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NEWS AND VIEWS

OPINION

# The mutational hazard hypothesis of organelle genome evolution: 10 years on

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**Why is there such a large variation in size and noncoding DNA content among organelle genomes? One explanation is that this genomic variation results from differences in the rates of organelle mutation and random genetic drift, as opposed to being the direct product of natural selection. Along these lines, the mutational hazard hypothesis (MHH) holds that 'excess' DNA is a mutational liability (because it increases the potential for harmful mutations) and, thus, has a greater tendency to accumulate in an organelle system with a low mutation rate as opposed to one with a high rate of mutation. Various studies have explored this hypothesis and, more generally, the relationship between organelle genome architecture and the mode and efficiency of organelle DNA repair. Although some of these investigations are in agreement with the MHH, others have contradicted it; nevertheless, they support a central role of mutation, DNA maintenance pathways and random genetic drift in fashioning organelle chromosomes. Arguably, one of the most important contributions of the MHH is that it has sparked crucial, widespread discussions about the importance of nonadaptive processes in genome evolution.**

*Keywords*: genome size, mitochondrial genome, mutation rate, nonadaptive evolution, plastid genome

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## Introduction

One decade ago, a compelling nonadaptive hypothesis was put forward by Lynch *et al.* (2006) to explain why animals and land plants, despite their similar effective population sizes, have drastically different mitochondrial genome architectures. Indeed, animal mitochondrial DNAs (mtDNAs) are among the smallest, most compact genomes of any genetic compartment, bottoming out at around 10 kilobases (kb) (Pett *et al.* 2011), whereas land plant mtDNAs can be >10 000 kb (Sloan *et al.* 2012a) and have orders of magnitude more noncoding nucleotides than their metazoan counterparts, and unlike animals, they can be sponges for introns, repetitive elements and foreign DNA (Rice *et al.* 2013). Lynch *et al.* (2006) argued that the genomic disparity between these two mitochondrial lineages, rather than being a direct product of natural selection, was a consequence of vastly different mutation rates. They reasoned that the very low mutation rates typical of land plant mitochondria (Palmer & Herbon 1988; Richardson *et al.* 2013) provide a more permissive environment for the accumulation of noncoding DNA and other genomic embellishments, such as RNA editing sites, as compared to animal mitochondria, which tend to have very high mutation rates (Haag-Liautard *et al.* 2008; Lynch *et al.* 2008). This hypothesis, now called the mutational hazard hypothesis (MHH), is an extension of earlier studies on bacterial and nuclear genomes (Lynch & Conery 2003) and based partly on the premise that 'excess' DNA of any kind is a mutational liability because it increases the potential and target size for harmful mutations, such as a mutation to an intronic splice site or one that generates a spurious regulatory element. Some have likened this concept to the Buddhist philosophy that the more possessions you own, the greater the chance for grief when you lose them (Bromham 2009).

The idea that organelle genomic architecture may be largely governed by mutation pressure has garnered much attention (Pérez-Brocal *et al.* 2006; Cuenca *et al.* 2010) and stimulated important scientific debate (Sloan *et al.* 2012a). This is partly because the nonadaptive nature of the MHH goes against some of the prevailing adaptive views of genome evolution (Lynch 2007a; Speijer 2010). But it is also because organelle genetic data are used in a wide range of analyses and disciplines, from medicine to forensics to phylogenetics, and have helped resolve major scientific questions, including the origins and diversification of eukaryotic life (Gray 2012; Keeling 2013). In other words, mitochondrial and plastid genome evolution is of general interest to biologists and a topic with far-reaching impacts.

Over time, the MHH has developed into a general theory for explaining the architectural diversity among all types of chromosome (Lynch 2007b), including plastid genomes. It holds that mutationally hazardous DNA has a greater tendency to accumulate in a system with a low mutation rate ($\mu$) and a small effective genetic population size ($N_e$), where levels of random genetic drift are high, rather than in one where $\mu$ and $N_e$ are large and drift is small. One of the challenges to testing this prediction is that $N_e$ and $\mu$ are difficult to measure, but insights into these two parameters can be gleaned from data on genetic variation. Silent-site

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genetic diversity within populations ($\pi_s$) provides an approximation of $2N_e\mu$ (with slight alterations needed depending on ploidy and mode of inheritance), between-species silent-site divergence ($d_s$) gives an entrée into $\mu$, and data on both $\pi_s$ and $d_s$ can be used to estimate $N_e$ (Kimura 1983; Lynch 2007b).

