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Retooling Spare Parts: Gene Duplication And Vertebrate Cognitive Complexity

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Abstract

Two new studies experimentally demonstrate how ancient genomic duplications of synaptic genes provided the substrate for diversification that ultimately expanded vertebrate cognitive complexity.

Population genetics is able to explain how random individual genetic mutations can ultimately result in considerable phenotypic diversification between species through a combination of selective forces and genetic drift¹. Although most mutations are effectively neutral, even those mutations with modest effects on fitness can become fixed in a lineage. Major components of fitness for motile animals include cognition and consequent behaviors. Ethologists have long noted similarities among sophisticated vertebrate behaviors; the tentative parsimonious explanation is that such behaviors are ancestral rather than derived². Indeed, both naturally occurring and transgenic animal models of disease have suggested that orthologous genes modulate aspects of these behaviors. Although most genes start their lives as redundant copies of other genes³, there has been little experimental exploration of the subsequent diversification of duplicated genes that could underlie complex vertebrate behaviors. These issues are particularly salient when one considers how evolution led to modern human cognitive and behavioral features. Enormous advances in our understanding of invertebrate and vertebrate genomes, especially those of many mammals, including our closest living relatives, the great apes and chimpanzees, provide new opportunities to explore these issues empirically. However, with a few exceptions^{4, 5}, most evolutionary comparisons have relied on statistical analysis and modeling based on oversimplified or unproven assumptions about population structure and genomic properties, rather than testing causality using modern experimental methods.

In this context, two studies in this issue of *Nature Neuroscience* use genetic manipulation in mice and cross-species cognitive phenotyping, including study of humans with parallel types of mutations, to provide experimental evidence for how two genome duplications that occurred in early vertebrates 550 million years ago subsequently permitted functional diversification of paralogs (similar genes in the same species that derive from a single ancestral gene) that modulate cognitive traits in modern vertebrates (Fig 1). In the first study, Nithianantharajah *et al.*⁶ used knockout mice to investigate how these duplications

permitted diversified functions of the paralogous *DLG* genes, which encode critical synaptic components. Furthermore, they studied phenotypes that can be measured across species, using a mouse touchscreen version of the Cambridge Neurophysiological Test Automated Battery (CANTAB) tests of human cognition^{7, 8}. Mutations in *Dlg2* had similar cognitive effects in mice and humans, suggesting that these functions of *Dlg2* arose sometime between 550 and 90 million years ago. This provides a striking example of similar behaviors that are modified in the same way by disruptions in orthologous genes. In a companion paper, Ryan *et al.*⁹ studied how the highly divergent C-terminal domains of paralogous GluN2 NMDA receptor subunits have functionally diversified at the levels of biochemistry, physiology and cognition. By swapping the highly divergent C-terminal domains of GluN2A and GluN2B in knock-in mice, they found differences in binding to two proteins encoded by the *Dlg* paralogs and different influences on long-term potentiation and a variety of cognitive functions.

Nithianantharajah *et al.*⁶ produced heterozygous and homozygous mouse knockouts of each of the four *Dlg* paralogs to compare their functions in four cognitive domains: conditioning or simple learning, complex learning, cognitive flexibility and response inhibition or control, and attention. Like flies and nematode worms missing both copies of *Dlg*, mice missing both copies of *Dlg1* die before birth. Notably, this is the only vertebrate paralog that contains all protein domains of the single invertebrate *Dlg*.

The researchers subjected mice without *Dlg2*, *Dlg3* or *Dlg4* and with just one copy of *Dlg1* to a battery of touchscreen tasks testing different cognitive functions in an approach similar to that used to test human cognition. The single copy of *Dlg1* was sufficient for normal performance in all tasks examined, but the mice missing both copies of any of the other paralogs showed deficits in some domains. For example, mice without *Dlg4* did not learn to touch a screen to receive a treat, nor did they differentially approach a signal indicating that a treat was imminent. Consequently, these mice were unable to complete subsequent tasks testing more advanced cognitive abilities. Unexpectedly, mice without *Dlg3* performed better than wild-type mice in tests of visual discrimination learning, extinction learning, and attention and response control. Nonetheless, because *Dlg3* has survived intact, its deletion is likely to have an overall deleterious effect on fitness. Mice lacking *Dlg2* had worse performance than wild-type mice in many of these same tests, and in other tests.

Intriguingly, people with *DLG2* mutations had similarly decreased performance on related touchscreen tests of visual discrimination and cognitive ability, visuo-spatial learning and memory, and attention. Three of these four people have schizophrenia, which has previously been associated with human mutations affecting the number of copies of *DLG2* present in the genome. The phenotypic similarities between people and mice with dysfunctional *Dlg2* provide another data point in a growing body of evidence that at least some molecular mechanisms underlying cognitive processes disrupted in schizophrenia are shared across species¹⁰. Although psychiatric disorders by definition afflict humans rather than animals, there are a growing number of transgenic animal models with construct, face and predictive validity for the phenotypes that can be measured in those species^{11, 12}. Of course, a perennial challenge of cognitive phenotyping is that the phenotypes of greatest clinical interest may not be assessable in other animals.

