Magnetically simulated displacements

In the experiments described in Fig. 3, the orientation arena was surrounded by a magnetic coil system that was used to control the field in which each lobster walked. The coil system consisted of two different independent four-coil systems arranged orthogonally[28]. Each coil measured 2.3 m on a side. Lobsters were restricted by a tether to an area in the centre of the coil defined by a horizontal circle of radius 25 cm. In this region, calculated[30] and measured deviations from perfect field uniformity were less than 0.5%. Each lobster was then tethered as before and tested in one of two magnetic fields. One field replicated magnetic conditions that exist at a location approximately 400 km to the north, whereas the other replicated a field at a location approximately 400 km to the south. The field used to approximate magnetic conditions at the location north of the test site had an inclination of 59.3° and a total intensity of 47.9 μT. The field simulating the location south of the test site had an inclination of 51.4° and total intensity of 42.8 μT. All magnetic field values were verified by three independent measurements with an Applied Physics Fluxgate Magnetometer (model 520A). The experimental fields were based on estimates provided by the International Geomagnetic Reference Field (IGRF) model, 2000 revision, for August 2001 (when the data were collected) using latitude 28.5° N, longitude 80.5° W for the northern site, and latitude 20.5° N, longitude 80.5° W for the southern site. Experiments were conducted in Long Key, Florida (latitude 24.8° N, longitude 80.8° W) where the measured inclination angle was 55.8° and the total field intensity was 45.3 μT.

Received 6 June; accepted 7 October 2002; doi:10.1038/nature01226.

1. Griffin, D. R. Bird navigation. *Biol. Rev.* **27,** 359–400 (1952).
2. Phillips, J. B. Magnetic navigation. *J. Theor. Biol.* **180,** 309–319 (1996).
3. Phillips, J. B., Adler, K. & Borland, S. C. True navigation by an amphibian. *Anim. Behav.* **50,** 855–858 (1995).
4. Wehner, R., Michel, B. & Antonsen, P. Visual navigation in insects: Coupling of egocentric and geocentric information. *J. Exp. Biol.* **199,** 129–140 (1996).
5. Walker, M. M. in *Orientation and Communication in Arthropods* (ed. Lehrer, M.) 187–213 (Birkhäuser, Basel, 1997).
6. Kramer, G. Experiments in bird orientation and their interpretation. *Ibis* **99,** 196–227 (1957).
7. Able, K. P. The concepts and terminology of bird navigation. *J. Av. Biol.* **32,** 174–183 (2001).
8. Able, K. P. Common themes and variations in animal orientation systems. *Amer. Zool.* **31,** 157–167 (1991).
9. Walcott, C. Pigeon homing: Observations, experiments, confusions. *J. Exp. Biol.* **199,** 21–27 (1996).
10. Gould, J. L. The map sense of pigeons. *Nature* **296,** 205–211 (1982).
11. Wiltschko, R. & Wiltschko, W. *Magnetic Orientation in Animals* (Springer-Verlag, Berlin, 1995).
12. Walcott, C. in *Orientation in Birds* (ed. Berthold, P.) 38–51 (Birkhauser, Boston, 1991).
13. Papi, F. Animal navigation at the end of the century: A retrospect and a look forward. *Ital. J. Zool.* **68,** 171–180 (2001).
14. Herrnkind, W. in *The Biology and Management of Lobsters* (eds Cobb, J. S. & Phillips, B. F.) 349–407 (Academic Press, New York, 1980).
15. Herrnkind, W., Van Derwalker, J. & Barr, L. Population dynamics, ecology, and behavior of spiny lobster, *Panulirus argus*, of St. John, U. S. Virgin Islands: Habitation and patterns of movements. Results of the Tektite program, vol. 2. *Sci. Bull., Nat. Hist. Mus., Los Angeles Co.* **20,** 31–45 (1975).
16. Creaser, E. P. & Travis, D. Evidence of a homing instinct in the Bermuda spiny lobster. *Science* **112,** 169–170 (1950).
17. Lohmann, K. J. *et al.* Magnetic orientation of spiny lobsters in the ocean: Experiments with undersea coil systems. *J. Exp. Biol* **198,** 2041–2048 (1995).
18. Fischer, J. H., Freake, M. J., Borland, S. C. & Phillips, J. B. Evidence for the use of magnetic map information by an amphibian. *Anim. Behav.* **62,** 1–10 (2001).
19. Lohmann, K. J. & Lohmann, C. M. F. Detection of magnetic field intensity by sea turtles. *Nature* **380,** 59–61 (1996).
20. Lohmann, K. J. Magnetic remanence in the western Atlantic spiny lobster, *Panulirus argus*. *J. Exp. Biol.* **113,** 29–41 (1984).
21. Groeneveld, J. C. & Branch, G. M. Long distance migration of South African deep-water rock lobster, *Palinurus gilchristi*. *Mar. Ecol. Prog. Ser.* **232,** 225–238 (2002).
22. Gregory, D. R. & Labisky, R. F. Movements of the spiny lobster, *Panulirus argus*, in south Florida. *Can. J. Fish. Aq. Sci.* **43,** 2228–2234 (1986).
23. Gould, J. L. Sensory bases of navigation. *Curr. Biol.* **8,** R731–R737 (1998).
24. Kirschvink, J. L., Jones, D. S. & MacFadden, B. J. *Magnetite Biomineralization and Magnetoreception in Organisms* (Plenum, New York, 1985).
25. Walker, M. M. *et al.* Structure and function of the vertebrate magnetic sense. *Nature* **390,** 371–377 (1997).
26. Lohmann, K. J., Cain, S. D., Dodge, S. A. & Lohmann, C. M. F. Regional magnetic fields as navigational markers for sea turtles. *Science (Washington DC)* **294,** 364–366 (2001).
27. Munro, U., Munro, J. A., Phillips, J. B., Wiltschko, R. & Wiltschko, W. Evidence for a magnetite-based navigational "map" in birds. *Naturwissenschaften* **84,** 26–28 (1997).
28. Lohmann, K. J. & Lohmann, C. M. F. Detection of magnetic inclination angle by sea turtles: A possible mechanism for determining latitude. *J. Exp. Biol* **194,** 23–32 (1994).
29. Zar, J. H. *Biostatistical Analysis* (Prentice Hall, Upper Saddle River, New Jersey, USA, 1996).
30. Kirschvink, J. Uniform magnetic fields and double wrapped coil systems: Improved techniques for the design of bioelectromagnetic experiments. *Bioelectromag.* **13,** 401–411 (1992).

