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Indirect selection of implicit mutation protocols

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A hypothesis that mutability evolves to facilitate evolutionary adaptation is dismissed by many biologists. Their skepticism is based on a theoretical expectation that natural selection must minimize mutation rates. That view, in turn, is historically grounded in an intuitive presumption that "the vast majority of mutations are harmful." But such skepticism is surely misplaced. Several highly mutagenic genomic patterns, including simple sequence repeats, and transposable elements, are integrated into an unexpectedly large proportion of functional genetic loci. Because alleles arising within such patterns can retain an intrinsic propensity toward a particular style of mutation, natural selection that favors any such allele can indirectly favor the site's mutability as well. By exploiting patterns that have produced beneficial alleles in the past, indirect selection can encourage mutation within constraints that reduce the probability of deleterious effect, thereby shaping implicit "mutation protocols" that effectively promote evolvability.

Keywords: mutation rate; variation; evolvability; indirect selection; protocols; simple sequence repeats

> [O]ne point, which has greatly troubled me; . . . what the devil determines each particular variation? What makes a tuft of feathers come on a cock's head, or moss on a moss-rose?[1]
>
> Charles Darwin

Charles Darwin's deep concern with such questions led him to predict, "A grand and almost untrodden field of inquiry will be opened, on the causes and laws of variation."[2] He surely would have appreciated the theme of this volume,[a] that the causes and laws of variation include some fascinating features of genome organization that actually encourage certain styles of mutation.

Historical emphasis on mutations as haphazard accidents

From the beginning of modern genetics, mutations have been regarded as errors in the transmission of hereditary information. And, as Calvin Bridges (one of the pioneers of *Drosophila* genetics) explained over ninety years ago, mistakes are unlikely to be advantageous.

> Any organism as it now exists must be regarded as a very complex physicochemical machine with delicate adjustments of part to part. Any haphazard change made in this mechanism would almost certainly result in a decrease of efficiency. . . . Only an extremely small proportion of mutations may be expected to improve a part or the interrelation of parts in such a way that the fitness of the whole organism for its available environments is increased.[3]

Despite its entirely intuitive origin, this conviction has been promulgated with remarkable consistency throughout the past century [emphasis added]:

- 1909, Francis Galton (Charles Darwin's cousin): "[T]he *vast majority* of mutations end up reducing the number of offspring."[4]
- 1930, J.B.S. Haldane (who helped establish the modern synthesis): "The *vast majority* of mutations are harmful."[5]
- 1937, Alfred Sturtevant (one of the pioneers of *Drosophila* genetics): "The *vast majority* of mutations are unfavorable."[6]
- 1989, John Maynard Smith (one of the founders of evolutionary game theory): "It is *common sense* that most mutations that alter fitness at all will lower it."[7]

[a]Effects of Genome Structure and Sequence on the Generation of Variation and Evolution. 2012. *Annals of the New York Academy of Sciences*. Volume 1267.

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- 2007, C. Baer *et al.* (reviewing recent analyses of mutation rate evolution): "[T]he *vast majority* of mutations with observable effects are deleterious."[8]

Yet such assurance is founded on surprisingly little data, primarily from experiments with radiation and chemical mutagens. But although artificially induced mutations are indeed predominantly deleterious, for mutations arising spontaneously under natural conditions the ratio of benefit to harm has never been realistically assessed.[9] Nevertheless, a presumed preponderance of deleterious mutations has informed most analyses of mutation rate evolution. As first argued by Alfred Sturtevant:

> [F]or every favorable mutation, the preservation of which will tend to increase the number of genes in the population that raises the mutation rate, there are hundreds of unfavorable mutations that will tend to lower it. Further, the unfavorable mutations are mostly highly unfavorable, and will be more effective in influencing the rate than will the relatively slight improvements that can be attributed to the rare favorable mutations. This raises the question—why does the mutation rate not become reduced to zero? No answer seems possible at present, other than the surmise that the nature of genes does not permit such a reduction. *In short, mutations are accidents, and accidents will happen.*[6] [emphasis added]

