Genetic robustness and functional evolution of gene duplicates

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ABSTRACT

Gene duplications are a major source of evolutionary innovations. Understanding the functional divergence of duplicates and their role in genetic robustness is an important challenge in biology. Previously, analyses of genetic robustness were primarily focused on duplicates essentiality and epistasis in several laboratory conditions. In this study, we use several quantitative data sets to understand compensatory interactions between Saccharomyces cerevisiae duplicates that are likely to be relevant in natural biological populations. We find that, owing to their high functional load, close duplicates are unlikely to provide substantial backup in the context of large natural populations. Interestingly, as duplicates diverge from each other, their overall functional load is reduced. At intermediate divergence distances the quantitative decrease in fitness due to removal of one duplicate becomes smaller. At these distances, yeast duplicates display more balanced functional loads and their transcriptional control becomes significantly more complex. As yeast duplicates diverge beyond 70% sequence identity, their ability to compensate for each other becomes similar to that of random pairs of singletons.

INTRODUCTION

Survival of biological systems crucially depends on robustness to harmful genetic mutations, i.e. genetic robustness, and to changes in environmental conditions (1–3). Two distinct mechanisms of genetic robustness have been previously discussed. First, alternative signaling and metabolic pathways provide an important mechanism for rerouting in many molecular networks (4,5). Second, a major role in genetic robustness is attributed to gene duplicates (1,6). Gene duplications are frequent in evolution and range in size from small-scale (SSD) to whole-genome events (WGD) (7,8). While in ~90% of the cases one duplicate is eventually lost in evolution (6), duplicated genes that remain in the genome can, at least partially, backup each other’s functions. Importantly, functional compensation by duplicates plays a significant role in buffering deleterious human mutations (9).

Genetic robustness due to gene duplicates is inherently tied to their functional divergence. Duplicates that acquire distinct molecular functions (MFs) are naturally unable to compensate for one another. In addition, even if MF is conserved, incomplete compensation between duplicates is possible owing to different expression patterns or dosage effects. Gene duplications are the major source of new genes (10) and several conceptual models of duplicates’ evolution have been proposed (11,12). In the neofunctionalization model one duplicate gains new functions, i.e. functions not associated with the ancestral gene, while the other duplicate retains the ancestral functions (10,13,14). In contrast, in the subfunctionalization model both duplicates become indispensable and are retained in evolution by partitioning the ancestral gene functions (15,16). Both these models imply an eventual loss of the ability of duplicates to fully substitute for each other. It is also likely that a significant fraction of duplicates are fixed and retained in genomes owing to selective advantages, such as dosage effects or condition-specific expression patterns, present from the moment of duplication (17,18). In cases of fixation due to a selective advantage, full compensation between duplicates is unlikely.

Even though full compensation between duplicates is not expected in the long term, the ability of duplicates to buffer deleterious mutations of their paralogs has been now demonstrated by several independent observations. These include a lower than expected fraction of essential genes with close duplicates (1), a paucity of pairwise epistatic interactions involving duplicated genes (19), and an excess of aggravating genetic interactions between paralogs (20,21). The contribution of duplicates...
to robustness has been primarily considered in the context of qualitative or quantitative growth phenotypes either in nutrient rich or in a small number of laboratory conditions (1,2,23). Although popular in experiments, these conditions are unlikely to approximate well a natural ‘milieu’ of living systems, which are constantly bombarded by a diverse array of environmental stresses and stimuli. Perhaps more importantly, even if there is a strong compensatory interaction between a pair of duplicates, an evolutionary relevant decrease in fitness can still persist—due to an incomplete buffering—after a damaging mutation in one of the duplicates (24). In the context of long-term evolution, there may not be much difference between mutations leading to the lethal phenotype and mutations associated with a fitness decrease substantially larger than the inverse of the effective population size (25,26). Given that typical population sizes of free-living microbial species are large (>10^6–10^8) (27), even a small fitness decrease can be effectively lethal for these organisms. Consequently, quantitative analyses of growth phenotypes, preferably in multiple environmental conditions, are necessary to understand the extent to which compensation between duplicates plays an important role in natural biological populations. Here we perform such an analysis and show that in the context of natural populations, genetic buffering mediated by duplicates is likely to be rare and, surprisingly, it is not a monotonic function of duplicates’ divergence.