Acknowledgements We thank K. Cohen, M. Piet and S. Peters for research assistance, and C. Lohmann and S. Johnsen for a critical reading of manuscript drafts. This work was supported by grants from the PADI Foundation and the National Science Foundation.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to L.C.B. (e-mail: LBoles@email.unc.edu).

Role of duplicate genes in genetic robustness against null mutations

Zhenglong Gu*†, Lars M. Steinmetz‡†, Xun Gu§, Curt Scharfe‡, Ronald W. Davis‡ & Wen-Hsiung Li*

** Department of Ecology & Evolution, University of Chicago, 1101 East 57th Street, Chicago, Illinois 60637, USA*
‡ Department of Biochemistry, and Stanford Genome Technology Center, Stanford University School of Medicine, Stanford, California 94305, USA
§ Departments of Zoology and Genetics, Iowa State University, Ames, Iowa 50011, USA
† These authors contributed equally to this work

Deleting a gene in an organism often has little phenotypic effect[1–5], owing to two mechanisms of compensation[4–10]. The first is the existence of duplicate genes: that is, the loss of function in one copy can be compensated by the other copy or copies. The second mechanism of compensation stems from alternative metabolic pathways, regulatory networks, and so on. The relative importance of the two mechanisms has not been investigated except for a limited study, which suggested that the role of duplicate genes in compensation is negligible[10]. The availability of fitness data for a nearly complete set of single-gene-deletion mutants of the *Saccharomyces cerevisiae* genome[11] has enabled us to carry out a genome-wide evaluation of the role of duplicate genes in genetic robustness against null mutations. Here we show that there is a significantly higher probability of functional compensation for a duplicate gene than for a singleton, a high correlation between the frequency of compensation and the sequence similarity of two duplicates, and a higher probability of a severe fitness effect when the duplicate copy that is more highly expressed is deleted. We estimate that in *S. cerevisiae* at least a quarter of those gene deletions that have no phenotype are compensated by duplicate genes.

