

Growth of Novel Epistatic Interactions by Gene Duplication

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Abstract

Epistasis has long been recognized as fundamentally important in understanding the structure, function, and evolutionary dynamics of biological systems. Gene duplication is a major mechanism of evolution for genetic novelties. Here, we demonstrate that genes evolved significantly more epistatic interactions after duplication. The connectivity of duplicate gene pairs in epistatic networks is positively correlated with the extent of their sequence divergence. Furthermore, duplicate gene pairs tend to epistatically interact with genes that occupy more functional spaces than do single-copy genes. These results show that gene duplication plays an important role in the evolution of epistasis.

Key words: epistasis, gene duplication, genetic innovation.

Introduction

Epistasis can have multiple interpretations (Phillips 2008). In this study, it refers to genetic interaction between genes, meaning that phenotypic consequences of a mutation in one gene may be modified by mutations in other genes (Boone et al. 2007; Phillips 2008). It may be either negative or positive, where the combination of mutations in two genes causes a greater or lesser phenotypic consequence, respectively, than expected from individual mutations (Dixon et al. 2009). Comprehensive understanding of epistasis is fundamental for many important biological issues such as the evolution of sex, speciation, pathway organization, and complex disease (Kondrashov 1982; Wagner et al. 1994; Barton and Charlesworth 1998; Sham 2001; Cordell 2002; Kelley and Ideker 2005; Otto 2007). In model organisms, illustrating epistatic interactions is also a powerful tool in dissecting functional linkages between genes (Avery and Wasserman 1992; Hartman et al. 2001; Kelley and Ideker 2005; Ma et al. 2008; Brady et al. 2009). Here, we investigated how gene duplication contributed to the evolution of epistasis in nature.

Gene duplication is one of the major mechanisms for the evolution of genetic novelties (Ohno 1970; Long et al. 2003). Novel functions of duplicate genes can be generated at different levels, including protein products of varied function, novel transcriptional regulation, and new partners in

protein–protein interaction networks (Zhang et al. 2002; He and Zhang 2005; Thomson et al. 2005; Hittinger and Carroll 2007; Li et al. 2010). It is well known that duplicate gene pairs can form negative epistasis due to their overlapping functions (Gu et al. 2003; Dean et al. 2008; DeLuna et al. 2008; Musso et al. 2008), but how gene duplication contributed to the growth of epistatic interaction networks remains unclear.

Recently, a genome-wide epistatic network was generated by assaying the fitness defect of double mutants in baker's yeast, *Saccharomyces cerevisiae* (Costanzo et al. 2010). In the study, ~5.4 million gene–gene pairs were screened, and those showing epistatic interactions were identified. The genes spanned all biological processes and represented an unbiased collection of epistatic interactions in a species. This genome-scale map of epistatic interactions provides a valuable opportunity to study the relationship between gene duplication and the evolution of epistatic interactions.

In our study, we investigated how epistatic interactions evolved as duplicate genes accumulated sequence divergence. We also analyzed functional distributions of epistatic-interaction partners for duplicate genes. Our results indicate a gradually evolutionary process to extend the functional diversity of duplicate genes by evolving novel epistatic interactions after gene duplication, providing an important picture for how epistasis evolves in nature. Our results also

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offer new evidence on how gene duplication contributes to the origin and evolution of genetic novelties, an important issue in evolutionary biology (Ohno 1970; Long et al. 2003; Teichmann and Babu 2004; Zhou et al. 2008; Innan and Kondrashov 2010).

Materials and Methods

Epistasis Data

Epistatic interactions were downloaded from Costanzo et al. (2010). In this study, 1,712 query genes were screened against 3,885 array genes in ~5.4 million gene–gene pairs. As epistatic interactions of query genes represent a more comprehensive picture of the overall epistasis for each gene at the genome level, in order to uncover a global view of how epistasis evolves, we investigated only the epistatic interaction of query genes. Both positive and negative epistasis data were included in the analysis. We used the intermediate cutoff for defining epistatic interaction between genes, which is the same criterion used by Costanzo et al. (2010). With a more stringent cutoff, as defined by Costanzo et al. (2010), we obtained similar results (supplementary table 1, Supplementary Material online).

Fungal Genome Sequence and Ortholog Identification

Sequences for the 42 sequenced fungal species were from Fitzpatrick et al. 2006. Using the InParanoid software package (Remm et al. 2001), orthologs between budding yeast and each of the other fungal species were identified.