George Williams ("widely regarded. . .as one of the most influential and incisive evolutionary theorists of the 20th century"[10]) did not mince words when he reiterated Sturtevant's argument in 1966:

> The fittest possible degree of stability is *absolute* stability. In other words, natural selection of mutation rates has *only one possible direction*, that of reducing the frequency of mutation *to zero*. . . .Evolution has probably reduced mutation rates to far below species optima, as the result of *unrelenting selection* for zero mutation rate in every population. . . .So evolution takes place, not so much because of natural selection, but to a large degree in spite of it.[11] [emphasis added]

Although challenged repeatedly in recent decades,[12–16] this conclusion remains influential. One recent review flatly declared, "the cost of fidelity is the generally accepted explanation for nonzero mutation rates in multicellular eukaryotes."[8]

Protocols for mutation

The contrary hypothesis, that facilitated variation is a true genomic function, also has deep historical roots. Darwin himself observed that "[s]ome authors believe it to be as much the function of the reproductive system to produce individual differences, or very slight deviations of structure, as to make the child like its parents."[17] In her eloquent preface to this volume's predecessor, Lynn Caporale took a more radical view based on consideration that every evolving lineage faces an ever-changing selective landscape:

> Far from clumsy stumblers into random point mutations, genomes have evolved mechanisms that facilitate their own evolution. These mechanisms . . .diversify a genome and increase the probability that its descendants will survive.[12]

More succinctly, "Life has evolved to evolve."[15] Indeed, a propensity to mutate within appropriate constraints could be a fundamental attribute of most genomes.

From the orthodox perspective rehearsed above, such assertions appear naive. But the keystone assumption of orthodoxy, that the vast majority of mutations are deleterious, ignores the multiplicity of distinct mutational mechanisms whose fitness-effect probabilities vary over differing genomic domains.[18] What if advantageous constraints could be imposed on certain mechanisms, to shift the overall impact of mutation toward a more favorable balance between benefit and harm? (The term "constraints" commonly implies limitations rather than creative opportunities, but constraints that suppress a sufficiently large number of unpromising possibilities can thereby increase the accessibility of more favorable alternatives.) The result would be implicit "mutation protocols," genomic patterns that permit localized increases in variability. Mutations that abide by protocol would not be random with respect to mechanism or genomic location. But mutations need not be accidental or haphazard to remain random in the sense required by classical Darwinian theory, that specific variants not be directed toward particular adaptive ends.

"Protocols" in this sense, as introduced by Csete and Doyle to address the robustness and fragility that characterize complex living systems, are "rules

designed to manage relationships and processes smoothly and effectively:"[19]

> Thinking in terms of protocols, in addition to genes, organisms, and populations, as foci of natural selection, may be a useful abstraction for understanding the evolution of complexity [cf. Ref. 13]. Good protocols allow new functions to be built from existing components and allow new components to be added or to evolve from existing ones, powerfully enhancing both engineering and evolutionary "tinkering."[19]

Examples of mutation protocols with undoubted adaptive value include antigenic variation in parasitic microorganisms[20] and hypervariability of vertebrate antibody genes.[21] But the "useful abstraction" of a mutation protocol is perhaps most clearly illustrated by a much simpler example, a "tuning knob" protocol implemented by simple sequence repeats.

