24081 NVE25265 NVE3616 NVE11021 NVE15117 NVE24970 NVE19991 NVE7481 NVE23123 NVE25496 NVE2252 NVE10731 NVE796 NVE5876 NVE11918 NVE13065 NVE21580 NVE1253 NVE14289 NVE3141 NVE17900 NVE7035 NVE17194 NVE4643 NVE5749 NVE21058 NVE11140 NVE24466 NVE26007 NVE23801 NVE8474 NVE4436 NVE16063 NVE4702 NVE24337 NVE26075 NVE18553 NVE2340 NVE8236 NVE20445 NVE22417 NVE12771 NVE5351 NVE10227 NVE10095 NVE6295 NVE459 NVE24336 NVE21893 NVE647 NVE11119 NVE339 NVE12366 NVE8902 NVE12655 NVE18293 NVE19543 NVE15604 NVE12992 NVE23664
30_32Sdown	15	NVE19460 NVE4730 NVE7512 NVE23153 NVE10075 NVE10189 NVE22411 NVE24607 NVE2217 NVE2187 NVE4672 NVE7446 NVE8461 NVE24899 NVE5385
20_32SCup	46	NVE536 NVE9930 NVE18990 NVE6235 NVE16630 NVE23792 NVE21102 NVE8783 NVE10180 NVE17463 NVE9886 NVE16799 NVE13685 NVE15287 NVE21823 NVE13315 NVE2064 NVE17138 NVE7318 NVE22379 NVE5576 NVE16400 NVE11344 NVE9193 NVE2964 NVE16052 NVE2618 NVE21133 NVE18224 NVE2682 NVE14220 NVE15088 NVE1614 NVE23120 NVE1808 NVE13301 NVE20844 NVE24003 NVE2965 NVE21332 NVE10804 NVE25581 NVE18683 NVE18753 NVE529 NVE17346
20_30SCup	51	NVE2049 NVE6310 NVE20133 NVE19732 NVE23810 NVE7851 NVE6685 NVE7522 NVE24795 NVE15195 NVE16891 NVE9071 NVE6609 NVE19183 NVE857 NVE45 NVE13747 NVE1939 NVE882 NVE16086 NVE25536 NVE10325 NVE14800 NVE22414 NVE10173 NVE15615 NVE15529 NVE26074 NVE9787 NVE8075 NVE21950 NVE23458 NVE4723 NVE3406 NVE23726 NVE5154 NVE19184 NVE16286 NVE10911 NVE20233 NVE23813 NVE21972 NVE10888 NVE25569 NVE4141 NVE19863 NVE7823 NVE24162 NVE3509 NVE9125 NVE4658