Duplicate Gene and Singleton Identification

We identified duplicate genes and singletons in a genome based on an all-against-all BlastP alignment (Altschul et al. 1997). Duplicate genes were defined according to criteria that the alignable region between two genes is longer than 50% of both genes and the E -value is less than 10^{-10} . These stringent criteria were used to avoid false-positive predictions even though some known duplicate genes (such as some duplicate copies that were formed in whole genome duplication [WGD]) were excluded from the list. Singletons were defined as proteins that do not have any other homologous proteins in the genome with E -values of <0.1 . All possible duplicate pairs were studied. We obtained similar results for duplicate gene pairs from two gene families (data not shown).

We further grouped duplicate gene pairs and singletons into two categories, respectively. For duplicate genes, group I (82 pairs) includes those duplicate pairs that have negative epistasis, indicating possible functional overlap and genetic redundancy, between each other. Group II (1,005 pairs) includes those duplicate pairs that do not show negative epistasis between duplicate copies. For single-copy genes, type I

singletons include those genes whose orthologs don't have duplicate genes in any of the 42 sequenced fungal species (368 genes) and type II singletons include those genes whose orthologs have duplicate genes in other fungal species (194 genes).

In order to see how epistatic interactions evolved after gene duplication, first, we calculated the correlation between the numbers of epistatic interactions with the level of sequence divergence for duplicate gene pairs (supplementary table 1, Supplementary Material online). Then we classified all duplicate gene pairs into five groups according to the levels of sequence divergence between duplicate genes: very low (47 duplicated pairs with E -values $<10^{-200}$); low (40 pairs with E -values $>10^{-200}$, and $<10^{-150}$); medium (43 pairs with E -value $>10^{-150}$ and $<10^{-100}$); high (136 pairs with E -values $>10^{-100}$ and $<10^{-50}$); and very high (821 pairs with E -values $>10^{-50}$ and $<10^{-10}$). When we grouped duplicate gene pairs according to their amino acid identities, we obtained similar results (supplementary fig. 3, Supplementary Material online). Ribosomal duplicate gene pairs do not affect our conclusions (data not shown).

Functional Association Analysis

Physical Interaction

We downloaded all protein–protein interactions in budding yeast from the SGD website (<http://www.yeastgenome.org/>, Accessed 22 March 2011) and compared the number of protein interactions linking epistatic interaction partners of duplicate gene pairs (1,087 pairs) versus singletons (562 genes). The average numbers of protein–protein interactions among epistatic interaction partners of duplicate gene pairs and singletons were 322 and 175, respectively. However, duplicate gene pairs have more epistatic interaction partners than single-copy genes. To exclude the impact of gene-number difference, for each duplicate gene pair and singleton, we respectively normalized the number of protein–protein interactions among their epistatic-interaction partners by the total number of all possible gene pairs among these partners.

Transcriptional Coregulation

Microarray data generated under varied experimental conditions (<http://www.weizmann.ac.il/home/barkai/Rewiring>, Accessed 22 March 2011) were downloaded (Ihmels et al. 2005). The data set compiles microarray gene expression under multiple conditions and contains 1,011 data points for each gene. Pair-wise correlation coefficients for gene expression among the studied genes were calculated using R. For each duplicate gene pair and singleton, we calculated the average correlation coefficients of gene expression between each pair of their epistatic-interaction partners.

