

EVOLUTION

Illusions of conservation

If a sequence or property appears to be conserved across evolutionary time it is often interpreted as being functionally important. But can apparently conserved patterns be produced by neutral or indirect forces? New simulations of regulatory element evolution show that they can.

Enhancer elements often contain clusters of transcription factor (TF)-binding sites, and clustered and/or overlapping sites can be conserved among species. This has been attributed to selection on the spatial organization of binding sites, but functional effects of binding-site position have been shown in only a few cases.

Lusk and Eisen explored how binding-site arrangements arise by simulating *Drosophila melanogaster*

enhancer evolution. They started with synthetic enhancers comprised of TF-binding sites randomly positioned in DNA. The enhancers were subjected to mutations of a similar type and rate to those observed in *D. melanogaster*, and only sequences that retained a threshold number of binding sites continued to the next mutational step. The authors found that the loss and gain of binding sites in the simulation was consistent with the rates of turnover estimated from *D. melanogaster* comparative genomics.

Lusk and Eisen then included binding sites for TFs that overlap in their sequence specificity. They found that overlapping binding sites occurred almost twice as frequently as expected by chance. Once overlapping sites appear, they disappear slowly, even if there is no functional advantage to having sites that overlap. However, in comparative genomic analyses it will seem that selection is acting to preserve them.

The authors also observed that individual binding sites tend to move closer together. This property was dependent on a bias for deletions over insertions — which is known to exist in *Drosophila* species — being included in the simulation. So, binding-site clustering can be driven by deletion in the spacers between sites, rather than

by selection for clustered sites. And because the effect is greatest for older sites, clustered sites will seem to be preferentially conserved — another evolutionary illusion.

The authors then ran the simulation with the compositional constraints (that is, the number and type of binding sites) of a real enhancer, *even-skipped* stripe 2. The simulation produced a similar number of clustered and overlapping sites to the real enhancer sequence, which showed that models to explain the evolution of this enhancer need not involve selection for spatial arrangement of binding sites.

There is some evidence that a bias towards small deletions rather than insertions exists in many species, so this model of regulatory element evolution could be a general explanation for clustered sites. Furthermore, this study suggests that the influence of non-adaptive forces on apparent conservation should be considered in other comparative and functional genomics analyses.

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ORIGINAL RESEARCH PAPER Lusk, R. W. & Eisen, M. B. Evolutionary mirages: selection on binding site composition creates the illusion of conserved grammars in *Drosophila* enhancers. *PLoS Genet.* **6**, e1000829 (2010)

FURTHER READING Marguiles, E. H. & Birney, E. Approaches to comparative sequence analysis: towards a functional view of vertebrate genomes. *Nature Rev. Genet.* **9**, 303–313 (2008)