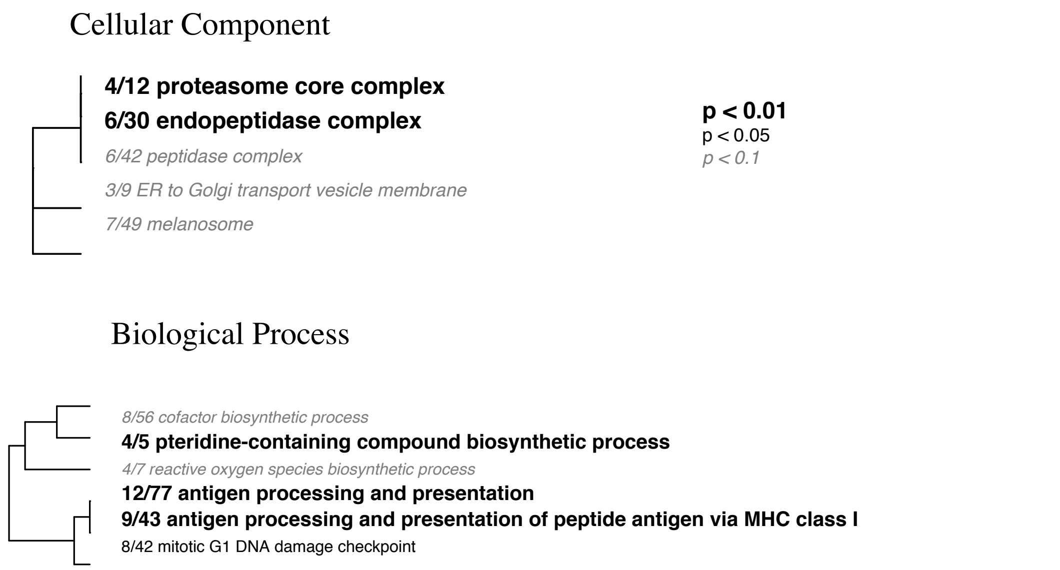

Cellular Component
p < 0.01
p < 0.05
p < 0.1
22/44 cell-cell adherens junction
51/126 cell-cell junction
71/196 cell junction
10/18 cell-cell contact zone
21/60 cell-substrate junction
48/116 anchoring junction
72/231 perinuclear region of cytoplasm
4/5 dendrite membrane
27/57 leading edge membrane
67/239 intrinsic component of plasma membrane
60/141 cell leading edge
22/65 ruffle
33/66 lamellipodium
13/21 neuron spine
54/129 dendrite
124/349 cell projection part
7/14 dendritic shaft
34/95 axon part
17/46 site of polarized growth
111/313 neuron projection
60/173 axon
11/27 perikaryon
67/197 cell body
57/169 synapse part
18/50 postsynaptic density
75/213 synapse
5/5 spectrosome
9/19 fusome
38/121 apical part of cell
5/7 photoreceptor inner segment
10/20 basal part of cell
7/14 basal plasma membrane
81/263 plasma membrane region
21/54 actin-based cell projection
13/28 microvillus
10/22 myosin complex
49/128 actin cytoskeleton
6/11 filamentous actin
11/27 actomyosin
11/27 cortical cytoskeleton
60/172 cytoplasmic region
25/60 cell cortex part
26/73 membrane region
16/30 sarcolemma
11/27 ion channel complex
5/8 voltage-gated calcium channel complex
35/101 plasma membrane protein complex
18/50 I band
12/24 ciliary transition zone
7/12 photoreceptor connecting cilium
37/92 ciliary part
51/134 cilium
15/21 photoreceptor outer segment
37/121 cell surface
59/198 cytoplasmic vesicle part
43/139 vesicle membrane
6/11 cytoplasmic vesicle lumen
30/86 secretory granule
44/133 secretory vesicle
11/25 early endosome membrane
27/84 early endosome
15/31 cell division site
18/50 midbody
12/24 extracellular matrix component
27/70 nuclear membrane
Molecular Function
6/7 ion channel binding
36/97 calcium ion binding
93/250 cytoskeletal protein binding
49/119 actin binding
25/46 actin filament binding
5/7 spectrin binding
51/158 protein-containing complex binding