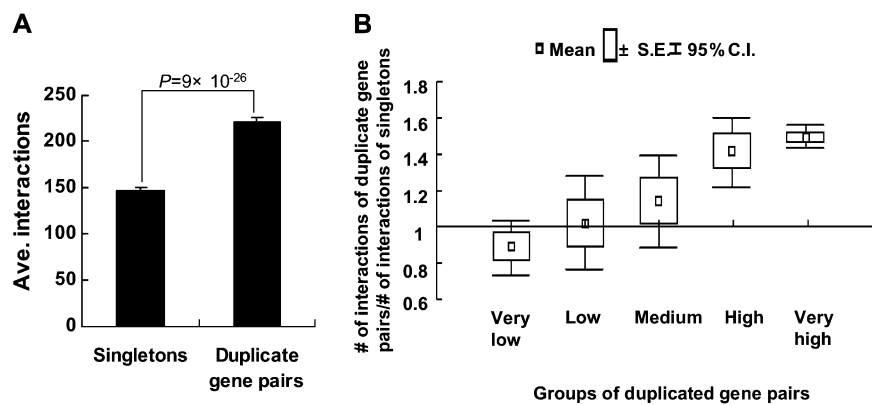


FIG. 1.—Evolution of epistasis by gene duplication. (A) Duplicate gene pairs have significantly more epistatic interaction partners than do singletons. (B) The distribution for the number of epistatic interactions for duplicate gene pairs with different levels of sequence divergence. All duplicate gene pairs were divided into five groups according to the levels of sequence divergence between duplicate genes: Very low (E -value $< 10^{-200}$), Low (E -value $> 10^{-200}$ and $< 10^{-150}$), Medium (E -value $> 10^{-150}$ and $< 10^{-100}$), High (E -value $> 10^{-100}$ and $< 10^{-50}$), and Very high (E -value $> 10^{-50}$ and $< 10^{-10}$). The number of epistatic interactions for duplicate gene pairs in each group was divided by the average number of epistatic interactions for singletons (analysis of variance: $F = 8.80$, $P = 5.5 \times 10^{-7}$, degrees of freedom = 4, SE, standard error; CI, confidence interval).

Functional Diversity Analysis

The data for the gene ontology (GO) terms, number of environmental perturbation conditions under which gene-deletion mutants show fitness defect and cellular sublocalization of each gene in *S. cerevisiae* were downloaded from SGD (<http://www.yeastgenome.org/>, Accessed 22 March 2011). For each duplicate gene pair or singleton, we calculated the average number of functional proxies (GO terms, phenotypes, and localizations) for their epistatic-interaction partners by counting the total number of each functional proxy occupied by their epistatic-interaction partners and dividing this by the respective number of epistatic-interaction partners.

Results and Discussions

Gene Duplication Leads to More Epistatic Interactions

In order to investigate how gene duplication contributes to the evolution of epistasis, we compared the number of epistatic interactions of duplicate genes and singletons. Consistent with previous results (Costanzo et al. 2010), on average, individual duplicate genes have fewer epistatic interactions than single-copy genes (data not shown). As we are interested in how gene duplication contributes to the growth of epistatic interaction network at the genomic level, the sum of epistatic interactions for a duplicate gene pair, including the original epistatic interaction partners that were inherited from the common ancestor before the duplication occurred, and the epistatic interactions that have been gained since duplication, reflect the real number of epistatic interactions after duplication. As shown in figure 1A, the number of epistatic interactions for duplicate gene pairs is significantly larger than that of single-copy genes (fig. 1A). Similar conclusions still hold when a more stringent cutoff

for epistatic interactions was used, and positive and negative epistasis were analyzed separately (supplementary fig. 1, Supplementary Material online).

Using a protein–protein interaction network, He and Zhang (2005) showed that after gene duplication, duplicate copies can have rapid “subfunctionalization” accompanied by prolonged “neofunctionalization” during evolution. In order to test if the model is also true for the evolution of epistatic interaction after gene duplication, we grouped duplicate gene pairs into five categories based on their level of sequence divergence. As shown in figure 1B, the sum of epistatic interactions for closely related duplicate gene pairs is similar to the number of epistatic interactions for single-copy genes. Furthermore, the number of epistatic interactions in each group is positively correlated with the level of sequence divergence between duplicate gene pairs (analysis of variance: $P = 5.5 \times 10^{-7}$). There is also an overall correlation between sequence similarity and combined interaction degree for individual duplicate gene pairs (supplementary table 1, Supplementary Material online).

Our results suggest that for closely related duplicate genes, subfunctionalization might be important in determining the number of epistatic interaction partners for two duplicate copies. The sum of epistatic interactions gradually increases when duplicate gene pairs evolved more sequence divergence. The ancestor of *S. cerevisiae* went through a WGD event around 100 Ma (Wolfe and Shields 1997). The WGD paralogs might have different effects on the epistatic interaction network from smaller scale duplication events. With the WGD paralogs removed, however, we still observed a similar pattern (supplementary table 1, Supplementary Material online), indicating that gene duplication can indeed lead to an increase in epistatic interaction in the yeast genome.

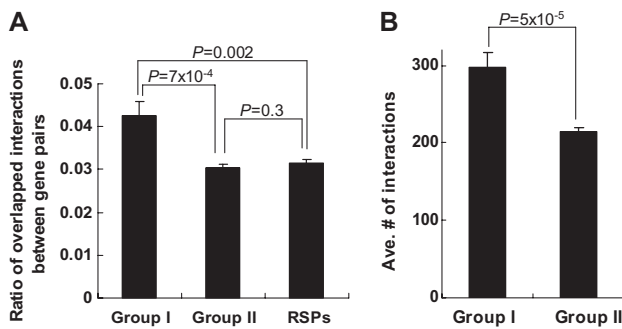


FIG. 2.—Functional redundancy and epistasis for duplicate genes. (A) Group I duplicate gene pairs (which have negative epistasis between two copies) share more epistatic interaction partners between two copies than group II duplicate genes (which do not have negative epistasis between two copies) and RSPs (1,000 randomly generated singleton pairs). The latter two are not significantly different. (B) The average number of epistatic interactions for group I duplicate gene pairs is significantly larger than that for group II duplicate gene pairs. Student's *t*-test was used for the comparison.

