GENETIC CONTROL OF THE EXPRESSION PATHWAY IN HUB GENES FROM *Saccharomyces cerevisiae*

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Benvolguts/des coordinadors/res de l'assignatura Treball Fi de Grau,

Autorizo na Laura Llobet i Reixach a l'entrega i presentació del seu Treball de Fi de Grau titulat "Genetic control of the expression pathway in hub genes from *Saccharomyces cerevisiae*" que ha realitzat sota la meva supervisió.

Atentament,

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ABSTRACT

The genetic bases of complex traits are a challenging matter of study, given the complexity of the underlying regulation. High-throughput technologies enable collecting data about cellular traits, such as gene expression, which acts as an intermediate molecular layer between genotype and phenotype. In this project, we carried out a statistical analysis to identify loci involved in regulation of gene expression (known as *expression quantitative trait loci* - eQTL), map them on the *Saccharomyces cerevisiae* genome and quantify their contribution by estimating the proportion of expression variance explained by them. We have compared data from six different experimental conditions to study the influence played by environment in genetic control of expression. In addition, we also calculate the degree of connectivity of our genes under study with all other genes of the data set, so correlation between connectivity and genetic control of gene expression can be analyzed. Our results indicate that genes with a strong genetic regulation of their expression levels show also a large degree of connectivity with other genes, becoming hubs in the underlying gene regulatory network. Additionally, they are characterized by a very homogenous eQTL map, despite environmental conditions; in contrast to those genes with a very poor genetic regulation, which show a very variable control of gene expression.

INTRODUCTION

Understanding the relationship between genotype and phenotype is very helpful for solving current biological problems, such as predicting disease risk in human population, improving productivity in farming and cattle raising, and predicting adaptative evolution.

Most heritable traits show continuous phenotypic variation, mainly due to underlying complex genetic regulation. It is a consequence of multiple interacting loci, with different allelic effects depending on environmental conditions; so it is not a trivial problem to handle with [1].

Genetic loci associated with quantitative heritable traits are known as *quantitative trait loci* (QTL). QTL mapping requires both phenotypic and genotypic data and it consists of determining if there is a statistically significant phenotypic difference among individuals with different genotype background. Once detected and localised, it is important to quantify the magnitude of such genetic effects. One of such magnitudes is the percentage of phenotypic variance explained by each QTL. It is important to keep in mind that variance explained by QTLs is often far from the percentage of the phenotypic variance with a genetic origin. This is known as “the missing heritability problem”, and is a current matter of debate in complex traits studies [2].

Recent technological developments in genome and transcriptome sequencing have enabled us to get new information about molecular intermediates between genotype and phenotype, which provide new insights into the genetic regulation of complex heritable traits. In 2002, Brem et al. [3] showed that cellular traits, such as gene expression, are inherited in a quantitative fashion as well, so studying genetic variants that affect gene expression is a good approach to increase statistical power to study genetic bases of complex traits, as well as to better understand underlying genetic regulatory mechanisms.

PROBLEM APPROACH AND OBJECTIVES

In a recent study by Tur et al. [5], where eQTLs were mapped to the yeast genome using data from a yeast cross published by Brem et al. [3], it was discovered that genes whose eQTLs explain 70% or more or their expression variance were highly connected in the functional network estimated from their expression profiles.

This project aims to study *expression QTL* (eQTL) in the *Saccharomyces cerevisiae* genome. Starting from a first data set of expression levels from 8382 genes in five different environments, eQTL mapping was restricted to a limited set of genes in order

to do a more simple and accurate analysis. However, we introduced data from them all, so we are able to elucidate the degree of connectivity of those we are studying, taking into account all 8382. We performed our analysis in 13 genes, involved in 3 different cellular pathways: 7 in mating regulation, 3 in daughter cells separation and 3 in leucine biosynthesis. They were chosen from those identified in a recent study by Tur et al. [5], whose eQTLs explain 70% or more of their expression variance and are located in a different chromosome from the linked gene. By doing so, it is intended to (i) understand the architecture of genetic regulation of expression of these genes more accurately, (ii) elucidate the role of environment in genetic expression and (iii) study implications of eQTLs in different cellular pathways.

MATERIALS AND METHODS

Strains and expression data

Experimental data was produced by Gagneur and colleagues [6]. They gathered information about expression of 8382 genes (both coding and non-coding) from an average of 35 individuals of *S.cerevisiae* in 5 different experimental conditions (glucose, low iron, rapamycin, ethanol and maltose). In order to be able to correlate a given phenotype with a determinate genotype, haploid organisms are recommended. So, in this kind of analysis, haploid spores from tetrads are used. This is made possible by doing crosses between haploid MATα and MATa strains, putting the resulting diploid through meiosis and sporulation, and physically dissecting the four ascospores from an ezimatically digested tetrad. Spores are allowed to germinate and the phenotypes of all segregants are determined [7]. Expression levels were estimated by tiling-array assays, in mid-exponential phase culture.

Segregants used in each experimental condition were chosen randomly from an initial data set of 159 individuals, which were obtained from Mancera and colleagues [8] data. These were derived from a cross of *S.cerevisiae* strains S96 (MATa ho:: lys5 gal2) and YJM789 (MAT ho::hisG lys2 gal2).

Regarding Tur et al. data, they used gene expression information by Brem et al. [9]. They used a yeast cross between BY4716, an isogenic to the the lab strain S288C, and the wild isolate RM11-1a.