No correlation was found between the sequence similarity of duplicate genes and the fitness effect of a null mutation in one of the two duplicates when functional data from the yeast *S. cerevisiae* was analysed previously[10]. It was therefore concluded that gene duplications contribute little to the ability of an organism to withstand mutations (genetic robustness), although they may be responsible for a small fraction of weak, null-mutation phenotypes[12]. Because this conclusion was based on only 45 duplicate genes, however, the issue deserves further investigation. Indeed, this conclusion is not supported by a limited analysis of a third of the genes in the yeast genome[1] and is contrary to the general observation of relaxed selective constraints after gene duplication[13,14].

From 5,766 yeast open reading frames (ORFs) for which we had a fitness measure of strains with a corresponding single-gene deletion[11], we found 1,509 duplicate (paralogous) genes. To avoid including pseudogenes and erroneously predicted genes, we subsequently analysed only genes that had been studied previously (that is, each had a gene name in the Saccharomyces Genome Database (SGD) in addition to its ORF name). This yielded 1,275 singleton genes, and 1,147 duplicate genes that had at least one paralogue elsewhere in the genome. We compared the frequency distribution of fitness for duplicate genes with that for singletons (Fig. 1a). We classified genes into four groups on the basis of the minimum fitness value for a strain across the five different growth conditions tested (Methods) including both fermentation and respiration, the main growth conditions of yeast.

The two distributions were significantly different ($P \ll 0.001$): duplicate genes had a significantly lower proportion of genes with a lethal effect of deletion (12.4% versus 29.0%) and a significantly higher proportion of genes with a weak or no effect of gene deletion

(64.3% versus 39.5%, the latter value is similar to a previous estimate of 43.6% (ref. 10); Fig. 1a). This comparison indicates that there is a significantly higher probability of functional compensation for a duplicate gene than for a singleton. We emphasize that 'compensation' here does not imply that the gene is dispensable in long-term evolution but means that the gene is dispensable in an individual under the conditions tested[6]. When the genes with a lethal effect of deletion were excluded from both collections, the difference between the two distributions remained significant ($P \ll 0.001$; Supplementary Table 1).

To see whether the above conclusion held for different growth environments, we compared the distributions of fitness (f) for duplicate and singleton genes under each of the five growth conditions studied[11]. The empirical cumulative distributions of f under the YPD growth condition (Methods) for duplicate genes and for singletons are shown in Fig. 1b. The Kolmogrov–Smirnov test indicated that the two distributions were significantly different ($P = 2.2 \times 10^{-16}$). The same conclusion held for the other four growth conditions (Supplementary Fig. 1).

If duplicate genes tend to compensate for each other's function, then a testable prediction is that deletions of duplicate genes should tend to show similar fitness effects. To avoid multiple comparisons within a gene family, independent (non-overlapping) duplicate gene pairs were selected randomly from each gene family. For each of the 418 duplicate gene pairs selected (with both copies having been previously studied), we computed the difference between the fitness effects of duplicate genes i and j (D_{ij}) and then obtained the mean of all D_{ij} values (D^*) for each growth condition. For comparison, 418 protein pairs were selected randomly from all previously studied genes and the D^* value was calculated as above. This procedure was repeated 100,000 times to derive a frequency distribution of the mean difference in fitness effects between genes for each studied condition. The mean value ($D^* = 0.193$) for duplicate genes was far lower than the mean value for random gene pairs ($P < 10^{-5}$) under the YPD growth condition (Fig. 2), confirming that duplicates tend to show more similar fitness effects of deletion than do random gene pairs. The same results held for the four other growth conditions (Supplementary Fig. 2).