It is important to stress, however, that these population genetic parameters can be impacted by a variety of factors, from species traits to inaccurate assumptions about the neutrality of silent sites to the misidentification of species. And the effects of these factors may be nonindependent, potentially introducing confounding covariation, not to mention that $N_e$ and $\mu$ are often estimated from the same data using the same theoretical assumptions, which can lead to the conflation of these two empirical measures and impede the ability to accurately discriminate hypotheses. It also is important to remember that there are many features that make molecular evolution in land plants, animals and protists distinctly different from one another. All of these factors need to be considered when evaluating the data in support or against the MHH and when considering the topic of genome evolution in general.

## Support for the mutational hazard hypothesis

Silent-site genetic variation data from mitochondrial and plastid genomes are accumulating for diverse plants and algae (Table 1). These data, in addition to improving our understanding of organelle genetics, have been used to assess the MHH, with certain analyses defending the hypothesis (e.g. Smith & Lee 2010) and others contesting it (e.g. Alverson *et al.* 2010) (Table 1). Some of the strongest support has come from studies of green algal plastid genomes.

For example, the plastids of chlamydomonadalean algae, such as *Volvox carteri*, *Pleodorina starrii* and *Tetrabaena socialis*, can harbour giant chromosomes (205–525 kb), parallel in size and structure to land plant mtDNAs (Smith *et al.* 2013; Del Vasto *et al.* 2015; Featherston *et al.* 2016). Based on the MHH, these inflated plastid DNAs (ptDNAs) should harbour little silent-site diversity, reflecting a low $N_e\mu$, which is exactly what has been observed. Population genetic studies of the ~525-kb ptDNA of *V. carteri* uncovered extremely low values of $\pi_s$ (~$6.5 \times 10^{-4}$), even when comparing isolates from as far away as India and Japan (Smith & Lee 2010). Moreover, there appears to be a negative scaling of organelle genome noncoding DNA content with $N_e\mu$ across the volvocine lineage as whole (Smith *et al.* 2013) (Fig. 1). More recently, a mutation accumulation study showed that the enlarged and repeat-dense plastid genome of *Chlamydomonas reinhardtii* has a relatively low mutation rate (~$9.2 \times 10^{-10}$ mutations/generation), skewed towards insertions vs. deletions and experiences substantially stronger levels of genetic drift relative to the nuclear

**Table 1** Data supporting or refuting the mutational hazard hypothesis

| | |
|---|---|
| Predictions of the MHH hypothesis | Lineages with expanded organelle genomes will tend to have lower organelle mutation rates ($\mu$) and smaller effective population sizes ($N_e$) than those with more compact organelle genomes |
| | Silent-site genetic variation between species ($d_s$) and within species ($\pi_s$), which can be used to approximate $\mu$ and $N_e\mu$, respectively, will scale positively with organelle genome compactness |
| Support for the hypothesis | Some of the most expanded organelle genomes ever observed, including the bloated ptDNAs of certain chlamydomonadalean algae (e.g. *Volvox carteri* and *Dunaliella salina*) and the giant mtDNAs of many land plants (e.g. *Liriodendron tulipifera* and *Magnolia stellata*), are predicted to have very low mutation rates and harbour little silent-site diversity |
| | Organelle genome streamlining within various lineages, including certain prasinophyte green algae, red algae, fungi and metazoans, often coincides with extremely high mutation rate estimates and elevated levels of $\pi_s$ |
| | For a few lineages, including volvocine green algae, organelle genome noncoding content scales positively with organism size and cell number, suggesting a relationship between $N_e$ and genome architecture (Fig. 1) |
| Challenges to the hypothesis | Mitochondrial genomes from the land plant genus *Silene* are currently the largest on record but can show extremely high levels of $d_s$ and moderate amounts of intraspecific silent-site diversity |
| | Many organisms with remarkably similar organelle genomic architectures have drastically different mutation rate estimates and/or levels of $\pi_s$ (e.g. the mitochondrial vs. plastid genomes of *Porphyra*) |
| | Organelle mutation rates are turning out to be much more dynamic and wide ranging within and among lineages and even across individual chromosomes, making it that much harder to correlate mutation rates with specific organelle genome architectures |
| | All else being equal, biparentally inherited organelle genomes should have a twofold greater effective population size than those that are uniparentally inherited and thus should also be more compact. Contrary to these expectations, there appears to be no significant difference in genome size or compactness between organelles that are biparentally inherited relative to those that are uniparentally inherited (Crosby & Smith 2012) |