Data availability

Expression data used in this project were downloaded from the ArrayExpress repository (http://www.ebi.ac.uk/arrayexpress/) under accession number E-MTAB-1398. Genotype data were obtained from the supplementary information available in the electronic version of Mancera et al. paper [8]

Processing of genotype data

Position of the markers in Mancera et al. data were specified in physical distances, but our analysis required genetic distances. So, we convert them following equivalence data available in Saccharomyces Genome Database (SGD, http://www.yeastgenome.org), where genetic distance vs. physical distances ratios for each chromosome are specified.

Moreover, there were some markers with missing genotypes or which were not informative that were removed using R/qtl package. At the end, our analysis was performed with the filtered data specified in Table 1.

eQTL mapping

eQTL mapping was conducted by single marker regression, which considers each marker individually. Individuals are split into groups according to their genotypes, and phenotype averages of each group are compared. Given that we analyse haploid spores from a tetrad, individuals have only two possible genotypes, just as in a backcross, so we perform our analyses as a backcross. This process is carried out using R/qtl package. [13]

Evidence of a QTL is measured by a LOD score: the $\log_{10}$ likelihood ratio comparing the hypothesis that there is a QTL at the marker to the hypothesis that there is no QTL at the marker. Larger LOD scores indicate stronger association between the marker and the phenotype. To assess which are statistically significant, we consider the global null hypothesis that there is no QTL anywhere in the genome. To test such hypothesis, a null distribution of the genome-wide maximum LOD scores has been derived by a permutation test. It consists of shuffling the phenotypes relative to the genotype data, getting a new data set, on which the QTL mapping method will be also applied. LOD score values corresponding to the top 5% of this distribution will be taken as the cutoff to consider a LOD score significant. Permutation tests are computationally demanding in R, so we ran these calculations in parallel by using packages *snow* and *rlecuyer.*

Calculation of percentage of variance explained by eQTLs

The proportion of variance of gene expression explained by eQTLs is calculated as the difference in unexplained variance between the null and alternative models, divided by the total phenotype variance, using the function `fitqtl()` from the R/qtl package.

In cases where we got more than 1 QTL per gene, we fit a multiple QTL linear model using again `fitqtl()` to assess the significance of each QTL, given the others. By doing so, we are able to elucidate if some of them are tagging the same causal variant and discard the redundant ones.

Calculation of degree of connectivity

Using each expression data set and the algorithm developed by Tur et al. [5] implemented in the R package `qpgraph`, we calculated the number of genes that are functionally related with each of the 13 genes we are analyzing in each experimental condition. This algorithm estimates the presence of a gene-gene functionally relationship from expression data adjusting for indirect effects. To enable this adjustment, q-order correlations are calculated from the entire expression data set, using q=75 (Brem et al.), q=23 (glucose), q=16 (low iron), q=14 (rapamycin), q=16 (ethanol) and q=15 (maltose). From the resulting q-order correlations, a network was estimated by a graph with gene pairs for which the null hypothesis of no q-order correlation was rejected 90% or more of the times it was tested.

Given that the number of connections we get depend on the sample size, and it is different for the 6 experimental conditions, we ranked them in each condition and calculated the percentile of genes below every observed degree of connectivity, so that we can compare results between different experimental conditions.

RESULTS

We performed eQTL mapping of each of the selected 13 genes with more than 70% of expression variance explained by their eQTL located in a different chromosome, as observed in Tur et al. data. It was carried out using data from 6 different eQTL experiments, which are described in Table 1. For each data set, we calculated the LOD score for determininig which markers tag the corresponding eQTL, the percentage of variance explained by them and the degree of connectivity of each gene with all the other ones in the functional network estimated from the expression data of the corresponding environment.

Results obtained in eQTL mapping assay, as well as the percentage of expression variance due to each eQTL and connectivity analyses are plotted in Figure 1, where colour, shape and size of dots represent the experimental condition, number of eQTLs and ranking in connectivity analysis, respectively. In addition all numerical data from these experiments are described in detail in Table S1, on the supplementary data.

Table 1 – Description of data used in our analysis. For all 6 experimental conditions, number of individuals, number of phenotypes available, and number of markers with available information are specified in corresponding columns. Brem et al. data [9] is which Tur et al. used in their analysis. Strains are derived from a cross of BY4716, isogenic to the lab strain S288C and the wild isolate RM11-1a. On the other hand, data from the other 5 environments are from Gagneur et al. data [6], which used S96 and YJM789 as parental strains in their cross.

	Number of individuals	Number of phenotypes (genes)	Number of markers
Brem et al.	112	6216	1857
GLUCOSE	46	8382	2188
LOW IRON	33	8382	1612
RAPAMYICIN	29	8382	1479
ETHANOL	32	8382	1591
MALTOSE	31	8382	1488

Most genes related to mating process have strong genetic regulation of their expression

If we focus on those genes related with mating regulation, we can observe that 6 of them (STE6, STE3, BAR1, MF(ALPHA)1, AFB1 and MFA2) show a very well conserved genetic regulation in all experimental conditions. In the *S.cerevisiae* strain used by Brem et al., a single eQTL was detected in chromosome III, at 96.56 cM. Using *Gagneur et al.* data we also found a single eQTL in all environment conditions, located in chromosome III as well, at a similar position (ranging from 91.98 to 98.77 cM). All eQTLs were detected with a remarkably high LOD score. Indeed, it is of notice that these 6 genes are the ones with the largest proportion of expression variance explained by their eQTLs: in all cases it scores 70% at least, and remarkably STE3 scores more than 90% in all our 5 growth mediums. In addition, they all are largely connected with all other set of 8382 genes we have studied, given that the vast majority ranks at the top 5% of those with more number of connections in the corresponding environment.