MATERIALS AND METHODS

Gene and protein sequences for *Saccharomyces cerevisiae*, *Saccharomyces paradoxus*, *Saccharomyces bayanus*, *Saccharomyces castelli*, *Saccharomyces mikatae*, *Saccharomyces kudriavzevi* and *Saccharomyces kluyveri* were obtained from the Saccharomyces Genome Database (SGD; http://downloads.yeastgenome.org/) and the study by Kellis *et al.* (28). Pairs of gene duplicates were identified by sequence homology between proteins within each genome using BLASTP (29). Only duplicates that were bidirectional best hits and could be aligned by >80% of each open reading frame’s sequence length were considered in our analysis (30). Following previous studies (1), we excluded ribosomal genes from the analysis owing to their high expression, dominant impact on growth and strong codon adaptation bias. Evolutionary distances between duplicated genes were estimated using the method of Yang and Nielsen (31) implemented in the PAML package (32); the use of other methods, such as maximum likelihood, to estimate Ka and Ks did not significantly change the observed patterns (Supplementary Figure S1A).

We used the data obtained by Hillenmeyer *et al.* (33) to measure the fitness contribution of duplicates across multiple environmental conditions and chemical perturbations. Using a *P*-value cutoff of 0.01, we obtained the number of experimental conditions for which a growth defect was observed for every single gene deletion mutant. We also analyzed quantitative growth measurements for double and single deletion yeast strains obtained from DeLuna *et al.* (34) and Costanzo *et al.* (35). Gene essentiality data was obtained from the study of Giaever *et al.* (36).

To functionally characterize duplicated genes, Gene Ontology (GO) (37) annotations were collected from SGD and Enzyme Commission (EC) annotations from the Comprehensive Yeast Genome Database (CYGD) (38). Transcription factor binding motifs used in our work were compiled from Kafri *et al.* (39) and the high-confidence predictions in Kellis *et al.* (28). We used protein localization data from Huh *et al.* (40), Codon Adaptation Index (CAI) calculations based on the data set by Lu *et al.* (41) and the annotation of protein complexes in CYGD.

RESULTS

Hillenmeyer *et al.* (33) quantified growth phenotypes of single-gene yeast deletion strains in a large collection of environmental conditions. The assembled data set contains ~5.5 million phenotypes of heterozygous and homozygous mutants in ~400 conditions. The sampled conditions represent 27 different environmental stresses and hundreds of perturbations with diverse chemical compounds. Environmental stresses comprised different growth media, media lacking specific vitamins or amino acids, as well as different pH and temperature regimes. This comprehensive collection of phenotypes allowed us to investigate in detail the diversification of duplicates’ functions and their contribution to genetic robustness in multiple conditions.

We first investigated how the average number of sensitive conditions, i.e. conditions with a significant growth decrease due to deletion of one duplicate, depends on sequence divergence (Ka) between the duplicated genes (Figure 1A and B). We considered the fraction of different conditions with a growth phenotype as a quantitative measure of compensation capacity for duplicates at various divergence distances. For close duplicates the average number of sensitive conditions is not significantly different from that of a random pair of yeast singletons (Figure 1B, horizontal line). Importantly, this result does not imply that random gene pairs and close duplicates are equivalent in terms of the similarity of their MF. As we demonstrate below, the observed pattern is likely due to a higher overall functional load of close duplicates. Here and throughout the article we use the term ‘functional load’ of a gene to characterize the average fitness decrease—across considered conditions—due to the gene deletion; we note that, based on the definition above, the functional load is not a measure of the total number of MFs a gene has, but it reflects the gene’s overall fitness contribution.