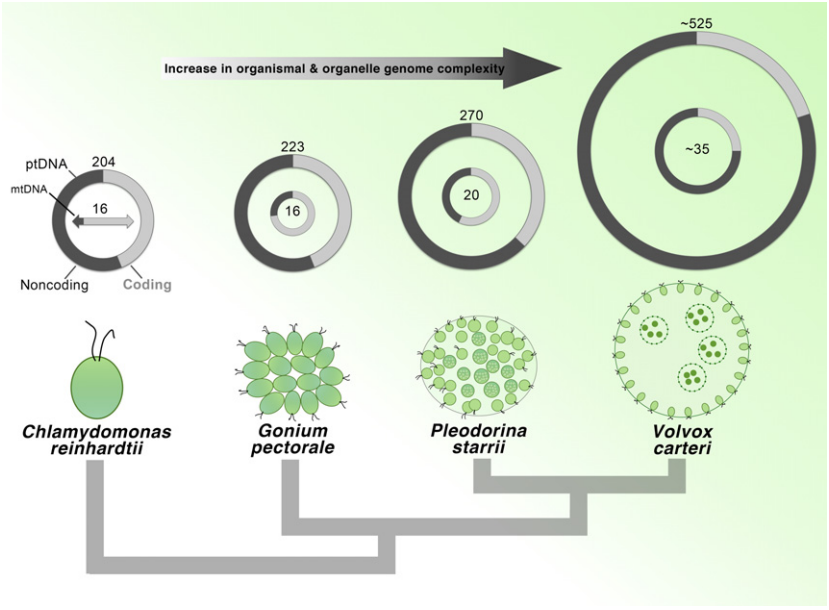


**Fig. 1** Positive scaling of organelle genome size and noncoding DNA content with organism size and cell number in volvocine green algae. Mitochondrial and plastid DNA maps (inside and outside, respectively) are shown for four volvocine species (genome size marked in kilobases). Genomes are drawn to scale within categories, but not when comparing between categories (i.e. ptDNA vs. mtDNA). All genomes are circularly mapping, with the exception of the *C. reinhardtii* mtDNA, which is linear with terminal inverted repeats. The proportion of coding vs. noncoding nucleotides is highlighted in light and dark grey, respectively. Image modified from Smith *et al.* (2013).

genome (Ness *et al.* 2016), which are features consistent with the MHH.

Members of the genus *Dunaliella*, like their close volvocine relatives, can have huge ptDNAs (285 to >375 kb), and also like some volvocine algae (Ness *et al.* 2016), they appear to have low plastid mutation rates (Del Vasto *et al.* 2015). Plastid genome sequencing of different *Dunaliella* species has revealed surprisingly low levels of $d_s$ for the ptDNA (<0.01 substitutions/synonymous site), about thirteen times smaller than those observed for the mtDNA (Del Vasto *et al.* 2015). The low rate of silent-site ptDNA substitution is in line with the MHH, but *Dunaliella* mitochondrial genomes are also expanded (Smith *et al.* 2010) and, therefore, following the MHH, one would have expected to find a low $d_s$ in the mtDNA as well, which was not the case (Del Vasto *et al.* 2015).

At the other end of the spectrum are the tiny plastid and mitochondrial genomes of many prasinophyte green algae (Lemieux *et al.* 2014). The ptDNA of *Ostreococcus tauri*, at 72 kb and 85% coding, is a paragon of compactness and consequently, one might anticipate it to have a higher mutation rate and/or effective population size than its chlamydomonadalean counterparts, which appears to be true. Population studies of *O. tauri* found relatively high levels of $\pi_s$ in its ptDNA (~$4 \times 10^{-3}$) (Blanc-Mathieu *et al.* 2013), much higher than those observed for *V. carteri*. Likewise, comparative analyses of closely related prasinophytes, such as *O. tauri* vs. *Ostreococcus* sp. RCC809 and *Micromonas pusilla* CCMP1545 vs. *Micromonas* sp. RCC299, found highly elevated silent-site substitution rates for the ptDNA, mtDNA and nuclear DNA (>0.8 substitutions/synonymous site) (Smith 2015), which is consistent with a high rate of mutation in these streamlined genomes.