In contrast, gene STE2 shows great differences among the six experimental conditions. A single eQTL in chromosome III (96.56 cM) was reported by Tur et al. However, in our analysis we found 2 eQTLs in both glucose and low iron environments, a single one when yeast were grown in rapamycin or ethanol, and none of them were they were grown in maltose medium. In glucose, low iron, rapamycin and ethanol conditions, one eQTL in chromosome VI was detected at 57.23, 44.57, 44.23 and 37.12 cM, respectively. Moreover, in glucose and low iron environments, another

eQTL was detected in chromosome III at 94.74 cM and 101.65 cM, respectively. Additionally, in these mediums STE2 show a high degree of connectivity. On the contrary, it is very low in rapamyicin, ethanol and maltose. In STE2, we didn't find so strong association between markers and eQTL as in previous six genes, given that LOD scores are notably lower, ranging from 5.1 to 6.56, and also the proportion of variance explained by them is more moderate: it scores between 52.01 and 82.33 %.

Expression of genes which control daughter cells separation is homogeneous among different conditions

Genes involved in daughter cells separation pathways (DSE1, SCW11 and DSE2) show very well conserved genetic regulation. For all three genes and all five growth mediums tested, a single eQTL has been detected with notably high LOD scores (5.74 – 19.56). In all cases, it is located in chromosome II at a very similar position, in a range from 165.04 to 172.66 cM, just as in Brem et al. strain, which it is reported to be in chromosome II, at 166.9 cM. They also show homogenous percentage of expression variance explained by such eQTLs, which scores between 57.38 and 85.88%. Similarly, from a connectivity point of view, they all rank between 90% and 95% in corresponding environments.

Genes which play a role in leucine biosynthesis are differently regulated depending on environment

Finally, genes involved in leucine biosynthesis (BAT1, LEU1 and OAC1) show a very variable genetic regulation, depending on growth medium and genetic background. Strains used by Brem et al. were reported to have an eQTL each, localised in chromosome III, at 39.27cM for BAT1 and 44.34 cM for LEU1 and OAC1, but in our analysis we got very different results. In glucose conditions, LEU1 and OAC1 show a single eQTL each, both in chromosome XV, at 169.86 cM and 166.39 cM, respectively. Proportion of variance explained by them is quite similar in both cases (42.44% in LEU1 and 38.68% in OAC1), as well as degree of connectivity (LEU1 ranks at 68.59%, and OAC1, at 51.81%). However, it is of notice that, comparing data with Brem et al. data, there is a notable difference in both percentage of variance (30%, at least) and degree of connectivity. In a maltose medium, one eQTL in chromosome VII (395.05 cM) is found for all three genes and, additionally, BAT1 shows a second eQTL in chromosome XVI (206.22 cM). Variance explained by them is around 70% both in BAT1 and OAC1, similar to data from Brem et al, but it scores only 41.6% in LEU1. Degree of connectivity is zero in both BAT1 and LEU1, and extremely low in OAC1.