25/41 calmodulin binding
8/11 myosin light chain binding
13/33 myosin binding
28/83 tubulin binding
24/61 microtubule binding
6/10 phosphatidylinositol kinase
5/7 1-phosphatidylinositol-3-kinase
9/12 calmodulin-dependent protein kinase
38/117 protein serine/threonine kinase
55/179 phosphotransferase, alcohol group as acceptor
26/67 small GTPase binding
12/30 Ras guanyl-nucleotide exchange factor
30/91 GTPase binding
14/30 Rho GTPase binding
89/282 enzyme binding
10/23 protein kinase C binding
33/107 nucleoside-triphosphatase regulator
18/52 GTPase activator
14/29 protein phosphatase binding
17/42 phosphatase binding
11/22 protein-containing complex scaffold
6/8 receptor signaling complex scaffold
36/108 protein domain specific binding
8/17 SH3 domain binding
11/27 phosphatidylinositol bisphosphate binding
4/5 phosphatidylinositol-3,4-bisphosphate binding
7/12 phosphatidylinositol-3,4,5-trisphosphate binding
30/86 phospholipid binding
14/38 phosphatidylinositol phosphate binding
8/15 phosphatidylinositol-3-phosphate binding
10/19 cadherin binding
8/11 alpha-catenin binding
24/59 cell adhesion molecule binding
13/16 actinin binding
11/27 beta-catenin binding
12/20 transmembrane receptor protein phosphatase
57/196 molecular transducer
16/39 thiolester hydrolase
18/34 protein tyrosine phosphatase
27/66 phosphoprotein phosphatase
4/5 non-membrane spanning protein tyrosine phosphatase
9/18 phosphoric diester hydrolase
4/6 phospholipase C
63/197 hydrolase, acting on ester bonds
43/108 phosphoric ester hydrolase
28/77 metal ion transmembrane transporter
12/31 calcium channel
18/44 divalent inorganic cation transmembrane transporter
13/20 voltage-gated channel
23/69 passive transmembrane transporter
6/8 voltage-gated calcium channel
8/15 potassium ion transmembrane transporter
4/5 H4 histone acetyltransferase
9/15 peptide N-acetyltransferase
14/32 acetyltransferase
13/23 N-acyltransferase