The two genes derived from a duplication should initially have the same function. In long-term evolution, the accumulation of mutations in both copies over time results in either functional loss in one copy or functional divergence between the two copies[15]. If gene duplication is important for genetic robustness, genes with close paralogues should be compensated for deletion more often than genes with only distant paralogues. To test this hypothesis, we focused on a set of duplicate genes that excluded ribosomal proteins because of their unusual properties, such as strong codon usage bias, very high expression, and severe fitness effects of null mutations. This set of duplicate genes was divided further into different groups on the basis of the nonsynonymous distance (K_A) of each gene to its most similar gene in the genome (defined as the gene with the smallest K_A value to the studied duplicate gene). Within each K_A interval, the frequencies of genes with different values of f were calculated. The fitness classification was based on the minimum fitness of a strain across all of the five tested media conditions, as in Fig. 1a. As expected under the above hypothesis, the proportion of genes with a weak or no effect decreased with K_A (correlation coefficient, $R = -0.95$; $P < 0.001$), whereas the proportion of genes that are lethal when deleted increased with K_A ($R = 0.94$, $P < 0.001$; Fig. 3). This observation is contrary to the previous conclusion that there is no correlation between K_A and the fitness effect of deleting a duplicate gene, which was based on a much smaller data set[10].

As the sequence similarity between duplicated genes decreases, their frequency of compensation will approach that for singletons. But even among duplicate genes with a K_A greater than 0.7 from their most similar paralogues in the whole genome, about 53% still had a weak effect or no effect when deleted (Fig. 3), which was significantly higher than the 39.5% of singletons that showed a weak or no effect of deletion (Fig. 1a, χ^2-test, $P < 0.01$), implying that the compensation effect might exist even for ancient duplicate genes. Nevertheless, the decreasing compensation effect between duplicate genes with K_A suggests that the functional divergence of duplicate genes is coupled to some extent with the divergence of their protein sequences.

Because expression level is used frequently to infer the activity and function of gene products, we tested whether deleting the copy

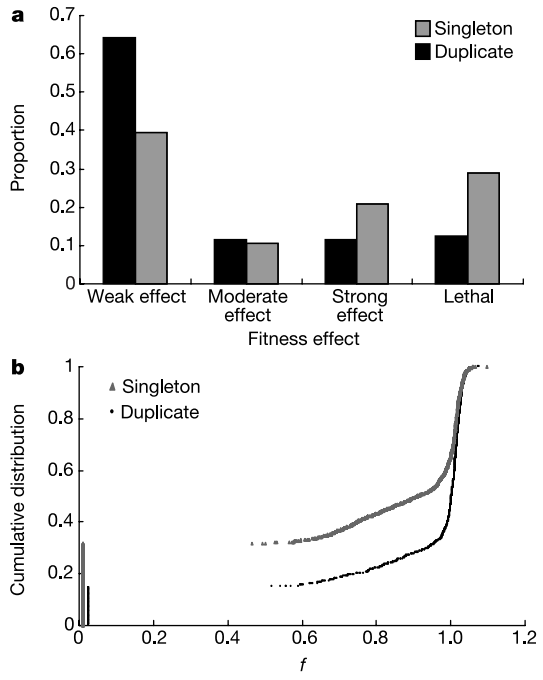

Figure 1 Distributions of fitness (f). **a**, Discrete distributions of f for singleton genes and for duplicate genes. The difference for the two distributions is significant (a contingency table test, $P \ll 0.001$) under YPD growth conditions. **b**, Empirical cumulative distributions of f for singleton genes and for duplicate genes. Data points with $f = 0$ have been shifted to $f = 0.025$ for duplicate genes and to $f = 0.01$ for singleton genes to distinguish between the two curves, but remained unchanged in the statistical analysis. The Kolmogrov–Smirnov test shows that the two distributions are significantly different ($P = 2.2 \times 10^{-16}$).

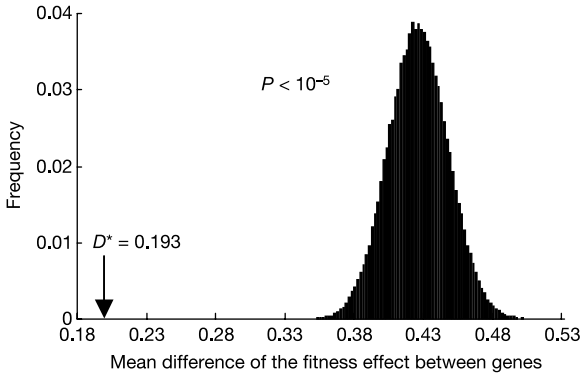

Figure 2 Distribution of mean fitness differences between randomly selected gene pairs (100,000 replicates each with 418 gene pairs) under the YPD growth condition. Arrow indicates the mean difference ($D^* = 0.193$) between duplicate genes.

