

LETTER

The Effect of Multifunctionality on the Rate of Evolution in Yeast

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Multifunctional genes are expected to evolve at lower rates because mutations in such genes that improve one function might often have deleterious effects on other functions. Here we tested for an association between multifunctionality and evolutionary rates in genes of *Saccharomyces cerevisiae*, and we find a highly significant negative correlation between the number of biological processes in which a gene is involved in and its rate of evolution. However, the magnitude of this effect is small, and the results do not support the notion that multifunctionality limits a gene's rate of evolution.

Introduction

The rate at which amino acid substitutions in a gene accumulate in the course of evolution depends on the fitness consequences of such substitutions. Genes in which many substitutions are beneficial will show high rates of evolutionary change, while genes in which most substitutions are deleterious will remain more conserved. One factor that potentially influences the fitness consequences of substitutions in a gene is the number of different processes the gene is involved in. If a gene is involved in many different processes (a multifunctional gene), then substitutions that are beneficial for one process might often be deleterious for other processes. The more processes a gene is involved in, the more likely that a beneficial effect in one process is offset by deleterious effects on other processes. Substitutions in multifunctional genes are thus expected to be more often deleterious in their net effect than substitutions in genes that are only involved in few processes. As a consequence, we expect multifunctional genes to evolve at lower rates.

This hypothesis is analogous to the hypothesis that pleiotropic genes (genes that affect many phenotypic traits) have low rates of evolution (Otto 2004). However, our hypothesis is formulated in terms of the number of biological processes in which a gene is involved rather than the number of phenotypic traits it affects. Here we tested whether genes of *Saccharomyces cerevisiae* that are involved in many biological processes evolve at lower rates than genes involved in only one or few processes. We retrieved the number of known biological processes for each gene from the Gene Ontology (The Gene Ontology Consortium 2002) project in the Saccharomyces Gene Database SGD (www.yeastgenome.org) for all genes for which at least one biological process was known ($N = 2292$). As a measure for the evolutionary rate, we used published data on the ratio of nonsynonymous to synonymous mutations (dN/dS') adjusted for codon bias to account for selection on synonymous sites (Hirsh, Fraser, and Wall 2005).

We found a moderate but highly significant correlation between the dN/dS' ratio and the number of biological processes (Spearman's $\rho = -0.087$, $df = 2291$, $P = 3 \times 10^{-5}$), indicating that multifunctional genes evolve at lower rates (fig. 1 and table 1). It has previously been reported that the rate of evolution of a gene correlates with its expression

level (Pál, Papp, and Hurst 2001), its dispensability (the fitness consequences of a deletion in this gene [Wall et al. 2005]), and its connectivity (the number of physical interactions the protein encoded by this gene is involved in [Fraser et al. 2002]). Therefore we checked whether the correlation between the dN/dS' ratio and the number of biological processes could be explained by a spurious correlation with any of these additional variables. Expression levels and connectivity both correlate negatively with the rate of evolution, but because they do not correlate with the number of biological processes (Wang et al. 2002; Jansen et al. 2003) (expression: Spearman's $\rho = 0.007$, $df = 2252$, $P = 0.723$, connectivity: Spearman's $\rho = -0.028$, $df = 1170$, $P = 0.313$), they cannot be responsible for the negative correlation between the number of biological processes and the rate of evolution reported here. Dispensability was shown to correlate positively with the rate of evolution (Wall et al. 2005) and correlates negatively with the number of biological processes (Spearman's $\rho = -0.096$, $df = 2208$, $P = 6 \times 10^{-6}$). However, a partial correlation analysis reveals that the correlation between number of biological processes and dN/dS' still holds after correcting for this effect (Spearman's $\rho = -0.069$, $df = 2210$, $P = 0.001$). In accordance with our hypothesis these results indicate that multifunctionality has an effect on evolutionary rates, and that this effect is independent of other biological parameters that previously have been reported to affect rates of evolution. However, the reported effect, although highly significant, is of a very small magnitude. Less than one percent in the ordinal variation of dN/dS' is explained by ordinal variation in our measures of multifunctionality. In other words, the rate at which a gene evolves is largely unaffected by the number of biological processes in which it is known to be involved.