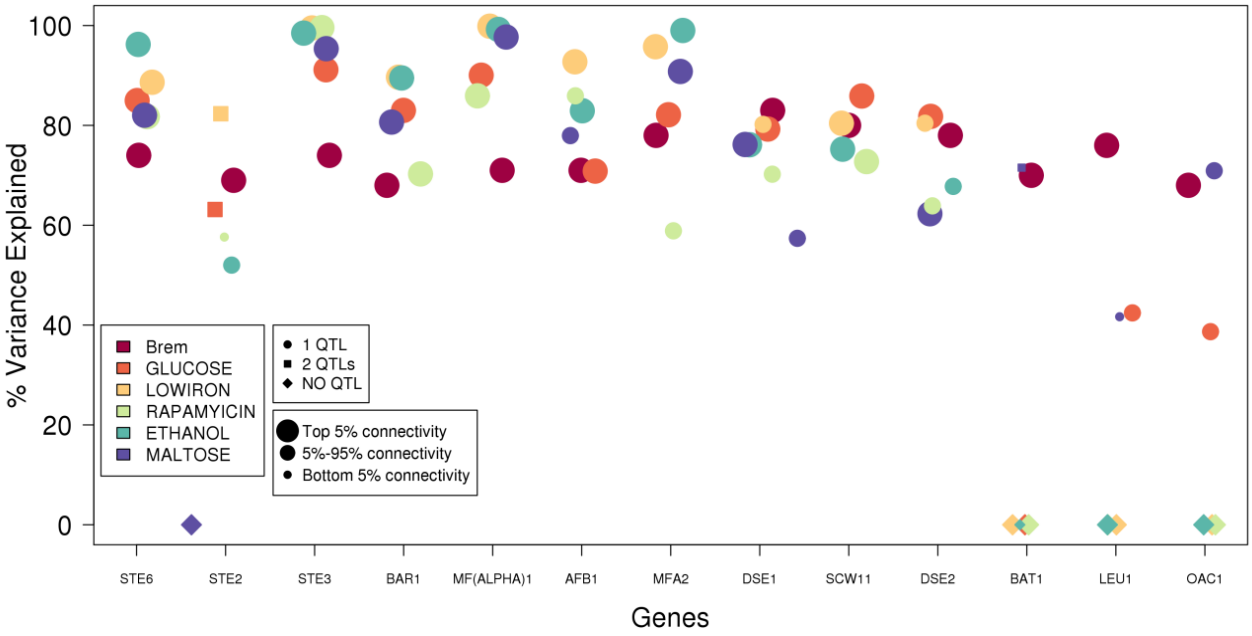

Figure 1 - Proportion of genetic expression variance explained by eQTL(s). For each of 13 genes that have been analysed, there are 6 dots plotted, corresponding to 6 different experimental conditions. Dot colour indicates the environment in which *S. cerevisiae* strains have been grown, dot shape reflects the number of eQTLs found, and dot size depends on the ranking position in the connectivity analysis. For all experimental conditions, 3 ranges have been established to classify the 13 genes of study: the largest dot represents those which have a degree of connectivity higher than 95% of other genes in the same environment, the medium one are for those which rank between 5% and 95%, and the smallest ones are those which rank at the bottom 5%.

Overall, it is observed that eQTL map pattern differs depending on the cellular pathway we focus on. Almost all genes involved in mating regulation, in all conditions, show a single eQTL in chromosome III at a very similar position. There is only a single exception, gene STE2, which shows high variability in its eQTL, depending on the environment. Genes related to daughter cells separation have a single eQTL in chromosome II, at almost the same position for all conditions studied. Finally, genes responsible for leucine biosynthesis show very variable eQTL maps. In Figure 2, differences between genes with different eQTL map pattern are shown.

Remarkable differences in genetic control of gene expression are seen among cellular pathways

If we analyze the dot distribution in Figure 1 there are some interesting features to highlight. The first seven genes are those involved in mating regulation, and in general, show quite homogenous cloud of dots: located at the top of the graphic, with similar shape and size. The only exception is gene STE2, the dots of which are more spread, with variable shapes and sizes. The following three genes are those related with daughter cells separation, and these do show extremely homogeneity. They are located

at slightly lower level than the previous ones, but they all show same shapes and sizes. The last three genes are those involved in leucine biosynthesis, and one feature to highlight is that only eQTLs have been found when yeast have been grown in mediums rich in carbohydrates (glucose and maltose). There is an exception in BAT1, where no eQTLs have been found in glucose conditions, but on the other hand we found 2 of them in maltose environment. Brem et al. strains have been grown in minimum medium, which also contains hydrates of carbon. Another important feature to highlight is that we don't find large points at the bottom of the figure, neither small ones at the top, indicating that those genes with a strong genetic regulation, given that their eQTLs explain a large proportion of their expression variance, are also those with a high degree of connectivity in the gene functional network.

In conclusion, notable differences can be noticed among genes belonging to the three different pathways, not only for the percentage of expression variance explained by their eQTLs, but also for the pattern in their eQTL maps. In addition, there is a correlation between strong genetic regulation and a high degree of connectivity, in all cases of study.