There might be alternative explanations for our observations. We argue that the overall reduction of epistatic interaction for individual duplicate genes occurred as a result of subfunctionalization after gene duplication. However, another possible explanation is that functional redundancy between duplicate genes masked the detection of epistatic interactions by double-gene knockout approach. Therefore to test this, we grouped duplicate gene pairs into two categories: group I duplicate gene pairs that have functional redundancy (defined as two duplicate genes forming negative epistasis) and group II duplicate gene pairs that do not have functional redundancy (Ihmels et al. 2007). Indeed, as we show in figure 2A, the group I duplicate gene pairs have more functional redundancy because they share significantly more epistatic interaction partners than the group II duplicate gene pairs ($P = 7 \times 10^{-4}$), which is consistent with a recent study (Li, Yuan, and Zhang 2010). Furthermore, comparing with random singleton pairs, the group I duplicate gene pairs share significantly more epistatic interaction partners ($P = 0.002$), whereas the group II duplicate gene pairs do not show any significant differences ($P = 0.3$). Interestingly, the average number of epistatic interactions for the group I duplicate gene pairs is even larger than that for the group II duplicate gene pairs ($P = 5 \times 10^{-5}$, fig. 2B), indicating that even if functional redundancy between duplicate gene pairs can mask epistasis detection for specific duplicate gene pairs, it might not be a general mechanism that can lead to reduction of epistatic interactions for duplicate genes.

Although epistatic interaction for individual duplicate gene is overall reduced, the sum of epistatic interactions for duplicate gene pairs is increased (fig. 1A). We argue that the overall increase of epistatic interaction for duplicate gene pairs occurred because novel epistatic interactions might be gained

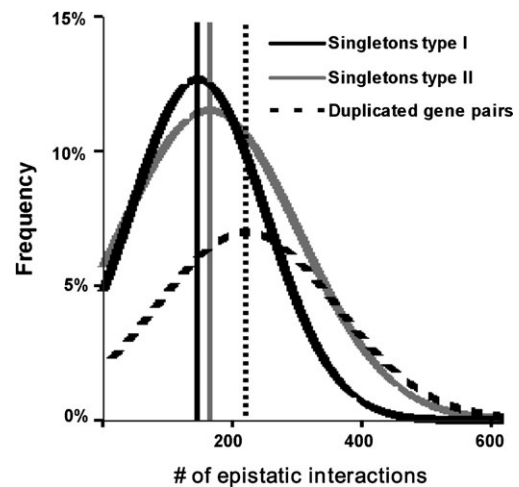


FIG. 3.—The distribution for the number of epistatic interactions for duplicate gene pairs and singletons. The black, gray, and dashed lines show the distributions for type I singletons (whose orthologous genes do not have duplicate genes in other fungi), type II singletons (whose orthologous genes have duplicate genes in other fungi), and duplicate gene pairs, respectively. The vertical lines show the average number of epistatic interactions for each distribution. The number of epistatic interaction partners for the type I and type II singletons are not significantly different.

after gene duplication. However, another possible explanation for this observation is that genes with more epistatic interactions tended to experience more duplication events. To investigate this possibility, we grouped single-copy genes into two categories: those genes whose orthologs in 42 sequenced fungal species don't have any duplicate genes (type I singletons) and those genes whose orthologs have duplicate genes in other fungal species (type II singletons). As shown in figure 3, the average number of epistatic interactions of duplicate gene pairs is significantly larger than that of both type I and type II single-copy genes (*t*-test, $P = 2 \times 10^{-8}$ and $P = 3 \times 10^{-5}$, respectively). Although the number of epistatic interactions for type II singletons is slightly increased in comparison with type I singletons, the difference is not statistically significant (*t*-test, $P = 0.84$), indicating that the number of epistatic interactions for a gene does not determine its propensity to duplicate.