Using data from a recent study that quantifies pleiotropy in *S. cerevisiae* based on phenotypic effects on growth in 21 different environments (Dudley et al. 2005), we further tested for an association between their measure of pleiotropy and rate of evolution (data not shown). Again we found a weak correlation (Spearman's $\rho = -0.115$, $df = 404$, $P = 0.021$), suggesting that pleiotropy also has a limited impact on a gene's rate of evolution. It is possible that a stronger connection between rates of evolution and multifunctionality or pleiotropy will emerge once new and more detailed measures of the latter are available. For the moment, we conclude that a connection between the multifunctionality or pleiotropy of a gene and its rate of

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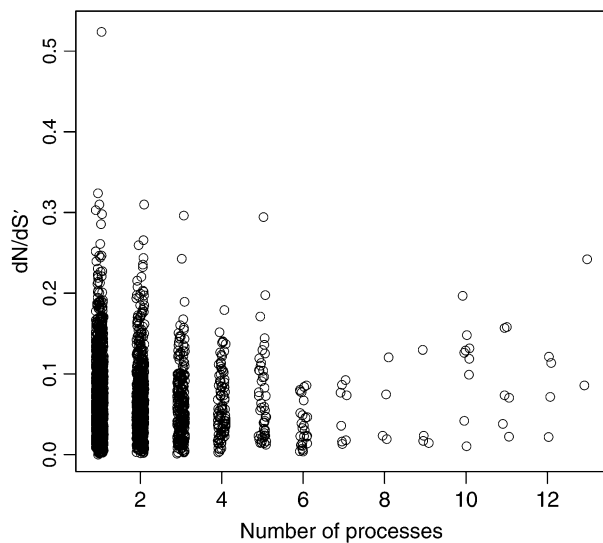


FIG. 1.—Scatter-plot showing the negative correlation between multifunctionality and the rate of evolution. Horizontal noise was added for better visibility.

evolution exists, but that there is no indication that this connection is strong enough to substantially constrain the rate of adaptation of a multifunctional gene.

Methods

Origin of Data

The number of biological processes according to the Gene Ontology (The Gene Ontology Consortium 2002) project for all genes was retrieved from the *Saccharomyces Genome Database* (www.yeastgenome.org, accessed June 24, 2005). The dispensability of a gene was defined as the growth rate of a strain in which this gene was knocked out. For essential genes, the growth rate was defined as zero. The list of essential genes was retrieved from the *Saccharomyces Genome Database* (www.yeastgenome.org, accessed June 9, 2004). The list of growth rate measures with knockout strains of yeast was taken from Steinmetz et al. (2002). The measure of pleiotropy from the Dudley study (Dudley et al. 2005) was taken from the file `pheno_data.xls`, worksheet `lowfinal` (data from http://club.med.harvard.edu/pheno/datafiles/pheno_data.zip, downloaded June 2, 2005). The connectivity data were taken from Jansen et al. (2003), downloaded at http://networks.gersteinlab.org/intint/supp_data/L_cut_PIT_600.tar. Expression data were taken from Wang et al. (2002) (HDA Data downloaded from <http://www-genome.stanford.edu/turnover/Yuleisupplementfile/table1.xls>).

Statistical Tests

Partial correlations were calculated following Sokal and Rohlf (1995).

Competing Interests Statement

The authors declare that they have no competing financial interests.

Table 1
The Number of Genes (*N*), Mean and Standard Error of Mean of the Rate of Evolution (*dN/dS'*) for Each Category of Number of Biological Processes

Number of Biological Processes	<i>N</i>	Mean <i>dN/dS'</i>	Standard Error of Mean <i>dN/dS'</i>
1	1215	0.074359	0.0014625
2	636	0.069217	0.0018424
3	238	0.062130	0.0027868
4	93	0.061537	0.0041539
5	47	0.070651	0.0014625
6	26	0.037103	0.0053245
7	8	0.051509	0.0120380
8	4	0.059410	0.0239008
9	4	0.046091	0.0278998
10	9	0.111224	0.0185698
11	6	0.086465	0.0237833
12	4	0.082033	0.0228565
13	2	0.163853	0.0780698

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