The tuning knob protocol

Simple sequence repeats (SSRs), in which a simple DNA motif is repeated several times in tandem (e.g., CACACACA, CAGCAGCAG, or CAATCAATCAAT), are a common feature in both prokaryotic and eukaryotic genomes. SSRs yield quantitative genetic variation that is both abundant and relatively safe. The following features are all well documented:[22]

- *SSRs implement a specific style of mutation.* They increase or decrease the number of repeating motifs, commonly by a single motif unit, without otherwise altering the repetitive pattern.
- *SSRs have high, site-specific mutation rates.* These are based on mechanisms that include replication slippage and unequal recombination. Mutation frequency at any particular site can be several orders of magnitude higher than the genome-wide average for base-pair substitution and depends on local sequence features such as motif length, number of repeats, and purity of repetition.
- *SSR mutations yield small, incremental phenotypic effects.* Although repeat variation has often been regarded as effectively neutral, numerous studies demonstrate that repeat-number variants can be causally associated with quantitative variation in gene function. (Extreme examples with pathological effects are also well known.)
- *SSR mutations are readily reversible.* Any stepwise change in the number of repeats can be undone by a step in the opposite direction.
- *SSRs are modular*. Each instance is intrinsically linked to a particular genomic site, has its own genetic effects and parameters for variation, and can evolve independently from any other repeat site.

These features, which together permit efficient incremental adjustment of gene function, have led to metaphorical characterization of SSRs as "evolutionary tuning knobs."[23]

Adjustment by tuning knob represents a huge improvement over haphazard accident. If a violin string is out of tune, a small random twist of its tuning knob has a much higher probability of improving the tone (about fifty percent) than would a sudden, accidental impact. A vast majority of such small adjustments should not be substantially deleterious, and the same knob can be repeatedly adjusted without risk to other structures. Of course incremental adjustability does not require a literal knob, just some physical configuration that can set a specific parameter value while also facilitating small changes of that value. For any SSR, the "setting" is a particular repeat-number allele, while "adjustment" is accomplished by reversible repeat-number mutations. As with a literal knob, no matter how often the repeat number changes, the SSR remains fundamentally adjustable.

Far from being a rare feature of special genes, the tuning knob protocol is ubiquitous. A typical eukaryotic genome contains hundreds of thousands of SSRs. They occur in a high proportion of genes and can be located in exons or introns, in transcribed or untranscribed regions, and in upstream or downstream regulatory regions.[22] Extended genes can include several distinct SSR sites within their regulatory and/or coding domains, so that practically any aspect of genetic function can be adjusted by associated SSR variation. And the tuning knob protocol can be implemented by a wide assortment of repeats based on many different sequence motifs. (Depending on motif and context, SSRs can also behave as reversible switches that turn gene function on or off, as in many bacterial contingency genes.[24])

Of course, not all random adjustments will be advantageous, so each site with an active protocol should impose some selective burden. But only mutations in the wrong direction will be deleterious, and since tuning knob mutations yield mostly small, quantitative phenotypic effects,[22] most deleterious mutations will be only slightly disadvantageous. Furthermore, as recognized by R. A. Fisher over eighty years ago, the proportion of "mutations of small effect" that are deleterious will be closer to fifty percent rather than a "vast majority," at least under unexceptional circumstances of suboptimal adaptation during environmental change.[25] Thus, the associated fitness cost can remain low and relatively constant even while a steady supply of new variants ensures reliable response to shifting selection pressures, including rapid replacement of potentially beneficial alleles that are lost to genetic drift. By contrast, the cost of reproducing without abundant selectable variation (i.e., with new variants appearing only as accidental, haphazard errors) can increase very quickly during extensive shifts of an adaptive peak, if a population must endure declining fitness while awaiting the appearance of each improbably beneficial "mistake."

The SSR mutation protocol bridges the gap between epigenetic responses that are rapid but not reliably inherited across generations and those arising from conventional single nucleotide substitutions that occur at a much slower rate but have much greater stability.[22] Tuning knob effects have been implicated as the basis for ongoing adaptation in several natural populations. For example, the number of threonine-glycine and serine-glycine dipeptide repeats in the *Drosophila* PER protein, encoded by a polymorphic hexanucleotide SSR in exon 5 of the *period* gene, influences sensitivity of circadian rhythm to temperature fluctuations.[26] Geographic and microgeographic clines in the frequencies of repeat-number alleles of this gene have been interpreted as evidence for adaptively fine-tuning this gene to local climate.[27] A similar association between geography and repeat variation has been reported for an avian clock gene.[28] In human populations, locally elevated mutation rates at a short thymidine repeat in the heart disease gene *MMP3* yield variation that experiences strong positive selection.[29] Experimental studies with yeast have directly demonstrated functional variability arising from intragenic tandem repeats[30] as well as advantageous transcriptional evolvability based on enrichment of SSRs in gene promotors.[31]