Biological Process

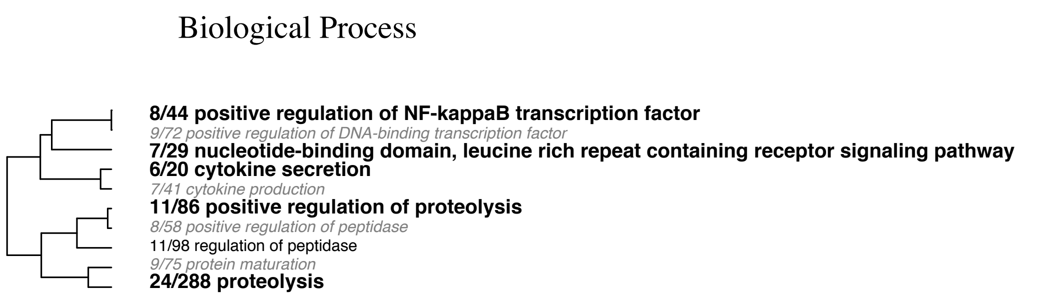
Supplemental Figure 1: GO enrichment terms for the blue module from WGCNA



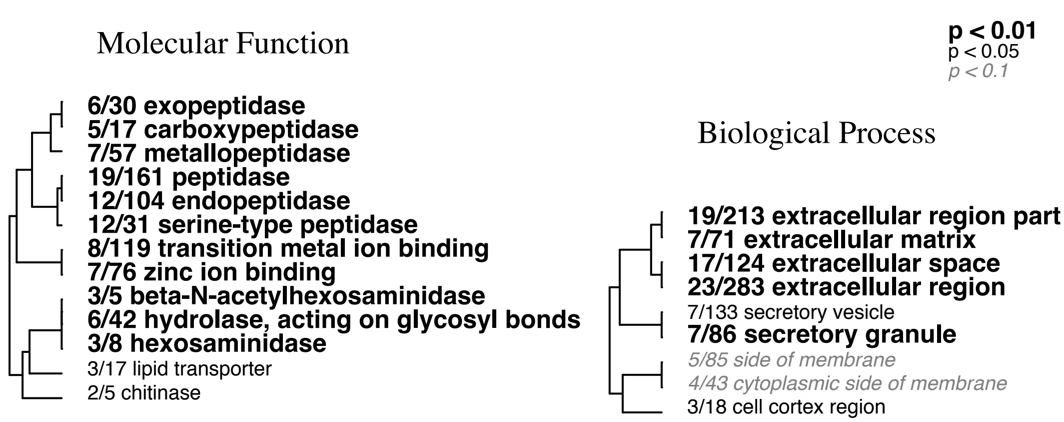
Supplemental Figure 2: GO enrichment terms for the brown module from WGCNA