Relative silent-site substitution rate data now exist for a variety of other plastid-bearing protists, including those with primary or secondary plastids as well as nonphotosynthetic taxa (Riisberg & Edvardsen 2008; Hua *et al.* 2012; Smith & Keeling 2012; Smith *et al.* 2012; Pochon *et al.* 2014). These data suggest that in algae, there is a tendency for mtDNA to have higher rates of silent-site substitution than the ptDNA and nuclear DNA, potentially reflecting a higher mutation rate in the mitochondrion (Smith 2015). Under the MHH, this would help explain why for some algae the mitochondrial genome is smaller and more compact than the plastid and nuclear counterparts, but see Smith *et al.* (2012) for an opposing view.

## An increasingly complex picture of organelle mutation rates

Organelle genetic variation data from the angiosperm genus *Silene* paint a different picture of the MHH than those from various algae. Some *Silene* species have enormous mitochondrial genomes (>10 000 kb), but contrary to the MHH, these genomes appear to have high rates of mutation and show no obvious indications of long-term decreases in $N_e$. Comparisons of near-complete *Silene* mtDNAs exposed massive (and sometimes genome-wide) accelerations in silent-site substitution rates, with the biggest mitochondrial genomes having the highest $d_s$ (and presumably highest μ) (Sloan *et al.* 2012a), which runs counter to the MHH. Mitochondrial genetic diversity within *Silene* species can also be high (Sloan *et al.* 2012a,c), potentially reflecting a high $N_e\mu$. Studies of *Silene vulgaris*, for instance, revealed unexpectedly large intraspecific diversity in mitochondrial genome sequence, structure and content (Sloan *et al.* 2012c). However, the patterns of intraspecific variation can be complex, with some mitochondrial genes containing many silent-site polymorphisms and others being invariable (Sloan *et al.* 2012c), suggesting

that mutation rates vary widely across *Silene* mitochondrial chromosomes. These trends are also complicated by very low levels of $\pi_s$ in the mtDNA of some species, including the ~6700-kb mitochondrial genome of *Silene noctiflora*, for which comparisons of isolates from across North America and Europe yielded a silent-site diversity of $\ll$0.001 (Sloan *et al.* 2012a), which is compatible with the MHH.

Interpreting the MHH using data from other angiosperm lineages can be equally ambiguous. The inflated mitochondrial genomes of *Liriodendron tulipifera* (tulip tree) and *Magnolia stellata* have extraordinarily low genome-wide silent-site substitution rates (Richardson *et al.* 2013), upholding the MHH as well as the more traditional view that land plant mitochondrial genomes evolve slowly. Conversely, *Cucurbita pepo* (zucchini) has a much larger mtDNA but a significantly higher synonymous substitution rate than its close relative *Citrullus lanatus* (watermelon), implying that the relationship between a low mutation rate and a large mtDNA is decoupled in the Cucurbitaceae (Alverson *et al.* 2010).

Other analyses have found even greater variation in $d_s$ among land plant organelle DNAs, making it that much more challenging to correlate mutation rates with specific organelle genome architectures. For angiosperms alone, there is a 5000-fold range in the absolute rate of synonymous site substitution among explored mitochondrial genomes (Richardson *et al.* 2013), and up to a 340-fold range for genes within a single mtDNA (Zhu *et al.* 2014). Complicating things further are the high rates of horizontal gene transfer in many land plant mtDNAs (Mower *et al.* 2012). The enormous 3900-kb mitochondrial genome of *Amborella trichopoda*, for instance, contains six genome equivalents of foreign mtDNA, acquired from green algae, mosses and other angiosperms (Rice *et al.* 2013). Such rampant horizontal gene transfer can contribute to the substitution rate variation observed within and among land plant mtDNAs and can also result in genomic expansion and general genomic upheaval (Mower *et al.* 2012).

The mitochondrial silent-site substitution rate range is almost as staggering for animals (Hellberg 2006; Oliveira *et al.* 2008) and various protist lineages (Smith 2015). Plastid genomes, too, show an astonishing range of $d_s$ both within and across lineages (Sloan *et al.* 2012b; Pochon *et al.* 2014; Wicke *et al.* 2014; Smith 2015), but the range does not appear to be as extreme as that found in mitochondrial or nuclear genomes. Together, these findings underscore the potential for massive variation in organelle mutation rate among and within lineages and the fact that organelle genomes with similar sizes, structures and contents can have very different rates of mutation. Although providing no clear resolution to the MHH, these data have led to more attention being paid to the role that DNA replication and repair have in shaping mitochondrial and plastid chromosomes.