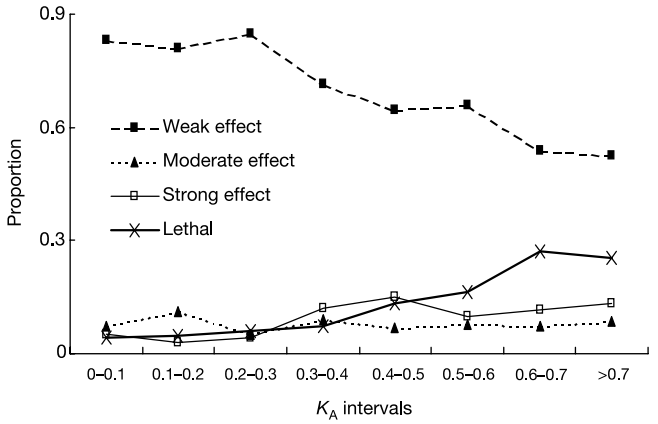

Figure 3 Relationship between protein distance and fitness effect of deletion. Protein distance is measured by the K_A of each gene to its most similar paralogue in the genome. The proportion of genes with a weak effect of deletion decreases with K_A ($R = -0.95$, $P < 0.001$), whereas the proportion of genes with lethal effect increases with K_A ($R = 0.94$, $P < 0.001$).

of a duplicate gene that is more highly expressed would have a stronger fitness effect than deleting the copy with lower expression. We considered only duplicate gene pairs with different fitness effects of deletion (that is, those in which the relative fitness difference (as defined by $|(f_i - f_j)/[(f_i + f_j)/2]|$) for genes i and j was larger than 5%, or those for which one of the two duplicate copies is essential and the other is not). Expression (absolute transcript abundance) was estimated using available data measured by Affymetrix microarray experiments[16]. We used the fitness effects of the duplicate genes measured under the YPD growth condition in this analysis because the expression levels were also measured under the YPD growth condition[16]. Deleting the duplicate gene that has higher expression indeed tended to have a larger fitness effect (Table 1). For example, in 72 of the 98 gene pairs where the deletions had different fitness effects, the stronger fitness effect was seen in the more highly expressed gene.

The high frequency of genes that have weak or no fitness effects of deletion among single genes, as well as among duplicate genes (Fig. 1a), indicates that the fitness effect of a gene deletion is also affected by factors other than copy redundancy. Whereas some genes may show null-mutation phenotypes only under experimental conditions that differ from the five growth conditions tested here, a fraction of weak, null-mutation phenotypes among singletons might be due to compensation through alternative pathways or network branching, as suggested previously[10]. The relative importance of the compensating mechanism through functionally redundant duplicate genes can be estimated roughly as follows. If we assume that the extra proportion of genes with a weak or no fitness effect of deletion in duplicate genes when compared with the proportion for singleton genes is due to copy redundancy (64.3% for duplicates, 39.5% for singletons; difference 24.8%; Fig. 1a), this will give the lower bound of the contribution of gene duplication to genetic robustness. The proportion of this contribution (G) is estimated to be 23% because for 284 genes (1,147 duplicates × 24.8%) out of a total of 1,241 genes that are robust against deletion (1,147 duplicates × 64.3% + 1,275 singletons × 39.5%), the robustness can be attributed to gene duplication. The upper bound can be estimated by assuming that all of the genes with a weak or no fitness effect of deletion in duplicate genes are due to copy redundancy. This gives an upper estimate of 59% for G because 738 duplicate genes (1,147 duplicates × 64.3%) and 503 singleton genes (1,275 singletons × 39.5%) showed a weak or no fitness effect of deletion (Fig. 1a).