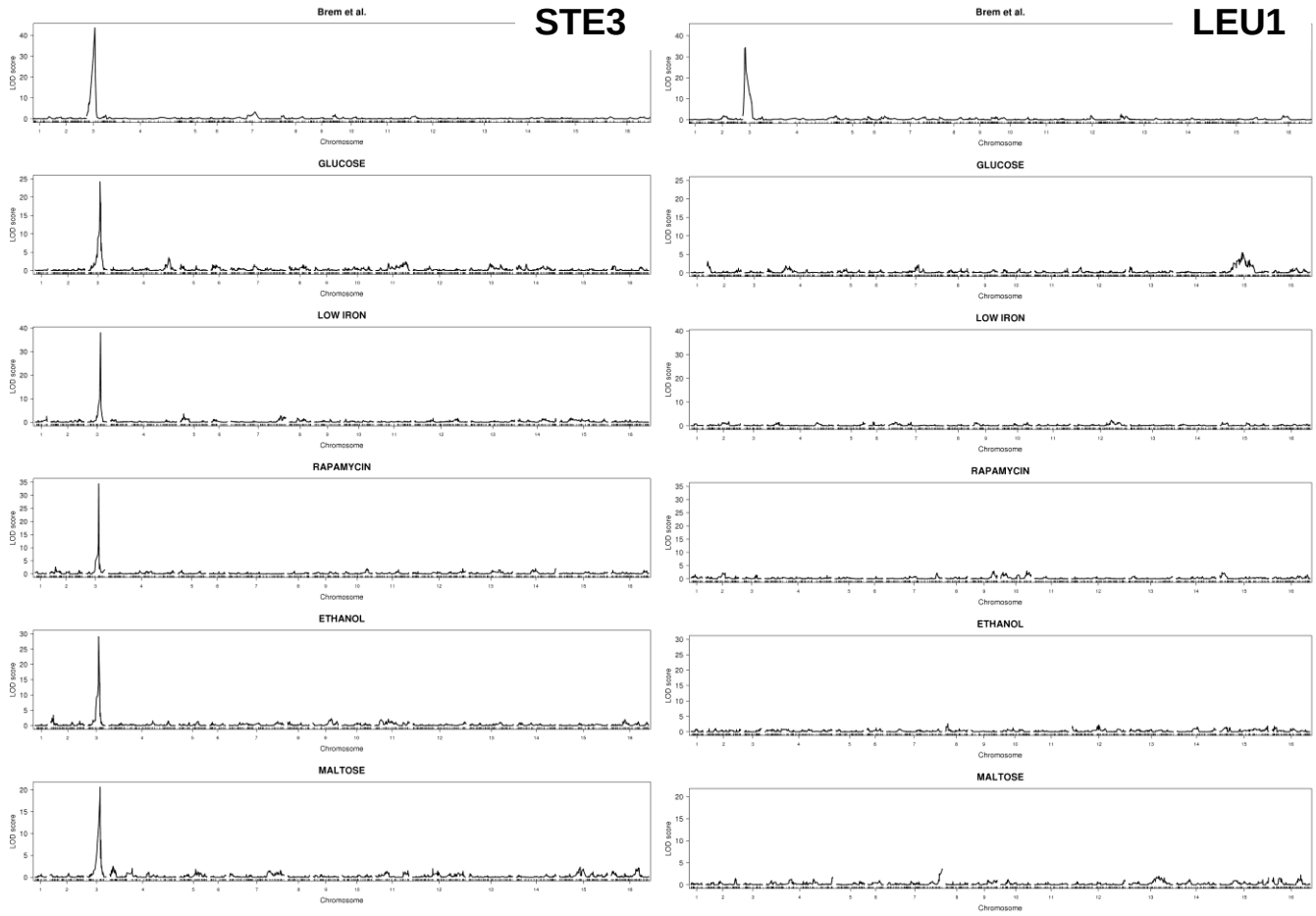

Figure 2 – LOD score profile along all *S. cerevisiae* genome. In these two panels, LOD scores for each marker analyzed is plotted. Panel on the left corresponds to STE3 gene, which belongs to mating regulation pathway, and it shows a very homogeneous eQTL map for all experimental conditions. In contrast, panel on the right corresponds to LEU1 gene, which belongs to leucine biosynthesis pathway and shows a very variable map.

DISCUSSION AND CONCLUSIONS

The genetic bases of gene expression play an important role in gene regulation underlying most large-scale phenotypes. Yet, mapping and interpreting the genetic components of expression variance is difficult due to the fact that gene expression is a high-dimensional multivariate molecular phenotype. A way to approach this complexity is to think of genes as forming a network of functional associations with direct and indirect effects, some of which with a genetic origin. In Tur et al. data was observed that those genes with a high number of associations also showed a very strong genetic control of their expression. In this project, we elucidated which of these genes preserve such a strong genetic regulation in different environmental conditions and the importance of it in different cellular pathways

The architecture of gene networks has been studied by calculating degree of connectivity of each gene given all the others in each corresponding environment. We could observe that, similar to Tur et al. analyses, those genes that are related to a higher number of genes also present a stronger genetic regulation of their expression. We could think that this may be because of the higher number of connections they have, but we have realised that in general, all genes have one single eQTL, two at maximum. So that, we have to conclude that eQTL of genes with a higher degree of connectivity have a stronger effect than those related with genes connected with just a few.

Regarding the role of environment in the control of genetic expression, we have observed that in the major part of cases we have studied, genetic regulation is similar among all different experimental conditions, so it does not have a very strong effect in this subset of genes under strong genetic regulatory control. However, genes involved in biosynthesis of leucine aminoacid, show remarkable differences. In these cases, we only found genetic regulation when yeasts were grown in mediums rich in hydrates of carbon, like glucose and maltose, but not in ethanol, rapamycin or low iron conditions.

Comparing data obtained from eQTL mapping in each of three cellular pathways analysed, it is clearly seen that cellular pathways that contain genes with a very strong genetic regulation, show a single eQTL in all conditions which maps at a very similar position in all cases, whereas those genes with a very poor genetic control of their expression show a very variable map of their eQTLs. So, we can conclude that cellular pathways where control of genetic expression is crucial possess very well conserved eQTLs, while those pathways in which genetic expression is not so important show more variable eQTLs.

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Table S1 - Results of eQTL mapping analysis. For each gene and experimental condition we analysed, it is reported the information of corresponding eQTL(s). It is reported the chromosome and the genetic position (in centimorgans) where are located, as well as the LOD score obtained in the analysis and the percentage of expression variance explained by them. In cases where more than one eQTL have been found, we report the variance explained by all together. Those situations where no eQTLs have been found are marked with dashes (-). In the last column, number of connections of each gene taking into account the whole amount of 8382 is reported. Given that this number may be very variable depending on experimental conditions, we also report the ranking position of each within the corresponding environment, so they are easier to compare (e.g, a gene with a degree connectivity of 25 which ranks at 99% means that such gene is connected with 25 other genes and this connectivity is larger than the 99% of other genes in the corresponding experimental condition).