## Do DNA maintenance processes shape organelle genome architecture?

The MHH was built partly on certain assumptions about the patterns of organelle mutation rate across eukaryotes and how they relate to specific genomic features, such as genome size (Lynch *et al.* 2006; Lynch 2007b). However, as discussed above, the mutational landscape of organelle DNA has proven to be much more varied and wide ranging than previously thought. Not only does this further complicate the question of how mutation rate relates to organelle genome architecture, it also raises new questions about what is responsible for the massive variation in organelle mutation rate.

Mutation rate is a reflection of the underlying DNA maintenance machinery. The replication and repair of mtDNA and ptDNA is almost entirely dependent on nuclear-encoded, organelle-targeted proteins, many of which have a complex evolutionary history, having been co-opted or acquired through lateral gene transfer (Sloan & Taylor 2012; Oldenburg & Bendich 2015). In plants and algae, certain components of the DNA maintenance machinery are dual targeted to the mitochondrion and plastid, and even when this is not the case, some mitochondrion-targeted proteins have closely related paralogs performing parallel functions in the plastid (Sloan & Taylor 2012). The existence of these shared or parallel components can explain why some species have similar rates of mutation in both their mitochondrion and plastid (Smith & Keeling 2015). Moreover, the fact that there is significant variation in the organelle DNA maintenance machinery across eukaryotes can account for the broad spectrum of observed organelle mutation rates. Differences in organelle DNA replication and repair may also be responsible for variation in organelle genome architecture (Smith & Keeling 2015).

Recent studies detected what appear to be different types of double-strand break DNA repair occurring in land plant mitochondria, some of which might be resulting in the expansion of noncoding regions (Fig. 2). By comparing mitochondrial genome sequences of *Arabidopsis thaliana* ecotypes and other closely related angiosperms, Christensen (2013, 2014) showed that intergenic sites have a much higher rate of substitution (including insertions/deletions and rearrangements) than synonymous sites, which is a feature that has largely gone unnoticed in previous work. It is not known why substitution rates differ so significantly between these two kinds of so-called neutral sites, but Christensen (2013, 2014) proposed that there is greater selection on synonymous vs. intergenic sites in land plant mtDNAs, which ultimately impacts how these sites are repaired.

One explanation is that natural selection results in the accurate repair of mutations to synonymous sites (and coding mtDNA as a whole), whereas mutations to more neutrally evolving intergenic DNA are repaired using error-prone processes, such as break-induced replication, which can cause genomic expansion, rearrangements and chimeric gene production (Fig. 2) (Christensen 2014; Wynn & Christensen 2015). If true, this could elegantly account for some seemingly contradictory features of land plant mtDNAs, including the low substitution rate often observed for genes, the massive enlargement and sequence

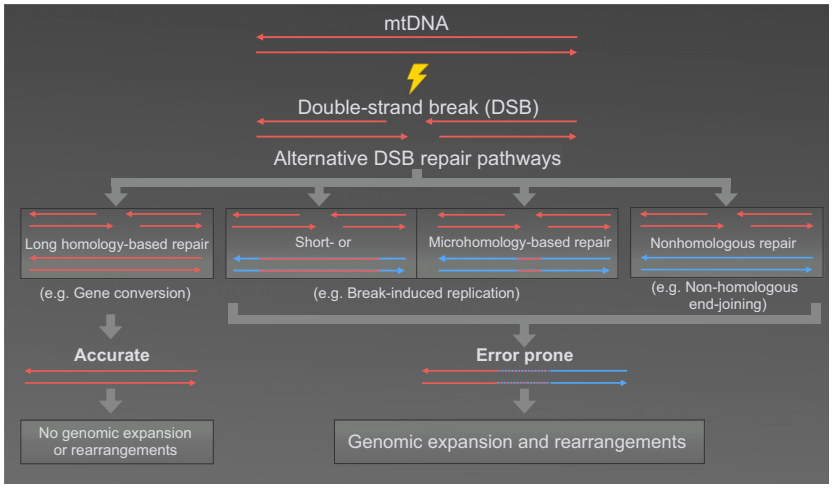


**Fig. 2** Hypothetical pathways for repairing double-strand breaks in land plant mitochondrial genomes. DNA damage leads to (or is converted into) double-strand breaks (DSB), which can then be repaired by alternative pathways, some which depend on the availability of a template molecule. Long homology-based repair mechanisms, such as gene conversion or homologous recombination, can be very accurate and do not normally result in genomic expansion or rearrangements. Short-, micro- or nonhomology-based repair pathways, such as nonhomologous end-joining or microhomology-mediated break-induced replication, are error prone and can often lead to genomic expansion and rearrangements. Figure based on Christensen (2013, 2014).

divergence of intergenic regions, and why sometimes very high rates of $d_s$ coincide with extreme genomic inflation (Christensen 2013, 2014). Whatever the cause, the observed disparity in $d_s$ between synonymous and intergenic sites means that previous studies, including those of Lynch *et al.* (2006), which used mostly gene sequences for measuring silent substitutions, likely underestimated land plant mitochondrial mutations rates.