As our estimates of G were based on only about a third of all genes in the yeast genome owing to the stringent criteria used originally to define singletons and duplicate genes (Methods), we repeated the calculations on more genes by using a less stringent criterion to group genes. We divided the whole set of yeast genes into singletons and duplicate genes as follows: a protein was defined as a singleton if it did not have a hit with any other proteins in a FASTA[17] search of the whole yeast proteome (6,357 ORFs) with $E = 0.01$ (E is the expected number of hits by chance); otherwise, it was a duplicate gene. This procedure led to 3,249 duplicate genes and 3,108 singletons. We then selected only genes that have been studied previously: 2,240 duplicate genes and 1,567 singletons, for which 2,063 and 1,477, respectively, had an associated fitness measurement. By applying the above procedure to these two sets of duplicate genes and singletons, we estimated G to be between 0.21 and 0.67; these two values are not very different from the above lower and upper bounds, respectively. In conclusion, the analyses in this study provide strong evidence for the importance of duplicate genes in genetic robustness against null mutations.

Table 1 **Relationship between expression and fitness effect of null mutation in duplicate genes***

Relative expression	Number of genes with a larger fitness effect of deletion		
	Different effect†	One lethal	Similar effect†
High	72‡	50‡	125
Low	26	12	108
Total	98	62	233

*Only gene pairs for which both copies have been studied previously were included.
†Two duplicate genes i and j were said to have different fitness effects if $|(f_i - f_j)/[(f_i + f_j)/2]| > 0.05$, but similar fitness effects if otherwise.
‡Significant at $P < 0.001$.

Although our estimates are compatible with the view that interactions among unrelated genes rather than duplicate genes are the main cause of genetic robustness against mutations[10,18], two additional factors need to be considered. First, because we have considered only five growth conditions, it is possible that when a gene deletion showed no effect in any of these conditions it was not due to compensation by other genes but was because the gene deleted was not related to the growth conditions used. Intuitively, when more growth conditions are studied, both the proportion of duplicate genes and the proportion of singletons that show only a weak or no effect of deletion on growth rate will decrease. Indeed, the two proportions were 70.9% and 49.2% when only the YPD growth condition was considered (data not shown), but became 64.3% and 39.5% when the five growth conditions shown in Fig. 1a were used. The decrease is larger for singletons than for duplicate genes, probably because duplicate genes have on average a stronger overlap in function than do singletons and so can compensate each other in a wider range of conditions. For this reason, our lower bound of 23% for the relative contribution of duplicate genes to compensation for null mutations is likely to be an underestimate. Second, a singleton in this or other studies could actually have one or more paralogues in the genome that cannot be detected by the criteria used but still overlap in function. Thus, gene duplication might be the ultimate origin of functional compensation for some 'singletons'. In conclusion, whether the contribution of gene duplication to genetic robustness is really less important than interactions among unrelated genes is an issue that remains to be resolved by further studies. □

Methods

Fitness measurements

Fitness measurements were obtained from a high-throughput study[11] that measured the growth of each strain of a nearly complete collection of yeast single-gene-deletion mutants under both fermentable and non-fermentable (respiratory) growth conditions. We studied five growth media: YPD (1% Bacto-peptone (Difco), 2% yeast extract and 2% glucose), YPDGE (0.1% glucose, 3% glycerol and 2% ethanol), YPE (2% ethanol), YPG (3% glycerol) and YPL (2% lactate). Each strain contained the precise homozygous

diploid deletion of 1 of 4,706 ORFs in the yeast genome. We calculated the fitness values for each media condition as the extent of survival and reproduction (fitness) of the deletion strain relative to the pool of all strains grown and measured collectively. Fitness values (f) of 1.0 indicate no difference between a single strain and the pool average for that condition; $f < 1.0$ indicates that the strain is less fit, and $f > 1.0$ indicates that the strain is more fit than the pool average. In addition, we added to our analysis 1,060 ORFs that each had a lethal effect when deleted and assayed in YPD; we used only lethal deletions that could be inferred as lethal from both of the two studies conducted[11,19]. We divided all genes into four groups according to the f value as follows: (1) if $f > 0.95$ for all five media conditions[11], the deletion has a weak or no fitness effect in all conditions; (2) if $0.8 \leq f_{min} < 0.95$, where f_{min} is the smallest f value for all five growth conditions, the deletion has a moderate effect; (3) if $0 < f_{min} < 0.8$, the deletion has a strong effect; and (4) if the deletion is lethal, we set $f = 0$.