GENE		**EXPERIMENTAL**	**eQTL**				**Degrees of connectivity**
Name *Cellular pathway*	**Chr**	**CONDITION**	**Chr**	**Position (cM)**	**LOD score**	**Variance explained (%)**	(Ranking within environment - %)
STE6 *Mating regulation*	XI	Brem et al	III	96,56	53,90	74,00	25 (99,97)
		GLUCOSE	III	94,74	18,93	84,97	42 (97,98)
		LOW IRON	III	98,70	15,59	88,64	41 (97,83)
		RAPAMYICIN	III	96,60	10,71	81,75	38 (97,04)
		ETHANOL	III	91,98	22,77	96,22	37 (97,40)
		MALTOSE	III	98,77	11,56	82,03	42 (95,89)
STE2 *Mating regulation*	VI	Brem et al	III	96,56	43,10	69,00	20 (98,58)
		GLUCOSE	III	94,74	5,45	63,15	24 (92,68)
			VI	57,23	6,22		
		LOW IRON	III	101,65	6,56	82,33	13 (86,89)
			VI	44,57	5,44		
		RAPAMYICIN	VI	44,23	5,41	57,63	0 (0)
		ETHANOL	VI	37,12	5,10	52,01	1 (0)
		MALTOSE	-	-	-	-	6 (65,77)
STE3 *Mating regulation*	XI	Brem et al	III	96,56	43,70	74,00	23 (99,47)
		GLUCOSE	III	94,74	24,21	91,14	43 (98,22)
		LOW IRON	III	98,70	38,07	99,51	38 (97,18)
		RAPAMYICIN	III	96,60	34,43	99,58	44 (97,84)
		ETHANOL	III	91,98	29,07	98,48	40 (97,88)
		MALTOSE	III	98,77	20,66	95,36	44 (96,22)
BAR1 *Mating regulation*	IX	Brem et al	III	96,56	41,40	68,00	21 (99,01)
		GLUCOSE	III	94,74	17,10	83,00	45 (98,46)
		LOW IRON	III	98,70	16,25	89,65	39 (97,49)
		RAPAMYICIN	III	96,60	7,64	70,27	31 (95,48)
		ETHANOL	III	91,98	15,66	89,50	35 (96,91)
		MALTOSE	III	98,77	11,06	80,66	41 (95,64)

MF(ALPHA)1 *Mating regulation*	XVI	Brem et al	III	96,56	40,80	71,00	20 (98,59)
		GLUCOSE	III	94,74	23,05	90,05	41 (97,84)
		LOW IRON	III	98,70	47,39	99,87	38 (97,18)
		RAPAMYICIN	III	96,60	12,34	85,92	36 (96,74)
		ETHANOL	III	91,98	33,69	99,22	39 (97,76)
		MALTOSE	III	98,77	22,86	97,70	38 (94,81)
AFB1 *Mating regulation*	XII	Brem et al	III	96,56	47,70	71,00	19 (98,19)
		GLUCOSE	III	94,74	12,31	70,84	28 (94,16)
		LOW IRON	III	98,70	18,80	92,74	34 (96,26)
		RAPAMYICIN	III	96,60	12,34	85,92	23 (92,67)
		ETHANOL	III	91,98	12,28	82,93	35 (96,91)
		MALTOSE	III	98,77	10,18	77,95	34 (93,40)
MFA2 *Mating regulation*	XIV	Brem et al	III	96,56	36,50	78,00	25 (99,67)
		GLUCOSE	III	94,74	17,20	82,13	45 (98,47)
		LOW IRON	III	98,70	22,65	95,76	39 (97,49)
		RAPAMYICIN	III	96,60	5,60	58,88	24 (93,25)
		ETHANOL	III	91,98	32,10	99,01	40 (97,88)
		MALTOSE	III	98,77	16,06	90,80	36 (94,09)
DSE1 *Daughter cells separation*	V	Brem et al	II	166,69	43,40	83,00	13 (94,41)
		GLUCOSE	II	165,04	15,72	79,26	38 (97,12)
		LOW IRON	II	168,88	11,60	80,20	23 (93,17)
		RAPAMYICIN	II	172,66	7,63	70,22	21 (91,68)
		ETHANOL	II	167,66	9,95	76,12	27 (94,63)
		MALTOSE	II	167,99	9,66	76,17	36 (94,09)
SCW11 *Daughter cells separation*	VII	Brem et al	II	166,69	40,00	80,00	15 (96,06)
		GLUCOSE	II	165,04	19,56	85,88	39 (97,33)
		LOW IRON	II	168,88	11,69	80,43	24 (93,51)
		RAPAMYICIN	II	172,66	8,19	72,75	26 (93,93)
		ETHANOL	II	167,66	9,70	75,23	27 (94,63)
		MALTOSE	II	167,99	5,74	57,38	32 (92,68)
DSE2 *Daughter cells separation*	VIII	Brem et al	II	166,69	37,20	78,00	10 (90,27)
		GLUCOSE	II	165,04	17,02	81,80	33 (95,76)
		LOW IRON	II	168,88	11,69	80,43	21 (92,46)
		RAPAMYICIN	II	172,66	6,41	63,88	19 (90,54)
		ETHANOL	II	167,66	7,87	67,78	21 (92,43)
		MALTOSE	II	167,99	6,57	62,30	38 (94,81)

BAT1 *Leucine biosynthesis*	VIII	Brem et al	III	39,27	28,50	70,00	18 (97,67)
		GLUCOSE	-	-	-	-	4 (51,81)
		LOW IRON	-	-	-	-	4 (58,15)
		RAPAMYICIN	-	-	-	-	3 (46,37)
		ETHANOL	-	-	-	-	0 (0)
		MALTOSE	VII XVI	395,09 206,22	6,58 4,01	71,53	0 (0)
LEU1 *Leucine biosynthesis*	VII	Brem et al	III	44,34	34,40	76,00	21 (99,01)
		GLUCOSE	XV	169,86	5,52	42,44	7 (68,59)
		LOW IRON	-	-	-	-	4 (58,16)
		RAPAMYICIN	-	-	-	-	3 (46,37)
		ETHANOL	-	-	-	-	1 (0)
		MALTOSE	VII	395,09	3,63	41,67	0 (0)
OAC1 *Leucine biosynthesis*	XI	Brem et al	III	44,34	27,80	68,00	16 (96,75)
		GLUCOSE	XV	166,39	4,88	38,68	4 (51,81)
		LOW IRON	-	-	-	-	4 (58,16)
		RAPAMYICIN	-	-	-	-	12 (85,21)
		ETHANOL	-	-	-	-	15 (89,10)
		MALTOSE	VII	395,09	8,31	70,91	2 (26,28)