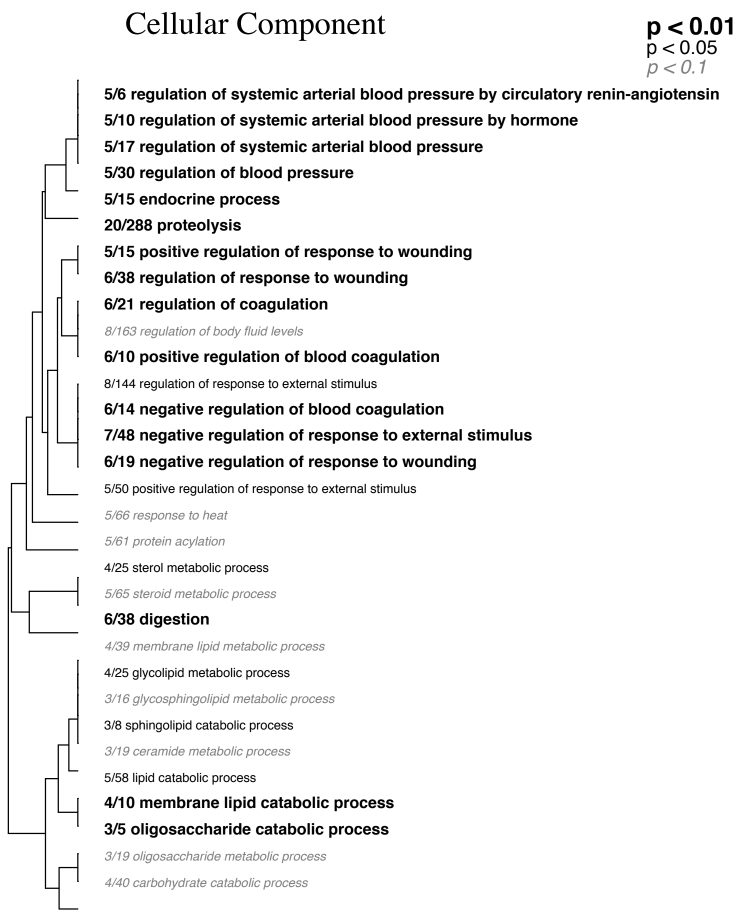


Supplemental Figure 3: GO enrichment terms for the cyan module from WGCNA



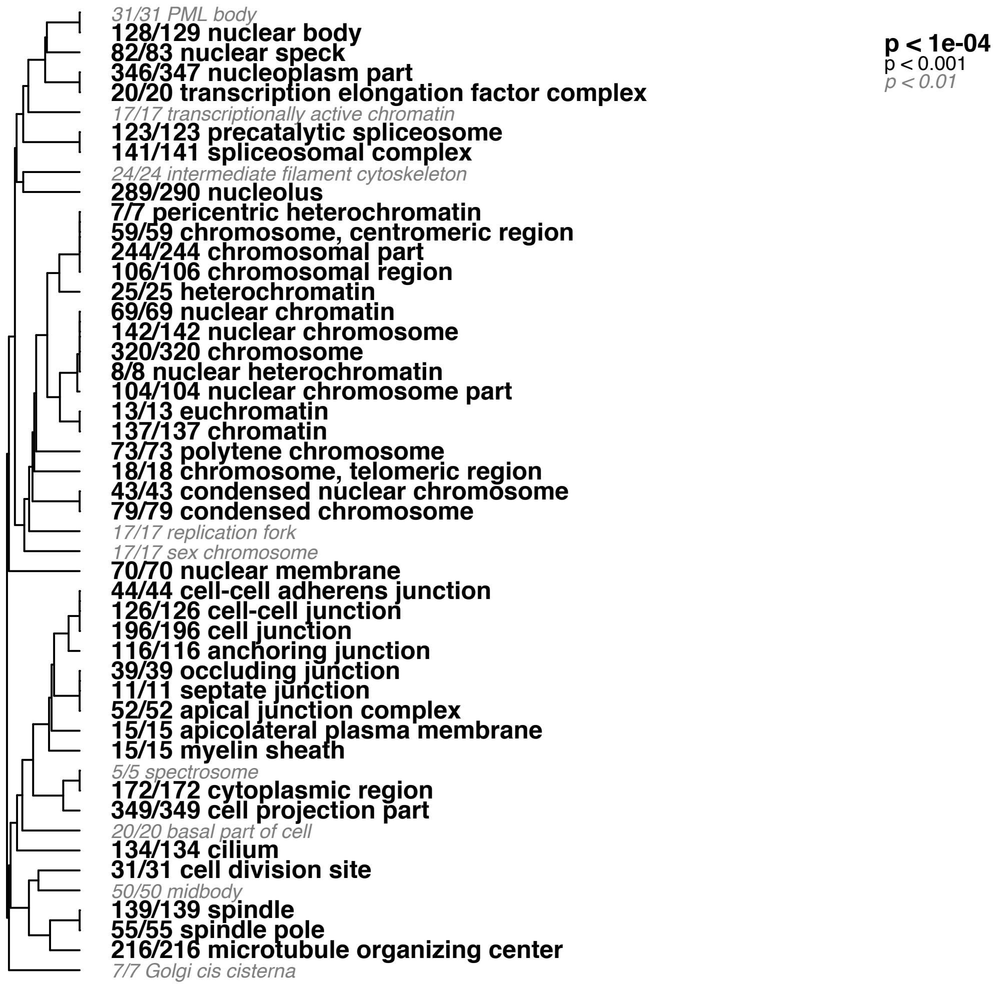
Supplemental Figure 4: GO enrichment terms for the green module from WGCNA