There are other means of diversification besides changes in the genomic sequence of a protein's coding exons. Previously reported evidence suggests more recent evolution involving regulation of transcription of the *DLG*s specifically in the human lineage. For example, hippocampal expression of *DLG4* has changed since the split with chimpanzees 6 million years ago¹³. Likewise, a splicing factor predicted to target *DLG2* and *DLG4* has been upregulated in the human lineage relative to macaques and chimps¹³. As most transcriptional changes may be neutral¹⁴, a network approach to gene coexpression may better capture larger-scale neurological changes such as differences in cell-type composition. However, these non-genetic molecular approaches to evolutionary phenomena also face other challenges, such as effects of dietary changes or exposure to only recently ubiquitous toxins, which might effect methylation, gene expression or splicing.

Although the evolutionary angle in each of these studies is fascinating, such work also has an important translational aspect. It is more tractable to study specific intermediate cognitive endophenotypes¹⁵ in patients and animal models than to directly tackle broad syndromic neuropsychiatric diagnoses in patients. The cross-species adaptation of the CANTAB used here allows straightforward genetic dissection of specific cognitive functions altered in neuropsychiatric disorders. Layers of genetic, biochemical and physiological investigations of such endophenotypes will provide a more holistic understanding of these disorders and ultimately help us choose more appropriate disease models.

Ryan *et al.*⁹ investigated the effects of the extensive divergence that has occurred between the C-terminal domains of NMDA receptor subunits GluN2A and GluN2B since their birth in the second round of early vertebrate genome duplication. Knock-in mice with the C-terminal domain of one gene replacing the C-terminal domain of the other were given eight cognitive tests to identify functions for which the proper C-terminal domains of each protein are required. These tests spanned three classes of behavior: learning; emotion and motivation; and motor skills. Mice lacking either GluN2A, GluN2B or either of their C-terminal domain performed worse in every test. In contrast, both knock-in mice had normal phenotypes in tests of reversal, associative and motor learning, suggesting that both intact genes are simultaneously required to properly modulate this trait, and that this functional divergence is reflected by their sequence divergence. Both knock-ins performed poorly, however, in a test of impulsivity, suggesting that both intact genes are simultaneously required to properly modulate this trait, and that this functional divergence is reflected by their sequence divergence. Thus, in the C-terminal domain of these two genes, there are recently derived regulators of impulsivity, but not of reversal, associative and motor learning. At first blush, this might seem paradoxical, as impulsivity might be assumed to be a simpler trait than learning. These data suggest, to the contrary, that later sequence changes in vertebrates may have further elaborated certain aspects of impulse control.

As regions of the protein outside the C-terminal domains exhibit relatively high conservation (60–89% similarity), Ryan *et al.*⁹ assumed as the basis for interpreting the remaining results that all components of the gene except the portion encoding the C-terminal domain are functionally equivalent. Others have shown that deletions of the C-terminal domains of either GluN2A (homozygously) or GluN2B (heterozygously) lead to similar behavioral deficits. Here, Ryan *et al.*⁹ reasoned that, if the C-terminal domain of GluN2A

cannot substitute for that of GluN2B on a given task, the C-terminal domain of GluN2B uniquely modulates that phenotype. Also, because they found that the C-terminal domain of GluN2B could substitute for that of GluN2A on several behavioral tasks, they inferred that the *GluN2B* gene not only gained new functionality but also retained some of the ancestral GluN2 function shared by GluN2A. However, with even the modest divergence in the other regions of the protein, it remains possible that the new functionality of the GluN2B requires cooperative interaction with these other regions.

Of course, there must be biochemical and physiological mechanisms mediating these genes' different influences on behavior. The authors found that the divergent C-terminal domains differentially bind to core NMDA receptor proteins and have different effects on synaptic long-term potentiation. It is not certain whether these biochemical and physiological mechanisms underlie the particular behavioral differences observed, but a mutation that fundamentally disrupts long-term potentiation is likely to have a profound effect.

Together, these papers provide the first experimental evidence that gene duplication and divergence have influenced the evolution of cognition. Of course, many genes generally affect any given cognitive trait, and some genes affect many traits. Given the complexity of behavior, some genes likely interact in non-additive ways, and considerably more work needs to be done to understand the complex neural intermediates between biochemical activity and behavior. Nonetheless, these two studies provide a powerful rubric for investigations into how genomic duplication events affected synaptic function and led to an expansion in cognitive complexity.

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Figure 1. How drift and selection can result in functional diversification

Nithianantharajah and colleagues⁶ found functional diversification among the four *Dlg* paralogs in vertebrates and compared this function to the known function of the single invertebrate *dlg*. Here we present a hypothetical scenario of how such genes could diverge following the two rounds of genome duplication. As the ancestral gene had a critical function, purifying selection continues to act on the invertebrate gene, keeping the population frequency of deleterious mutations low. A drifting mutation, coupled with a change in environment, gives this gene a new, added function, after which it continues to operate under purifying selection. *Dlg1* has some new, nonfunctional mutations fixed owing to genetic drift, and is only acted on by purifying selection when the other three paralogous genes have lost their core ancestral function. *Dlg4* undergoes two periods in which beneficial mutations are positively selected to fixation, separated by an epoch of purifying selection on the first newly derived function. The gene loses its core ancestral function through genetic drift of a deleterious mutation. *Dlg2* loses its ancestral function and gains new mutations by genetic drift until a beneficial gain-of-function mutation rapidly sweeps to fixation, at which point the gene is acted on by purifying selection for its new function. *Dlg3* has multiple mutations fixed owing to drift and one beneficial mutation conferring a new

function swept to fixation. After 550 million years of evolution, these genes have diversified, diverging from one another both in sequence and in function.