Genetic control of the expression pathway in hub genes from *Saccharomyces cerevisiae*

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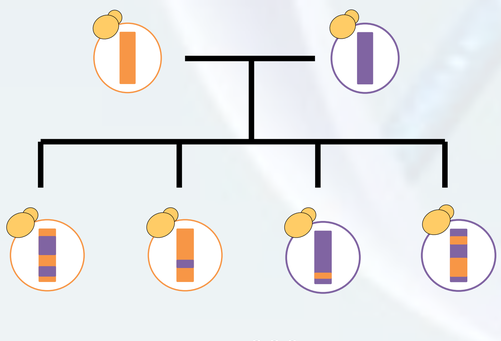
Treball de fi de grau, Biologia Humana. Universitat Pompeu Fabra (2014)

INTRODUCTION

The genetic bases of complex traits heritability are a challenging matter of study, given the complexity of genetic regulation underlying it. High-throughput technologies enable collecting data about cellular traits, such as gene expression, which acts as an intermediate molecular layer between genotype and phenotype. In this project, we carried out a statistical analysis to identify loci involved in regulation of gene expression (known as *expression quantitative trait loci* - eQTL), map them on the *Saccharomyces cerevisiae* genome and quantify their contribution by estimating the proportion of expression variance explained by them. In addition, we also calculate the degree of connectivity of our genes under study with all other genes of the data set. By doing so, it is intended to:

- Understand the architecture of genetic regulation of expression of these genes more accurately
- Elucidate the role of environment in genetic expression
- Study implications of eQTLs in different cellular pathways

METHODOLOGY



EXPRESSION PROFILING

In order to compare among different environmental conditions and genotype backgrounds, we used data from two sources

- **Gagneur et al, 2013:** data about yeast grown in 5 different mediums: glucose, low iron, rapamycin, ethanol and maltose.
- **Brem et al, 2005**: yeast grown in minimum medium.

GENOTYPING

The corresponding genotypes for expression data were obtained from:

- **Mancera et al, 2008**: yeast cross between S96 x YJM789
- **Brem et al., 2005:** yeast cross between BY4716 x RM11-1a

eQTL mapping

It was conducted by single marker regression, using R/qtl package. Evidence of a QTL is measured by a LOD score, the larger it is, the stronger is the association between the marker and the phenotype.

We performed our analysis in 13 genes, involved in 3 different cellular pathways: 7 in mating regulation, 3 in daughter cells separation and 3 in leucine biosynthesis. They were chosen from those identified in a recent study by Tur et al. [5], whose eQTLs explain 70% or more of their expression variance and are located in a different chromosome from the linked gene