Do these new data on nucleotide substitution and DNA repair in land plants refute the MHH? Yes, because counter to the MHH they imply that excess DNA has accumulated in land plant mitochondria not because it has a 'low mutational liability' but because *it is* a mutational liability – it has a high mutation rate and the modes for repairing those mutations cause further genomic expansion. However, like the MHH, the model of Christensen (2013, 2014) is nonadaptive, in that land plant mtDNA expansion is the result of mutations that are fixed through random genetic drift. It remains to be seen whether differences in DNA repair are responsible for the enlargement or contraction of other types and lineages of organelle genome, such as the ptDNAs of *Volvox* and *Dunaliella*. Up to now, genetic variation studies on large ptDNAs have looked mostly at synonymous sites rather than intergenic ones (Smith 2015). Finally, the model of Christensen (2013, 2014), unlike the MHH, cannot explain why land plant mitochondrial genomes are prone to RNA editing, horizontal gene transfer and intron accumulation, but that aside, these new insights on organelle DNA repair have arguably given the biggest blow yet to the MHH, but not a mortal blow. The MHH may be operating in a limited set of circumstances. For example, there could be a threshold mutation rate at which it is the most relevant force for organelle evolution and the best explanation.

## Conclusions: bringing nonadaptive hypotheses to the forefront

As biologists, we have a propensity for seeing the natural world in an adaptive light. There is increasing evidence, however, that much of the biological complexity and variation found at the cellular and molecular levels are a consequence of nonadaptive processes. It might be easy to see the hand of natural selection in shaping the snout of an anteater or the streamlined feathers of an eagle, but explaining the origins of massive mitochondrial genomes or an extensive and costly organelle RNA editing infrastructure is not so straightforward.

Perhaps one of the most enduring and important contributions of the MHH is that it has sparked crucial, widespread discussions about the importance of nonadaptive processes in genome evolution. This, in turn, has introduced many scientists, instructors and students to nonadaptive reasoning in evolutionary explanations and brought more attention to the frailty of adaptive hypotheses for the origins of genomic complexity (Lynch 2007a,b). The MHH is not alone in highlighting these issues.

Organelle genomes, given their proclivity towards unconventional architectures and modes of expression, have had a long history of changing the way biologists view genetics and evolution as well as a reputation for dividing researchers along 'adaptive' vs. 'nonadaptive' lines (Lukeš *et al.* 2009; Speijer 2010). Some of the most compelling nonadaptive evolutionary theories that exist today have come from studies of organelle DNA, including the 'codon-capture' hypothesis (Osawa & Jukes 1989), a well-regarded neutral explanation for codon reassignment, which incorporates bias mutation and a stage where a

codon disappears entirely from the genome. Similarly, constructive neutral evolution (CNE) (Stoltzfus 2012), which is a one-directional, ratchet-like process whereby neutral (or slightly deleterious) mutations that result in increased complexity are fixed by random processes, such as genetic drift, is primarily based on observations of organelle RNA editing (Covello & Gray 1993).

Some of the arguments about organelle genome evolution are similar and connected to the recent controversy surrounding the Encyclopedia of DNA Elements (ENCODE) project (2012) and its definition of 'functional DNA' (2012), which equated the biochemical activity of a genomic site (e.g. transcription) with function (Kellis *et al*. 2014). Many researchers have subsequently spoken out against such a broad and arguably erroneous definition of function (Doolittle 2013; Graur *et al*. 2013), stressing the importance of distinguishing 'between what a genomic element does (its causal-role activity) from why it exists (its selected-effect function)' (Graur *et al*. 2015). The ongoing disputes about ENCODE have reignited the broader debate as to whether genome complexity is primarily the result of adaptive processes or the neutral accumulation of slightly deleterious changes – a question that is also at the heart of the MHH.

Aspects of the MHH and other neutral hypotheses for genome evolution may prove to be incorrect, as some already have. But the idea that nonadaptive processes play a central role in shaping genomes is here to stay, and future work will likely only reinforce the importance of mutation, DNA maintenance and random genetic drift in genome evolution.

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