Interestingly, the number of sensitive conditions initially drops as duplicates diverge, decreasing about 30% at the distances corresponding to Ka ≈ 0.1 (Ks ≈ 1, see Supplementary Figure S2A and B). As duplicates diverge further, the average number of sensitive conditions increases again, reaching the average for a random pair of yeast singletons at Ka ≈ 0.25. The trend shown in
Figure 1B is not sensitive to the P-value cutoff used to determine the significance of the growth decrease observed in mutant strains (Supplementary Figure S3). A similar trend was also observed for the average growth decrease (functional load), measured either by log ratios or Z-scores across all tested conditions (Supplementary Figure S4A and B). Bin-free analyses of the data (Supplementary Figures S1B and C and S2B) also revealed a smaller fitness cost due to the loss of duplicates at intermediate distances (Ka \(\approx 0.1\)).

Because most actively growing wild-type yeast populations are diploid (42), we mainly focused our analysis on heterozygous mutant strains. The patterns of functional compensation for heterozygous and homozygous mutants are similar when multiple-drug resistance genes, as defined by Hillenmeyer et al. (33), are not considered (Supplementary Figure S4C). The trends also remain similar when only environmental perturbations are analyzed in the homozygous experiments (Supplementary Figure S4D). We also checked that the observed compensation patterns due to closest duplicates are not significantly influenced by additional, i.e. more diverged, paralogs (Supplementary Figure S4E). This lack of significant compensation by diverged duplicates results in an approximately linear relationship between the number of sensitive conditions per yeast protein family and the
family size (Supplementary Figure S5). Finally, the observed compensation patterns were not affected by removal of gene pairs with a high CAI (Supplementary Figure S6A), suggesting that the observed trend cannot be explained by expression-based constraints on the rate of duplicate sequence evolution (Ka) (43) or high expression levels of certain duplicates.

It is interesting to compare the ability of duplicates to buffer mutations leading to any detectable growth decrease beyond a given fitness threshold (Figure 1B) and their role in protecting against the no-growth phenotype, i.e. the likelihood to observe essential genes in duplicate pairs. In Figure 1C, using data from the study by Giaever et al. (36), we show the fraction of essential duplicates as a function of their divergence. In agreement with previous studies (1,22,23) we found that the fraction of essential genes remains low and approximately constant for close duplicates, and increases substantially only at divergence distances corresponding to Ka > 0.4. Notably, this pattern is qualitatively different from the quantitative fitness data from DeLuna et al. (34) and the synthetic genetic array (SGA) data from Costanzo et al. (35). In these studies, the authors performed quantitative growth measurements of yeast strains with individual and simultaneous deletions of duplicates. Using the single deletion phenotypes from the DeLuna et al. (Figure 2A) and Costanzo et al. studies (Figure 2B), we observed fitness profiles similar to the one obtained based on the data from Hillemeyer et al. (Figure 1B) as a function of Ka, with smaller phenotypic effects at intermediate distances. Interestingly, the overall functional load of duplicate pairs, measured by the phenotype of double deletions, indeed substantially decreases with their divergence (Figure 2C and D). This result suggests that while close duplicates are more likely to have similar functions, their higher functional load makes complete compensation less likely. Because the overall functional load of duplicates remains approximately constant for Ka > 0.15, the higher fraction of detectable growth phenotypes at these distances is likely due to a decreased ability for functional compensation as duplicates diverge. Compensation between duplicates quantified by the presence of aggravating interactions between duplicate pairs decreases as a function of sequence divergence (Figure 2E and F) [see (21)].

Besides a smaller overall functional load, it is possible that duplicates at intermediate distances have other properties that favor genetic robustness. To explore this possibility, for each duplicate pair, we looked at the gene with the largest and the gene with the smallest number of sensitive conditions (Figure 3A). Notably, while the duplicate with more conditions (Figure 3A, more sensitive duplicate) follows the average trend for all duplicates (Figure 1B), the duplicate with fewer conditions (Figure 3A, less sensitive duplicate) shows a steady gain in the number of conditions as a function of Ka. Consequently, the functional load of close duplicates, measured by the number of sensitive conditions, is different, and this difference becomes significantly smaller as the genes diverge (Figure 3B. Pearson’s r = −0.64, P = 7 × 10^{-4}, see also Supplementary Figure S2C). Close duplicates with the larger number of sensitive conditions also show a higher evolutionary constraint, evaluated by the normalized ratio of nonsynonymous to synonymous substitutions per nucleotide site, Ka/Ks (Wilcoxon Signed Rank test P = 7 × 10^{-3}, Figure 2C). This result agrees with previous reports of asymmetric evolution of duplicates in the context of co-expression, genetic interaction and protein–protein interaction networks (19,44,45). The observed asymmetry in the functional load between close duplicates can make buffering difficult. For example, if the less sensitive duplicate is expressed only under specific environmental conditions.