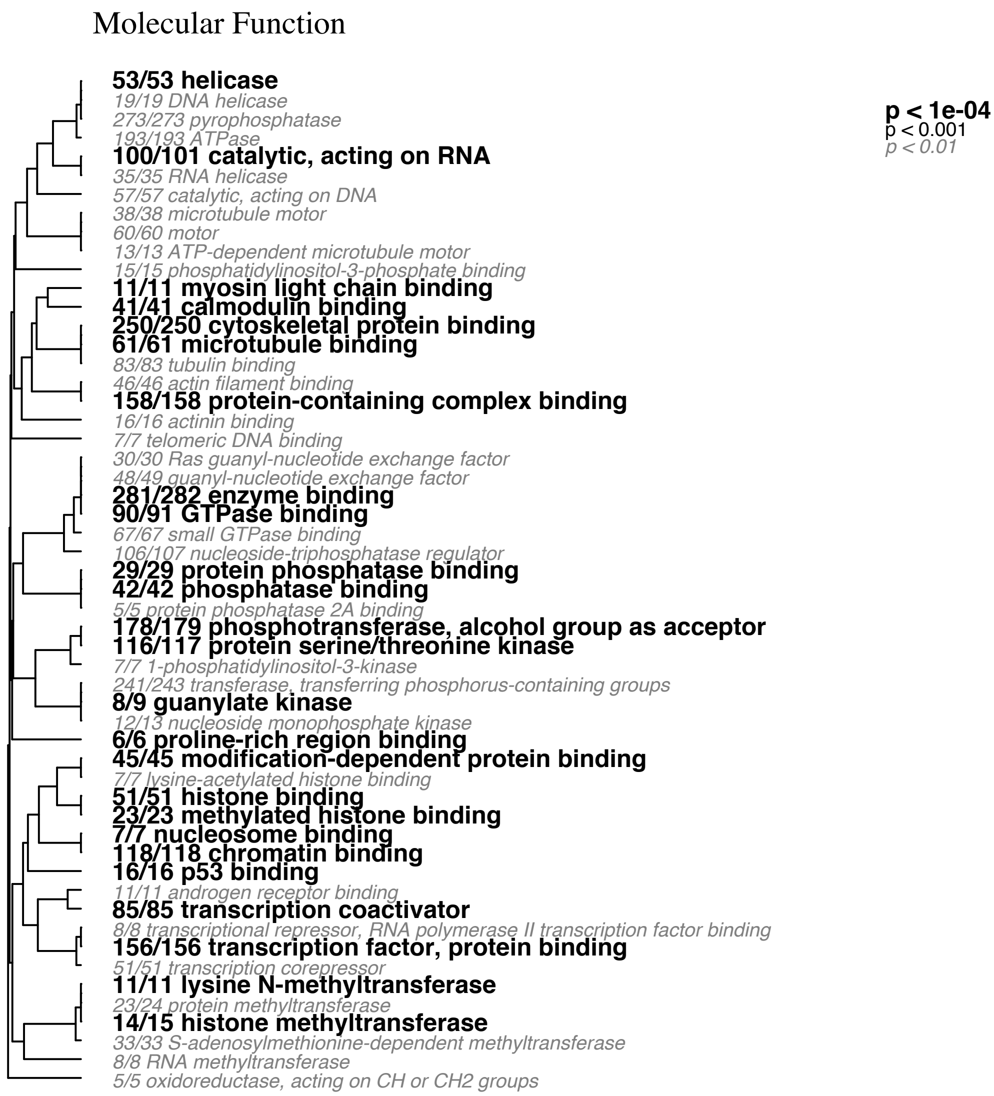


Supplemental Figure 5: GO enrichment terms for the green yellow module from WGCNA