Indirect selection

To the extent that SSRs (as well as other prolific sources of variation) have been recognized as important contributors to adaptive evolution, this role has been generally perceived as fortuitous. But if a mutation protocol can increase the speed or effectiveness of adaptation—that is, if systematically constrained variation offers a more efficient route to discovery of beneficial variants—then perhaps, in contrast to traditional orthodoxy, locally elevated mutation rates can also be indirectly favored by selection.[32–35]

To see why selection of mutation protocols must necessarily be indirect, we revisit our metaphor. A violinist's audience listens not for the presence of tuning knobs but for music that is played in tune. Yet applause for a tuneful performance must also, indirectly, be applause for the tunable violin. Similarly, the agencies of natural selection, conceived as acting on individual organisms, cannot directly discriminate between better and worse mutation protocols. Selection sees only how well each particular phenotype is suited for its immediate environment. Thus, only particular genetic parameters (i.e., individual repeat-number alleles) are selected directly, not any associated potential for adjustability. Nevertheless, whenever selection favors a particular SSR allele, that selection must also, indirectly, favor the mutation protocol that is implicit in motif repetition.

Just like direct selection, indirect selection acts in every generation upon every allele; it is no less effective because it is indirect. Furthermore, while a direct selective sweep based on a single beneficial allele can indirectly establish an incipient SSR throughout a population, the incipient protocol can be suppressed only very gradually as direct selection eliminates deleterious mutations as they arise, one allele at a time. Thus an effective mutation protocol can be favored over the long term even when most associated mutations are not advantageous (i.e., even when direct selection would seem to oppose its mutagenicity), as long as fitness-enhancing alleles arise often enough to override the associated direct cost of deleterious mutations.

The processes that create, maintain, and eliminate an SSR are closely related. Single base-pair substitutions, insertions, or deletions can convert a nonrepetitive sequence into a short SSR or introduce interruptions into a preexisting SSR; interruptions can be eliminated by subsequent slippage mutations that extend and shorten a repeat tract. The balance over time among all such mutations, together with the effects of selection on particular alleles, will determine the evolutionary duration ("life cycle") of the SSR.[36] Maximally stable alleles should outcompete tuning knob alleles only when variation associated with the protocol is consistently disadvantageous for very many generations, in which case orthodox theory correctly predicts that selection should push mutation rates downward until costs of replication fidelity balance lost reproductive capacity from deleterious mutations. But the only circumstance necessary for the tuning knob protocol to prevail is a selective environment in which constrained variation is occasionally advantageous.

Indirect selective advantage has been documented for the SSR-based mutability of contingency loci in pathogenic prokaryotes.[24,37] But confidence in orthodoxy has hindered appreciation for this example's broader significance:

> Interestingly, unstable sequence features such as tandem repeats tend to be found disproportionately near and within [bacterial contingency loci], where they enhance variability and remain linked to new beneficial mutations. Contingency loci provide our best example of evolvability-as-adaptation, but their relevance to the general question of whether evolvability-as-adaptation is a major missing component of evolutionary theory seems rather limited. . . . It is indeed attractive to suppose that the most important evolutionary feature of organisms—their very capacity to evolve and adapt—is itself an adaptation, but this is probably only true in highly restricted circumstances.[38]

Circumstances in which SSR mutability is advantageous can seem "highly restricted" only if one ignores the ubiquity of SSRs throughout most eukaryotic genomes, their functional integration into a high proportion of genes, and the conservation of some variable SSR sites across long expanses of evolutionary time. Taken together, these all suggest that the SSR mutation protocol, shaped by indirect selection, could indeed play a major role in organisms' "very capacity to evolve and adapt."[22,23,31–35]