To further explore the mechanism behind the observed backup patterns, we analyzed the functional diversification of yeast duplicates as a function of their sequence divergence (Ka). First, for genes encoding metabolic enzymes we calculated the fraction of gene pairs with conserved EC numbers (Figure 4A); the conservation of EC numbers indicates that corresponding proteins catalyze identical biochemical reactions. Second, we calculated the fraction of shared GO terms describing protein MF for all duplicates (Figure 4B). Both measures showed that the MF of yeast duplicates typically starts to substantially diverge only at about Ka > 0.4. The timing of this divergence approximately coincides with a significant increase in the fraction of essential duplicates (Figure 1C). On the other hand, the significant changes in the number of quantitative growth phenotypes are observed when the MF of duplicates is usually still conserved.

A complementary analysis of transcription factor binding sites suggests that gene regulation plays an important role in establishing the observed compensation patterns. It was previously demonstrated that duplicated yeast genes have, on average, a higher number of cis-regulatory motifs than singleton genes (46). Using a comprehensive data set of ~150 known and predicted DNA binding motifs in yeast (28,39), we found that the average number of different motifs regulating a duplicate pair increases significantly at Ka ≈ 0.1 (Figure 4D, dashed line, Mann–Whitney U test, P = 0.06). At this divergence distance, the average number of different motifs per duplicate pair is more than twice the number of motifs for a pair of yeast singletons (Figure 4D, dashed horizontal line). The number of regulatory motifs increases both for the
duplicate with the highest and the duplicate with the smallest number of sensitive conditions (Supplementary Figure S8A and B). The increase in complexity of the duplicates regulation at $K_a \approx 0.1$ is also confirmed by a significant increase (Mann–Whitney U test, $P = 1 \times 10^{-3}$) at these distances of the number of transcription factor mutants (47) affecting duplicate gene expression (Figure 4D, solid line).

While the total number of DNA motifs regulating duplicates initially increases with divergence, the fraction of shared motifs [Supplementary Figure S9A, see also (48)], the overlap in GO terms describing biological processes (Figure 4C) and the overlap in cellular localization observed in fluorescence-tagging experiments (40) decrease (Supplementary Figure S8B). Such a pattern suggests that the increase in regulatory complexity allows duplicates to specialize for different biological processes while mostly preserving common MFs. The ability of duplicates with partially diverged regulatory regions to compensate for each other through expression...
changes of the intact gene was previously described by Kafri et al. (39,49). Also, the recent study by DeLuna et al. (50) showed that on deletion of one duplicate, expression changes of the remaining paralog are often need-based, i.e. they happen primarily when the corresponding function is required. Such regulatory backup circuits should, at least in some cases, enable functional compensation between homologs with different expression patterns in wild type. Notably, based on the data from recent study by Springer et al. (51), who measured the expression changes of yeast genes when one of two genomic copies was deleted in diploid cells, we observed a significant dosage response only for genes forming recently duplicated pairs (Ka < 0.15, Figure 4E). This suggests that genes with close duplicates are most responsive to dosage effects.

Finally, the patterns of diversification and functional compensation described above should correlate with the process of duplicate loss in evolution. We investigated the retention of yeast duplicates using the complete genomic sequences of seven species: S. cerevisiae, S. paradoxus, S. bayanus, S. castelli, S. mikatae, S. kudriavzevii and S. kluyveri. We calculated the number of remaining duplicates as a function of their sequence divergence (Figure 5, see also Supplementary Figure S10A for the corresponding relationships in individual yeast species). This analysis suggests that a relatively brief initial period of high duplicate loss (6) is followed by a long evolutionary period (Ka > 0.1) during which the average loss rate decreases >10-fold (red in Figure 5). Interestingly, the loss rate significantly decreases approximately at the divergence distance when duplicates become more similar in terms