Excluding these possible alternative explanations, our results indicate that gene duplication can indeed lead to more epistatic interactions. The overall evolution of epistasis by gene duplication might be explained by a model with an initial subfunctionalization in combination with a gradual neofunctionalization after gene duplication, a process that is similar to the evolution of physical interactions (He and Zhang 2005; Conant and Wolfe 2008; Marcussen et al. 2010). A recent study showed that the complementary loss and/or gain of epistatic interaction after gene duplication occurred asymmetrically between duplicate gene pairs (VanderSluis et al. 2010).

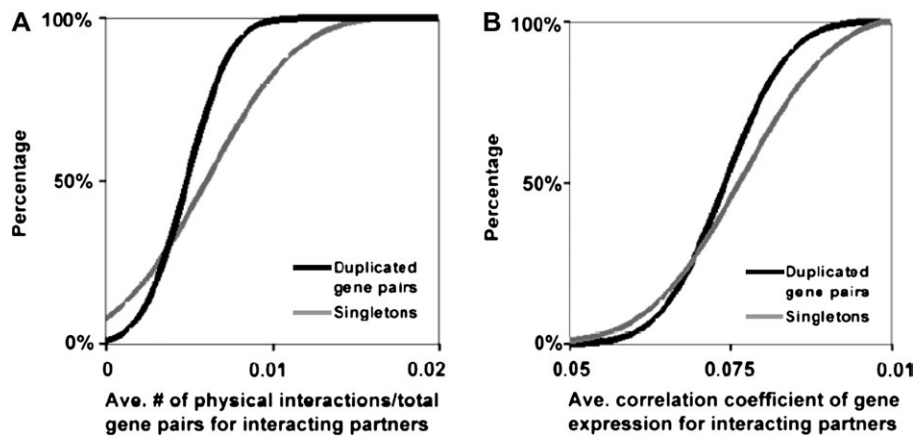


FIG. 4.—The functional distribution of epistatic-interaction partners for duplicate gene pairs (black line) and singletons (gray line). (A) In the protein–protein interaction network, the average numbers of protein–protein interactions among epistatic-interaction partners of duplicate gene pairs were compared with those for single-copy genes. As the numbers of epistatic-interaction partners are different for different genes, we normalized the observed number of protein–protein interactions by the total number of all possible gene pairs among epistatic-interaction partners. The cumulative distribution for the normalized protein–protein interaction connectivity is shown. (B) In gene coregulation network, the average correlation coefficients of pair-wise gene expression among epistatic-interaction partners for each duplicate gene pair or single-copy gene were calculated. The cumulative distributions of the average correlation coefficients are shown. Both distributions are significantly different between duplicate gene pairs and singletons.

Duplicate Gene Pairs Interact with Partners That Are More Diversely Connected

To further support our conclusion that gene duplication can, indeed, lead to the evolution of novel function by expanding the epistatic interaction network, we first investigated how epistatic-interaction partners of duplicate gene pairs and singletons are distributed. For this purpose, we compared two measurements, that is, physical interaction and transcriptional coregulation, among the epistatic-interaction partners of duplicate gene pairs and singletons. Both measurements indicate functional connections among genes. For each duplicate gene pair or singleton, we counted the number of protein–protein interactions among their epistatic-interaction partners and normalized this number by the total number of all possible gene pairs among these partners. As shown in figure 4A, the epistatic-interaction partners of duplicate gene pairs display significantly fewer protein–protein interactions than those of singletons (t -test, $P = 5 \times 10^{-7}$).

Consistent with this observation, the epistatic-interaction partners of duplicate gene pairs have significantly smaller correlation coefficients for gene expression among themselves than those of singletons (fig. 4B, t -test, $P = 7 \times 10^{-5}$). Our results indicate that the epistatic-interaction partners of duplicate gene pairs tend to have less functional association among themselves than those of singletons. Furthermore, these results are not affected by difference in functional distribution between duplicate genes and singletons (data not shown), indicating that gene duplication can, indeed, expand the epistasis network by interacting with partners that are more diversely connected.

Duplicate Gene Pairs Interact with Partners That Occupy Larger Functional Spaces

We further investigated how epistatic interactions expand the functional versatility of duplicate genes. Figure 1B shows that the connectivity of duplicate gene pairs in the epistatic interaction network increased as duplicate gene pairs diverged at the sequence level. As the connectivity of epistatic interactions is an important indicator for gene pleiotropy (Costanzo et al. 2010), the above observation implies that duplicate gene pairs can gradually evolve more functions after duplication. Indeed, comparison between duplicate pair and single-copy genes for their functional diversity parameters, including GO terms, the number of conditions under which gene-deletion mutants showed fitness defects and protein cellular localization confirmed this expectation (supplemental fig. 2, Supplementary Material online).