Identification of duplicate and singleton genes

An all-against-all FASTA[17] search was conducted for the whole set of *S. cerevisiae* protein sequences (downloaded from SGD, http://genome-www.stanford.edu/Saccharomyces/). A single-copy gene (that is, a singleton) was defined as a protein that did not hit any other proteins in the FASTA search with $E = 0.1$; this loose similarity search criterion was used to make sure that a singleton is indeed a singleton. (An even looser criterion $E = 1$ was also used in the definition of singleton genes but the results were essentially the same.) Duplicate genes were identified as described[20] except that the criterion of 80% alignable regions between protein sequences was reduced to 50%, because the 80% requirement can miss some duplicate genes. This relaxed criterion is still conservative for identifying duplicate genes; but because we wanted to detect the differences in fitness effects between real duplicate genes and singletons, we used stringent criteria to define duplicate genes and single genes. For each protein pair that met the homology criteria, the FASTA alignable regions were realigned using ClustalW[21] and the corresponding coding sequences were aligned on the basis of the protein alignments. The number of substitutions per synonymous site (K_S) and the number of substitutions per nonsynonymous site (K_A) between duplicate genes were estimated by the PAML package[22] using default parameters.

Estimation of gene expression

The Affymetrix microarray gene expression data were downloaded from a study investigating gene expression in response to environmental changes across the genome[16]. As controls, gene expression was measured at time zero in YPD before each environmental change was conducted. We averaged seven independent time-zero measurements to obtain an estimate of the absolute abundance of each messenger RNA transcript in YPD. Our data showed a very high correlation ($r = 0.95$, $P = 0$) to previous estimates of the absolute abundance of all transcripts in the yeast genome during YPD growth[23].

Received 29 July; accepted 16 September 2002; doi:10.1038/nature01198.

1. Winzeler, E. A. *et al.* Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285,** 901–906 (1999).
2. Cadigan, K. M., Grossniklaus, U. & Gehring, W. J. Functional redundancy: the respective roles of the 2 sloppy paired genes in *Drosophila* segmentation. *Proc. Natl Acad. Sci. USA* **91,** 6324–6328 (1994).
3. Saga, Y., Yagi, T., Ikawa, Y., Sakakura, T. & Aizawa, S. Mice develop normally without tenascin. *Genes Dev.* **6,** 1821–1831 (1992).
4. Gibson, T. J. & Spring, J. Genetic redundancy in vertebrates: polyploidy and persistence of genes encoding multidomain proteins. *Trends Genet.* **14,** 46–49 (1998).
5. Normanly, J. & Bartel, B. Redundancy as a way of life—IAA metabolism. *Curr. Opin. Plant Biol.* **2,** 207–213 (1999).
6. Brookfield, J. F. Y. Can genes be truly redundant? *Curr. Biol.* **2,** 553–554 (1992).
7. Nowak, M. A., Boerlijst, M. C., Cooke, J. & Smith, J. M. Evolution of genetic redundancy. *Nature* **388,** 167–171 (1997).
8. Tautz, D. Redundancies, development and the flow of information. *BioEssays* **14,** 263–266 (1992).
9. Maslov, S. & Sneppen, K. Specificity and stability in topology of protein networks. *Science* **296,** 910–913 (2002).
10. Wagner, A. Robustness against mutations in genetic networks of yeast. *Nature Genet.* **24,** 355–361 (2000).
11. Steinmetz, L. M. *et al.* Systematic screen for human disease genes in yeast. *Nature Genet.* **31,** 400–404 (2002).
12. Seoighe, C. & Wolfe, K. H. Yeast genome evolution in the post-genome era. *Curr. Opin. Microbiol.* **2,** 548–554 (1999).
13. Lynch, M. & Conery, J. S. The evolutionary fate and consequences of duplicate genes. *Science* **290,** 1151–1155 (2000).
14. Conant, G. C. & Wagner, A. GenomeHistory: a software tool and its application to fully sequenced genomes. *Nucleic Acids Res.* **30,** 3378–3386 (2002).
15. Ohno, S. *Evolution by Gene Duplication* (Springer, Berlin, 1970).
16. Causton, H. C. *et al.* Remodeling of yeast genome expression in response to environmental changes. *Mol. Biol. Cell* **12,** 323–337 (2001).
17. Pearson, W. R. & Lipman, D. J. Improved tools for biological sequence analysis. *Proc. Natl Acad. Sci. USA* **85,** 2444–2448 (1988).
18. Kitami, T. & Nadeau, J. H. Biochemical networking contributes more to genetic buffering in human and mouse metabolic pathways than does gene duplication. *Nature Genet.* **32,** 191–194 (2002).
19. Giaever, G. *et al.* Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418,** 387–391 (2002).
20. Gu, Z., Cavalcanti, A., Chen, F.-C., Bouman, P. & Li, W.-H. Extent of gene duplication in the genomes of *Drosophila*, nematode, and yeast. *Mol. Biol. Evol.* **19,** 256–262 (2002).
21. Thompson, J. D., Higgins, D. G. & Gibson, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22,** 4673–4680 (1994).
22. Yang, Z. & Nielsen, R. Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Mol. Biol. Evol.* **17,** 32–43 (2000).
23. Holstege, F. C. P. *et al.* Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95,** 717–728 (1998).