Additional mutation protocols

While the SSR-based tuning knob protocol supports adaptive adjustment of preexisting genetic functions, novel functions can emerge through opportunities provided by a "copy-and-paste" protocol implemented by transposable elements (TEs). TE activity can even be conveniently integrated with the tuning knob protocol. Some TEs use SSRs as sites for insertion (i.e., as "landing pads"[39,40]), while many TE insertions create new repeats at either end. Thus, if the action of a transposable element creates a new function, that function can come with ready-made tuning knobs for its adjustment. TEs are often disparaged as selfish or parasitic because their activity can disrupt gene function. Orthodoxy presumes that selection at the organism level must thus act in only one direction, toward suppression of TE mobility. But since TEs play an acknowledged role in genome evolution, creating permissive and possibly necessary conditions for adaptive innovation and diversification,[41–44] TEs might instead become "domesticated"[41] by indirect selection so potential harm is reduced while evolutionary utility is retained. An apparently selfish tendency toward dispersal throughout a genome can prolong the time available for such domestication, since a widely distributed TE stands a higher probability of producing at least one advantageous mutation, and hence becoming more firmly established, before its mutagenic potential can be suppressed.

The above examples of tuning knob and copy-and-paste protocols only hint at the potential consequences of indirect selection. Among the most familiar processes for generating systematic variation are those of sexual reproduction. Yet these have been explicitly excluded from textbook definitions of mutation (e.g., [emphasis added] "*mutation*: an *error* in replication of a nucleotide sequence, or any other alteration of the genome that is *not* manifested as reciprocal recombination"[45]) precisely because the vast majority of recombination products are *not* deleterious. Instead of being categorized as something distinct from mutation, meiotic recombination could be more productively conceptualized as perhaps the most sophisticated and highly organized of mutation protocols. If evolutionary processes can create and maintain sources of variation as elaborate as those of sexual

reproduction, despite their steep cost, then additional protocols for generating potentially advantageous variation can surely be expected. Intriguing possibilities include protocols that permit hypermutation at particular sites, such as genes mediating interspecies interaction;[46,47] protocols (in addition to retrotransposition) for copy number variation;[48] and protocols that organize recombination hotspots, coldspots, and inversions.[49–51] Recent demonstration of non-random variation of local mutation rates in *E. coli* (e.g., lower rates in highly expressed genes) suggests localized mutation-rate optimization based on mechanisms yet to be elucidated.[52] Eukaryotic genomes are unlikely to be any less sophisticated.

Counterarguments

Defenders of evolutionary orthodoxy have maintained that the hypothesis of advantageous mutation protocols is unnecessary:

> One might call this the evolvability-as-adaptation hypothesis. This hypothesis is intuitively appealing, but it has its own set of problems. For one thing, it assumes that variability—the capacity to generate new variation—is generally limiting to population persistence and success in nature. This assumption lacks empirical support. Most natural populations have large amounts of standing genetic variation and do not necessarily depend on *de novo* variation in order to adapt to environmental change. Directly observed rates of short-term evolution in natural populations often far exceed those inferred from the fossil record, and this too implies that there is ample capacity for adaptation in response to selection in most populations.[38]

But this argument fails by simply assuming the very phenomenon that mutation protocols help to explain: the reliable abundance of adaptively appropriate variation. A hypothesis that indirect selection might favor mutation protocols does not require that variability be generally limiting in most populations, only that the evident adequacy of standing variation might call for explanation beyond haphazard accident from imperfect replication. The concept of mutation protocols implies that serviceable variation abounds *because* effective mutation protocols have become thoroughly incorporated into most genomes.