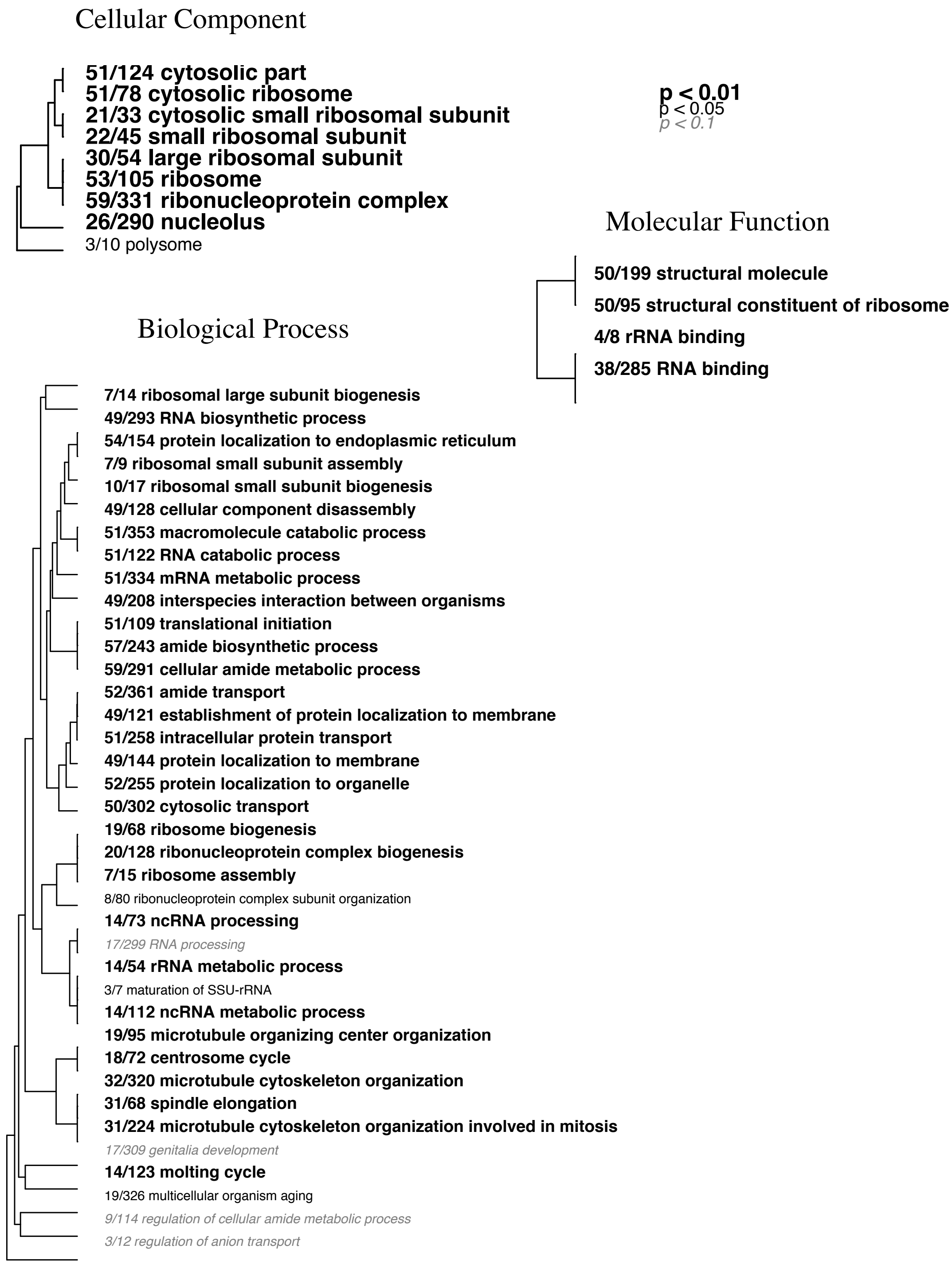
Cellular Component



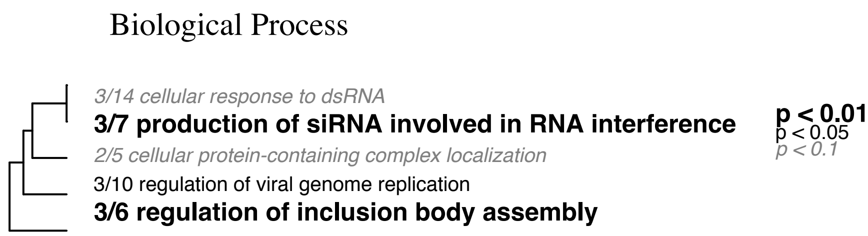
Biological Process



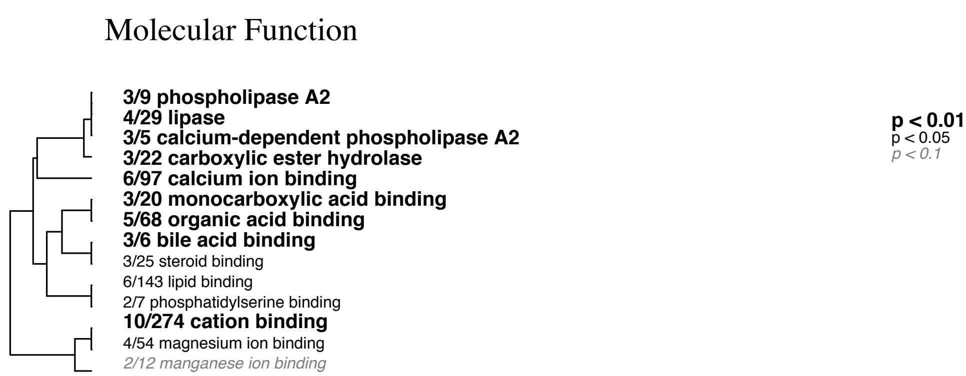
Supplemental Figure 6: GO enrichment terms for the grey60 module from WGCNA