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Differences in the number of sensitive conditions between duplicates. (A) The average fraction of sensitive conditions for the duplicates with the higher and lower number of sensitive conditions in each pair; Ka values represent sequence divergence between duplicates. The P-value is for the Mann–Whitney U test. (B) The relative difference in the number of sensitive conditions between duplicates as a function of their initial divergence; Ka values represent sequence divergence between duplicates. The relative difference was calculated as the absolute difference in the number of sensitive conditions between duplicates normalized to the total number of sensitive conditions for the pair (Spearman’s r = −0.60, P = 2 × 10⁻⁵; Pearson’s r = −0.64, P = 7 × 10⁻⁴). (C) The average Ka/Ks ratio for the paralogs with the largest (more sensitive) and smallest (less sensitive) number of conditions with a significant growth decrease. Ka/Ks ratios were calculated relative to orthologous sequences in S. bayanus. Only duplicates with Ka < 0.15 to each other were considered. The P-value is for the Wilcoxon signed rank test.
Figure 4. Diversification of duplicates function and regulation. (A) Fraction of metabolic duplicates sharing the same EC numbers; conservation of EC numbers indicates catalysis of identical biochemical reactions. (B) Fraction of GO MF terms shared between duplicates. (C) Fraction of GO Biological Process (BP) terms shared between duplicates. In panels B and C we considered only GO terms with a distance of three or more to the corresponding GO root hierarchy term. (D) Dashed line, the average number of different transcription factor binding motifs per duplicate pair. Transcription factor (TF) binding motifs were compiled from the studies of Kafri et al. (39) and Kellis et al. (28). Solid line, the average number of transcription factor deletions in *S. cerevisiae* that significantly affect the expression of duplicate genes. The data were obtained from the study by Hu et al. (47). For comparison we also show the average number of motifs and TF mutants affecting expression for random pairs of yeast singletons (horizontal dashed and solid lines); the *P*-values were calculated using the Mann–Whitney U test. (E) The average dosage compensation (responsiveness) of duplicates as a function of sequence divergence (Ka). The data for the average expression responsiveness was obtained from the work of Springer et al. (51). In that study, responsiveness was measured in diploid yeast strains as the Log2 ratio (perturbed versus normal) of expression changes for the remaining gene copy following deletion of the equivalent gene copy on a sister chromosome. The *P*-value was calculated using the Mann–Whitney U test. In all figures error bars represent the SEM.
In the figure, error bars represent the SEM. Phenotypes from the study by Hillenmeyer in small-scale experimental studies. Interestingly, based on the number of conditions with quantitative growth phenotypes from the study by Hillenmeyer et al. (33), and the quantitative growth measurements by Costanzo et al. (35), the duplicates annotated as redundant are not significantly different from all other yeast duplicates (Mann–Whitney U, $P = 0.13$ and $0.35$, respectively, Supplementary Figure S11). This demonstrates that, although many yeast duplicates indeed may show functional overlap in some laboratory conditions, their compensation properties will probably be significantly less important in large natural populations due to the ability of purifying selection to efficiently prune mutations causing even a small fitness decrease.

It is likely that several different factors contribute to the relative paucity of functional compensation between paralogs at small divergence distances. A significant fraction of duplicates are likely to be fixed owing to dosage effects (17), and functional compensation between such duplicates in the context of natural populations is unlikely. For example, the lack of significant compensation between histone pairs, HTA1-HTA2 and HHT1-HHT2, is likely to be a consequence of their role in maintaining proper histone levels in yeast cells. Gene dosage may explain the inability of some duplicates to backup each other, but it is unlikely to be the only explanation. We showed that even when all duplicate pairs with a high CAI (Supplementary Figure S6A) or pairs forming known protein complexes (Supplementary Figure S6B) are removed from the analysis, the patterns of functional compensation remain similar. Notably, genes with a high CAI have been also associated with higher frequencies of interlocus gene conversion (IGC) (53,54). While IGC can slow down the rate of duplicates sequence divergence (55), analyses based only on WGDs with no evidence of IGC [using data recently reported by Casola et al. (56)] revealed essentially identical compensation patterns (Supplementary Figure S12).