Supplementary Information accompanies the paper on *Nature*'s website (http://www.nature.com/nature).

Acknowledgements We thank M. Long, T. Oakley, D. Nicolae, K. Thornton, A. Deutschbauer and J. Zhang for discussions and comments. This work was supported by NIH grants to W.H.L and to R.W.D.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to W.H.L. (e-mail: whli@uchicago.edu).

Synaptic depression in the localization of sound

Daniel L. Cook*, Peter C. Schwindt*, Lucinda A. Grande† & William J. Spain*†‡§

* *Department of Physiology and Biophysics,* ‡ *Department of Neurology; and* § *Virginia Merrill Bloedel Hearing Research Center, University of Washington, Seattle, Washington 98105, USA*
† *VA Puget Sound Health Care System, Seattle, Washington 98108, USA*

Short-term synaptic plasticity, which is common in the central nervous system, may contribute to the signal processing functions of both temporal integration and coincidence detection[1–3]. For temporal integrators, whose output firng rate depends on a running average of recent synaptic inputs, plasticity modulates input synaptic strength and thus may directly control signalling gain[2] and the function of neural networks[1–4]. But the firing probability of an ideal coincidence detector would depend on the temporal coincidence of events rather than on the average frequency of synaptic events. Here we have examined a specific case of how synaptic plasticity can affect temporal coincidence detection, by experimentally characterizing synaptic depression at the synapse between neurons in the nucleus magnocellularis and coincidence detection neurons in the nucleus laminaris in the chick auditory brainstem[5]. We combine an empirical description of this depression with a biophysical model of signalling in the nucleus laminaris. The resulting model predicts that synaptic depression provides an adaptive mechanism for preserving interaural time-delay information (a proxy for the location of sound in space) despite the confounding effects of sound-intensity-related information. This mechanism may help nucleus laminaris neurons to pass specific sound localization information to higher processing centres.

In the avian auditory brainstem, each neuron of the nucleus laminaris (NL), like the medial superior olive in mammals[6,7], constitutes an azimuthal 'place code'[8] that is tuned to detect the coincidental arrival of excitatory postsynaptic potentials (EPSPs) generated by ipsi- and contralateral nucleus magnocellularis (NM) neuron action potential firing[9], which is phase-locked to sound waves arriving at each ear. The intensity of the sound, however, changes the likelihood that an NM cell will fire on a given sound wave cycle, such that increasing sound intensity increases the average NM firing rate and thus the average frequency of NM–NL EPSPs[10]. This increase of NM cell firing rate with sound intensity would be expected to increase the postsynaptic NL cell firing rate,