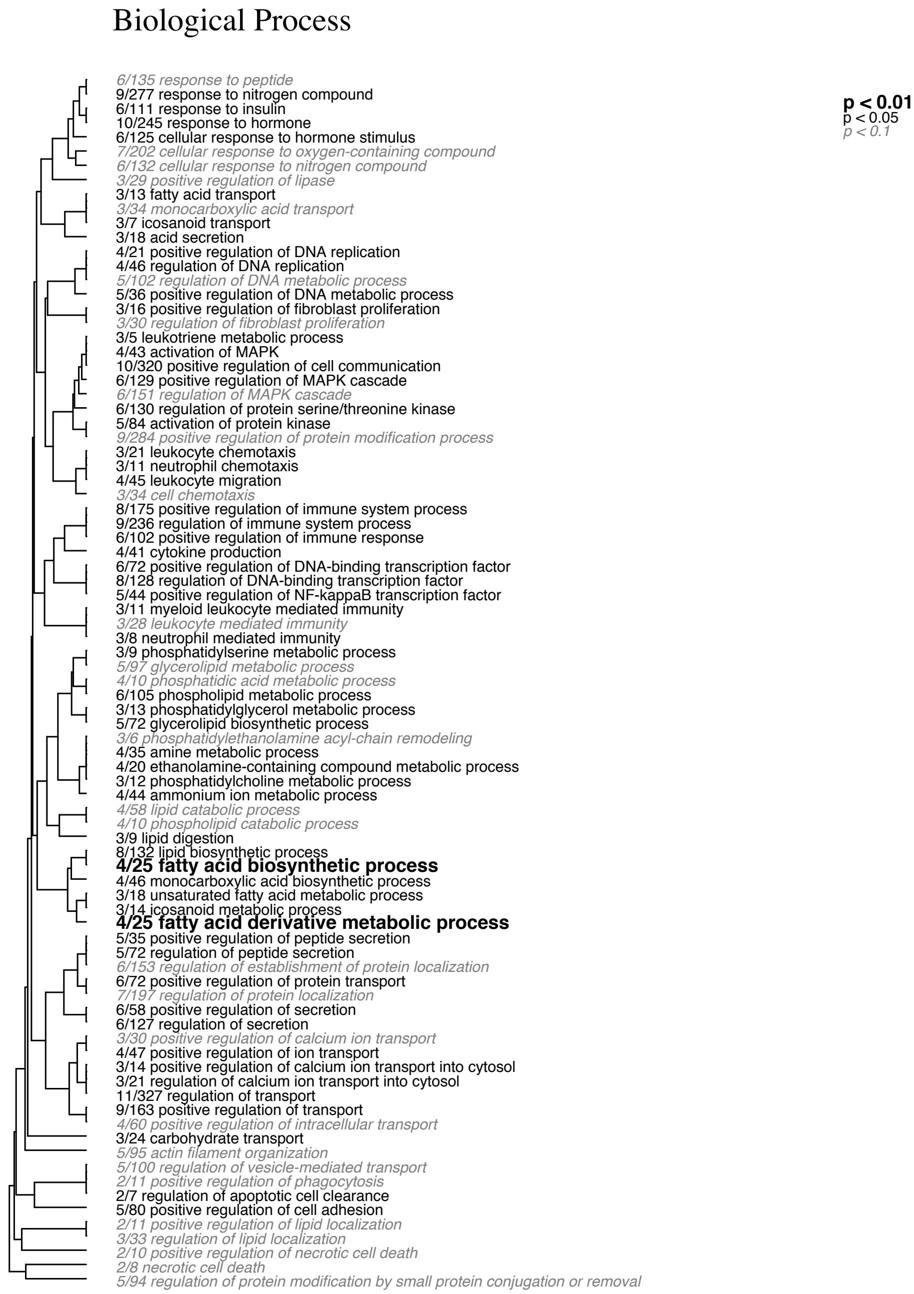
Supplemental Figure 7: GO enrichment terms for the light green module from WGCNA



Supplemental Figure 8: GO enrichment terms for the salmon module from WGCNA



Supplemental Figure 9: GO enrichment terms for the tan module from WGCNA



Supplemental Figure 9 (continued): GO enrichment terms for the tan module from WGCNA