Close duplicates are also less likely to compensate for each other probably owing to the aforementioned dichotomy in their functional loads (Figure 3A and B). Many close duplicates can be classified, based on their activity and breadth of expression, into a major and a minor functional isoforms. For example, the glyceraldehyde-3-phosphate dehydrogenase TDH1 is active under various stress conditions, while its isoenzyme TDH2 is used primarily during exponential growth (57). Similarly, the ubiquitin conjugating enzyme UBC4 is expressed during exponential growth, while its duplicate UBC5 is active during stationary phase (58). The difference in functional load for close yeast duplicates is also consistent with the asymmetric partition of functions, interactions and gene expression, observed between close duplicates in other organisms, for example, Arabidopsis and Human (45,59,60). This suggests that duplicate-dependent compensation in the context of natural populations may be limited in other species as well.

Our analysis suggests that a typical lifecycle of gene duplicates in yeast consists of several distinct evolutionary stages (11,12). In the first stage (at duplicate distances corresponding to $\text{Ka} < 0.05$), duplicates tend to have high overall functional loads and significant asymmetry in the number of sensitive conditions; both of these factors make complete compensation unlikely. The high functional load of close duplicates suggests that adaptive selection plays
an important role in their fixation. In the second stage (0.05 < Ka < 0.25), as duplicates diverge further, their overall functional load usually decreases. This may happen, for example, due to relaxation of the environmental conditions, which facilitated the original duplicate fixation. The vast majority of duplicates, likely the paralogs with relatively smaller functional loads (Figure 3C), are lost at this stage (Figure 5). Gene pairs that survive the period of high duplicate loss display more balanced functional loads and complex regulation; these gene pairs are usually retained for long evolutionary times in yeast genomes (Figure 5). Surviving duplicates can provide at least partial compensation at intermediate divergence distances and also serve as an important source of new protein functions. In the third stage (Ka > 0.3 or ~70% sequence identity), the lifecycle of duplicates is completed when their functional roles diverge, and their quantitative compensation properties become indistinguishable from those of random pairs of yeast singletons.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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REFERENCES
1. Gu,Z., Steinmetz,L.M., Gu,X., Scharfe,C., Davis,R.W. and Li,W.H. (2003) Role of duplicate genes in genetic robustness against null mutations. *Nature*, 421, 63–66.
2. Stelling,J., Sauer,U., Szallasi,Z., Doyle,F.J. III and Doyle,J. (2004) Robustness of cellular functions. *Cell*, 118, 675–685.
3. Wagner,A. (2005) Robustness and Evolvability in Living Systems. Princeton University Press, Princeton.
4. Wagner,A. (2000) Robustness against mutations in genetic networks of yeast. *Nat. Genet.*, 24, 355–361.
5. Papp,B., Pal,C. and Hurst,L.D. (2004) Metabolic network analysis of the causes and evolution of enzyme dispensability in yeast. *Nature*, 429, 661–664.
6. Lynch,M. and Conery,J.S. (2000) The evolutionary fate and consequences of duplicate genes. *Science*, 290, 1151–1155.
7. Wapinski,I., Pfeffer,A., Friedman,N. and Regev,A. (2007) Natural history and evolutionary principles of gene duplication in fungi. *Nature*, 449, 54–61.
8. Kellis,M., Birren,B.W. and Lander,E.S. (2004) Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature*, 428, 617–624.
9. Hsiao,T.L. and Vitkup,D. (2008) Role of duplicate genes in robustness against deleterious human mutations. *PLoS Genet.*, 4, e1000014.