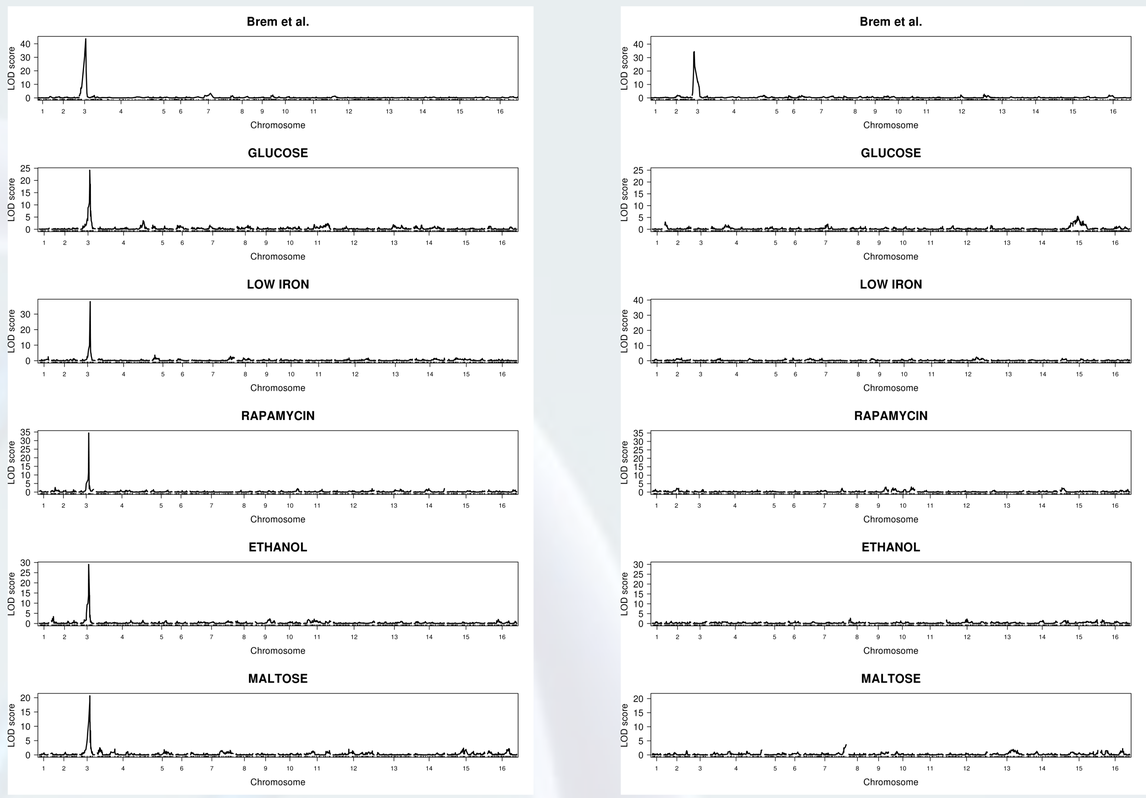
Magnitude of eQTLs

It is calculated as the difference in unexplained variance between the null and alternative models, divided by the total phenotype variance, using the function `fitqtl()` from the R/qtl package.

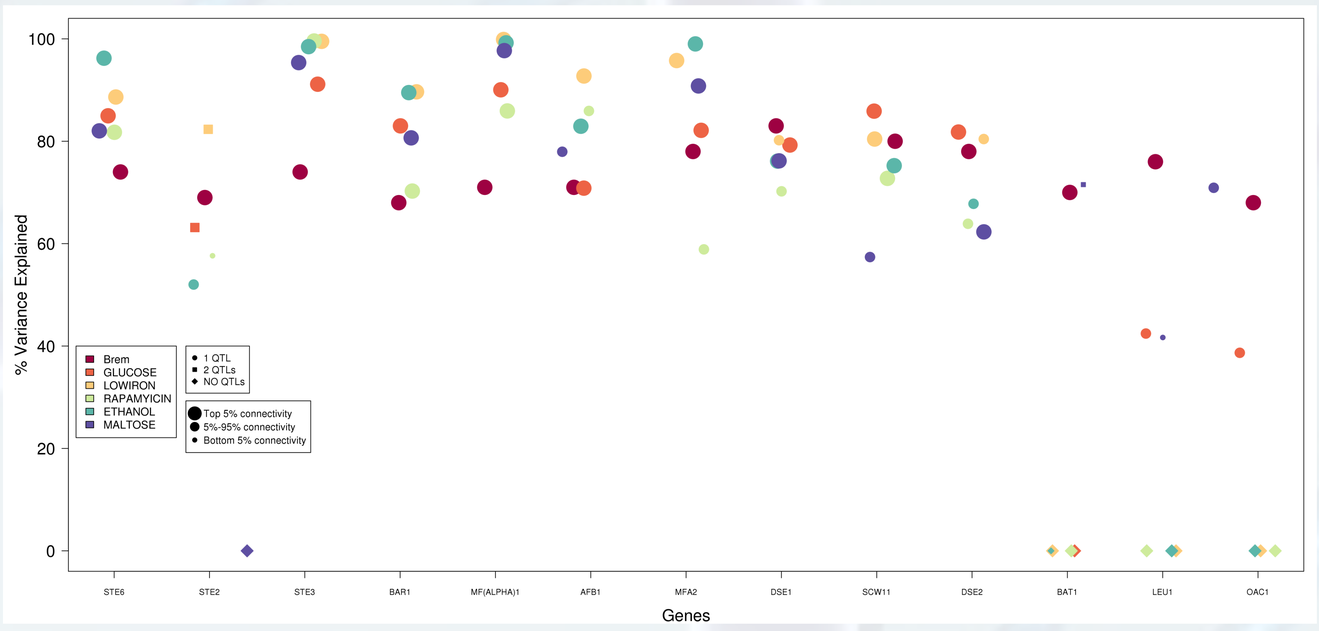
Degree of connectivity

Using each expression data set and the algorithm developed by Tur et al. implemented in the R package qpgraph, we calculated the number of genes that are functionally related with each of the 13 genes we are analyzing in each experimental condition.

RESULTS



LOD score profile along all *S. cerevisiae* genome. In these two panels, LOD scores for each marker analyzed is plotted. Panel on the left corresponds to **STE3** gene, which belongs to mating regulation pathway, and it shows a very homogeneous eQTL map for all experimental conditions. In contrast, panel on the right corresponds to **LEU1** gene, which belongs to leucine biosynthesis pathway and shows a very variable map.



Proportion of genetic expression variance explained by eQTL(s). For each of 13 genes that have been analysed, there are 6 dots plotted, corresponding to 6 different experimental conditions. Regarding the degree connectivity, 3 ranges have been established to classify the 13 genes under study: the largest dot represents those which have a degree of connectivity higher than 95% of other genes in the same environment, the medium one are for those which rank between 5% and 95%, and the smallest ones are those which rank at the bottom 5%.

CONCLUSIONS

In Tur et al. data was observed that those genes with a high number of associations also showed a very strong genetic control of their expression. In this project, we elucidated which of these genes preserve such a strong genetic regulation in different environmental conditions and the importance of it in different cellular pathways. Our main conclusions are:

- eQTLs of those genes with a higher degree of connectivity have a stronger effect than those related with genes connected with just a few.
- Environmental conditions do not influence genetic control of expression of genes involved in mating regulation or daughter cells separations. However, it may play a role in other subsets of genes, such as those involved in leucine biosynteshis.
- Cellular pathways where control of genetic expression is crucial possess very well conserved eQTLs across environments, while those pathways in which genetic expression is not so important show more variable eQTLs.

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