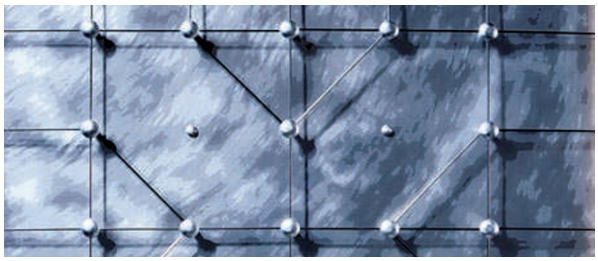
MOLECULAR EVOLUTION

Concealed connections

Many mutations are neglected by studies of molecular evolution — in particular, the non-synonymous protein changes that are not known to have functional or fitness consequences. A systematic study of the functional effects of non-synonymous amino-acid substitutions now suggests that a substantial portion of these variants are involved in hidden epistatic interactions.

In studies of sequence evolution, it is generally assumed that a substitution that is tolerated by one sequence will also be tolerated by a phylogenetically related sequence — that is, that sites evolve independently of each other. This assumption was put to the test in an experimental study in which 168 functionally neutral amino-acid substitutions that occur naturally in the *leuB* gene of *Pseudomonas aeruginosa* were each introduced into the homologous gene of *Escherichia coli*.



Of the 168 mutations that were substituted into *E. coli leuB* (which is involved in leucine biosynthesis), only 104 had no effect on protein function: of the remaining 64 substitutions, one enhanced protein activity and 63 compromised it. This result points to the existence of a large degree of interaction among mutations at different sites — an additive or multiplicative model based on these data would predict the activity of the *P. aeruginosa* protein to be impossibly low.

To identify specific epistatic interactions among the 168 variants, the authors created *E. coli leuB* double mutants between each of three strongly deleterious mutations (F37L, A94D and A284C) and each one of the remaining 167 mutations. In this screen, four compensatory mutations (three of which compensated only partially) were found for the F37L deleterious mutant, but not for the other two. Importantly, the screen allowed individually non-functional mutations to be connected with F37L in a network. Furthermore, all compensatory mutations were located at physically distant sites from F37L, suggesting that the search for compensatory mutations can also highlight new structure–function relationships.

Because of the flat fitness landscape of *E. coli leuB*, the fitness cost of any mutation is tiny, suggesting that cryptic epistasis arises by neutral evolution. In fact, the temporal order in which mutations occurred in the lineage leading to *E. coli* and *P. aeruginosa* supports the idea that the F37L-interacting mutations were built up by a 'nearly neutral' process; a series of slightly deleterious alleles would have been fixed by random drift, until the fixation of a fully compensatory mutation by positive selection.

The model developed in this study would suggest that many protein functions are locked in place by rampant epistasis, and that neutral mutations can provide rare opportunities for new functions to evolve. A more detailed characterization of gene–gene interactions would further our understanding of many research areas, from disease to speciation, and avoid potential errors in phylogenetic-tree reconstructions.

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ORIGINAL RESEARCH PAPER Lunzer, M., Golding, G. B. & Dean, A. M. Pervasive cryptic epistasis in molecular evolution. *PLoS Genet.* **6**, e1001162 (2010)

FURTHER READING Phillips, P. C. Epistasis — the essential role of gene interactions in the structure and evolution of genetic systems. *Nature Rev. Genet.* **9**, 855–867 (2008)