A related counterargument holds that "evolvability-as-adaptation must be the consequence of selection among populations rather than selection among individuals."[38] The widely accepted weakness of population-level selection (relative to individual selection, which by orthodoxy opposes mutability) then impugns the plausibility of selection for evolvability. But this argument depends on conflating evolvability *per se* with the underlying molecular and developmental protocols that confer evolvability.[35] Evolvability is indeed (by definition) a property of populations. But indirect selection does not require competition among populations based on differences in evolvability. Advantageous mutation protocols are selected indirectly (and powerfully) at the level of individual alleles and organisms, whenever direct selection favors a mutation that arises according to protocol.

The term "lineage selection" does offer a convenient shorthand for appreciating the relationship between indirect, organism-level selection of mutation protocols and the emergence of evolvability.[44] By increasing the efficiency of adaptation, an effective mutation protocol would increase a lineage's chance of enduring over time (i.e., the lineage analog of individual survival), while also boosting its prospects for adaptive radiation (i.e., the lineage analog of reproduction). Nevertheless, although lineage selection could plausibly reinforce the effects of direct or indirect selection acting at the conventional, organismal level,[53] actual competition among separate lineages is neither necessary nor sufficient to explain the establishment of mutation protocols. Just like any other trait that potentially affects fitness, each embodiment of a protocol must stand or fall based on how well its alleles support each individual's contribution to the next generation. Increasingly efficient evolvability emerges as an inevitable consequence when mechanisms of mutation become more advantageously constrained by effective mutation protocols.[33–35]

Implications for comparative genomics

The hypothesis presented in this essay—that indirect selection for the constrained variation of implicit mutation protocols has promoted faster and safer exploration of genome sequence space than would be possible by haphazard accident alone—carries an interesting corollary. Those sequence patterns that are most important for adaptive adjustment and evolutionary innovation might be among those that are most mutable and hence least

conserved over evolutionary time. Although "conservation equals function" is a reasonable expectation in certain contexts, most biologists also understand that many species-specific traits can contribute to fitness without being conserved at higher taxonomic levels. Comparative genomics should not focus exclusively on sequence conservation, precisely because unstable, evolutionarily labile sites could be especially relevant for understanding ongoing adaptation.[37,47,54] An exponential decline in the proportion of SSRs that are conserved over increasing phylogenetic distance has been interpreted as evidence for neutral evolution of such sites in the face of mutation pressure and drift.[55] But such a decline is also consistent with temporal variation in the circumstances for indirect selection at each particular SSR, for example, during periods of niche divergence or environmental change. Even so, some repeat sites have been deeply conserved.[55] One especially intriguing set of dinucleotide repeat sites is shared between human and opossom, even while mutations have churned the sites sufficiently to alter their motifs and thus obscure their homology in other species;[56] curiously, most of these sites occur within genes having neurodevelopmental roles.[57] Perhaps the simplest explanation for such persistently mutable sites could be their durable utility as a substrate for efficient adaptive adjustment of behavior.

Conclusion

A presumption that mutations are haphazard accidents, whose low probability of beneficial effect assures that selection must minimize mutation rates, is challenged by a significant body of data concerning several prolific sources of genetic change, notably simple sequence repeats, transposable elements, and sexual recombination. Just as life itself depends on ancient and strongly conserved metabolic protocols, such as those for using ATP and synthesizing proteins, so also could efficient adaptation in diverse and ever-changing environments depend on implicit "mutation protocols" that have become established throughout most genomes.[58] This hypothesis has broad implications, both for enduring controversies in evolutionary theory and for current computational analyses in comparative genomics. Studies in anatomy, physiology, and biochemistry have long relied on the assumption that organisms are adaptively "designed" by natural selection. We should not be surprised if the creative power of indirect selection parallels that of direct natural selection. Once released from the obsolete and misleading presumption that mutability cannot be advantageous, genetics should also advance by reverse engineering[19] those molecular mechanisms for mutation that not only serve immediate adaptation but also promote continuing evolvability.

Conflicts of interest

The author declares no conflicts of interest.

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