10. Ohno,S. (1970) *Evolution by Gene Duplication*. Springer-Verlag, Berlin, New York.
11. Conant,G.C. and Wolfe,K.H. (2008) Turning a hobby into a job: how duplicated genes find new functions. *Nat. Rev. Genet.*, 9, 938–950.
12. Innan,H. and Kondrashov,F. (2010) The evolution of gene duplications: classifying and distinguishing between models. *Nat. Rev. Genet.*, 11, 97–108.
13. Nadeau,J.H. and Sankoff,D. (1997) Comparable rates of gene loss and functional divergence after genome duplications early in vertebrate evolution. *Genetics*, 147, 1259–1266.
14. Didow,A. (1996) Genome duplications in the evolution of early vertebrates. *Curr. Opin. Genet. Dev.*, 6, 715–722.
15. Force,A., Lynch,M., Pickett,F.B., Amores,A., Yan,Y.L. and Postlethwait,J. (1999) Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, 151, 1531–1545.
16. Lynch,M. and Force,A. (2000) The probability of duplicate gene preservation by subfunctionalization. *Genetics*, 154, 459–473.
17. Kondrashov,F.A., Rogozin,I.B., Wolf,Y.I. and Koonin,E.V. (2002) Selection in the evolution of gene duplications. *Genome Biol.*, 3, RESEARCH0008.
18. Berghorsthorne,u., Andersson,D.I. and Rohtu,J.R. (2007) Ohno’s dilemma: evolution of new genes under continuous selection. *Proc. Natl Acad. Sci. USA*, 104, 17004–17009.
19. VanderSluis,B., Bellay,J., Musso,G., Costanzo,M., Papp,B., Vizcaíovumar,F.J., Baryshnikova,A., Andrews,B., Boone,C. and Myers,C.L. (2010) Genetic interactions reveal the evolutionary trajectories of duplicate genes. *Mol. Syst. Biol.*, 6, 429.
20. Ihmels,J., Collins,S.R., Schuldiner,M., Krogan,N.J. and Weissman,J.S. (2007) Backup without redundancy: genetic interactions reveal the cost of duplicate gene loss. *Mol. Syst. Biol.*, 3, 86.
21. Li,J., Yuan,Z. and Zhang,Z. (2010) The cellular robustness by genetic redundancy in budding yeast. *PLoS Genet.*, 6, e1001187.
22. Guan,Y., Dunham,M.J. and Troyanskaya,O.G. (2007) Functional analysis of gene duplications in *Saccharomyces cerevisiae*. *Genetics*, 175, 933–943.
23. Conant,G.C. and Wagner,A. (2004) Duplicate genes and robustness to transient gene knock-downs in *Caenorhabditis elegans*. *Proc. Biol. Sci.*, 271, 89–96.
24. Thatcher,J.W., Shaw,J.M. and Dickinson,W.J. (1998) Marginal fitness contributions of nonessential genes in yeast. *Proc. Natl Acad. Sci. USA*, 95, 253–257.
25. Gillespie,J.H. (1998) *Population Genetics, A Concise Guide*. Johns Hopkins Univ. Press, Baltimore.
26. Hartl,D. and Clark,A. (1997) *Principles of Population Genetics*, 3 edn. Sinauer Associates, Sunderland.
27. Lynch,M. (2006) Streamlining and simplification of microbial genome architecture. *Annu. Rev. Microbiol.*, 60, 327–349.
28. Kellis,M., Patterson,N., Endrizzi,M., Birren,B. and Lander,E.S. (2003) Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature*, 423, 241–254.
29. Altshuler,S.F., Madden,T.L., Schaffer,A.A., Zhang,J., Miller,W. and Lipman,D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, 25, 3389–3402.
30. Gu,Z., Cavalcanti,A., Chen,F.C., Bouman,P. and Li,W.H. (2002) Extent of gene duplication in the genomes of Drosophila, nematode, and yeast. *Mol. Biol. Evol.*, 19, 256–262.
31. Yang,Z. and Nielsen,R. (2000) Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Mol. Biol. Evol.*, 17, 32–43.
32. Yang,Z. (1997) *PAML*: a program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Biosci.*, 13, 555–556.
33. Hillemeyer,M.E., Fung,E., Wildenhaus,J., Pierce,S.E., Hoon,S., Lee,W., Proctor,M., St Onge,R.P., Tyers,M., Koller,D. et al. (2008) The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science*, 320, 362–365.
34. DeLuna,A., Vetsigian,K., Shoresh,N., Hegreness,M., Colon-Gonzalez,M., Chao,S. and Kishony,R. (2008) Exposing the fitness contribution of duplicated genes. *Nat. Genet.*, 40, 676–681.