More importantly, our results also show that the epistatic-interaction partners of duplicate gene pairs also occupied more functional spaces than the partners of single-copy genes: as shown in figure 5, after being normalized for the respective number of epistatic-interaction partners, the partners of duplicate gene pairs have significantly more overall GO terms (t -test, $P = 5 \times 10^{-27}$), show fitness defects after being deleted under more conditions of environmental perturbations (t -test, $P = 4 \times 10^{-32}$), and occupy more cellular localizations (t -test, $P = 3 \times 10^{-21}$) than the partners of singletons. In addition, figure 5 shows that the numbers of these functional diversity proxies for the epistatic-interaction partners of duplicate gene pairs are all positively correlated with the level of sequence divergence

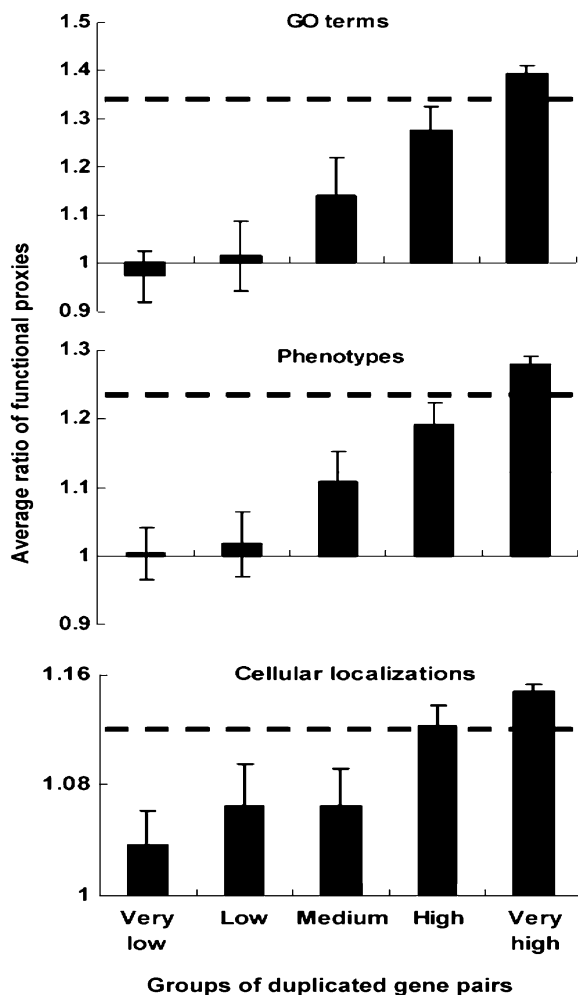


FIG. 5.—Duplicate gene pairs interact with partners that occupy larger functional space. The duplicate gene pairs were grouped as in figure 1B. The relative number of functional indexes for GO terms, environmental perturbation conditions under which gene-deletion mutants show fitness defect (labeled as phenotypes), and cellular localizations are shown for the epistatic-interaction partners of duplicate gene pairs. The number of each functional index for these partners was divided by that of singleton. The average and standard deviation of the normalized values for duplicate gene pairs in each category are shown. The dashed line represents the average value for all duplicate gene pairs.

between these gene pairs, indicating that gene duplication also contributed to the evolution of gene pleiotropy by acquiring epistatic interactions with functionally versatile partners.

Contribution of Gene Duplication to the Evolution of Epistasis

Our results provide a rough estimate of how much epistasis was contributed by gene duplication. If the ancestral genes before duplication had the same number of epistatic interactions

as those of single-copy genes, our results show that epistatic interactions increased ~46% after gene duplication. One caveat of this study is that we used current single-copy genes in the yeast genome as a baseline to infer the evolution of epistasis after gene duplication. Although the assumption is difficult to prove directly, it is reasonable because our results show that the number of epistatic interactions is not different between single-copy genes that do or do not have duplicate genes in other yeast species, indicating that the number of epistatic interaction for a gene does not determine its probability to duplicate. As gene duplication is prevalent in eukaryotic genomes, if what we have observed in yeast is true for other species, our results demonstrate that gene duplication plays an important role in the evolution of epistasis in nature.

Supplementary Material

Supplementary figures 1–3 and table 1 are available at *Genome Biology and Evolution* online (<http://www.gbe.oxfordjournals.org/>).

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