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REVIEW

Evolutionary origins and impacts of genome architecture in ciliates

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Genome architecture is well diversified among eukaryotes in terms of size and content, with many being radically shaped by ancient and ongoing genome conflicts with transposable elements (e.g., the large transposon-rich genomes common among plants). In ciliates, a group of microbial eukaryotes with distinct somatic and germ-line genomes present in a single cell, the consequences of these genome conflicts are most apparent in their developmentally programmed genome rearrangements. This complicated developmental phenomenon has largely overshadowed and outpaced our understanding of how germ-line and somatic genome architectures have influenced the evolutionary dynamism and potential in these taxa. In our review, we highlight three central concepts: how the evolution of atypical ciliate germ-line genome architectures is linked to ancient genome conflicts; how the complex, epigenetically guided transformation of germline to soma during development can generate widespread genetic variation; and how these features, coupled with their unusual life cycle, have increased the rate of molecular evolution linked to genome architecture in these taxa.

Keywords: ciliates; evolution; genome evolution; transposable elements; genome architecture

Introduction

Genomes are highly dynamic, undergoing constant modification by genetic and epigenetic processes, while also maintaining vigil against the spread of transposable elements (TEs).^{1–6} While TEs are often described as parasitic or selfish DNA that are assumed to proliferate at the expense of the host genome's fitness (i.e., by increasing genome instability),^{7,8} they remain essential and well-regulated genomic components, for example, possessing roles as centromeres and/or telomeres in *Dictyostelium discoideum* and *Drosophila melanogaster*, respectively.^{9,10} Maintaining some control over genome instability provides massive fitness benefits to the host genome/organism, and is linked to genome dynamism,^{11–17} which is exaggerated and well studied in pathogenic lineages (*Phytophthora infestans* and *Entamoeba histolytica*).^{11–13} A dramatic example is the separation of germ-line and somatic genomes, which provides the means to

protect the heritable genome while reaping the benefits of a highly dynamic and responsive soma.

Distinct somatic and germ-line genomes are found in diverse lineages across the eukaryotic tree of life and are best understood in multicellular eukaryotes, where they are partitioned into separate tissues (e.g., pollen in plants, eggs in animals, and spores in fungi). However, in single-cell ciliates, these two genomes are found in dimorphic nuclei: a diploid, transcriptionally silent germ-line micronuclear genome (MIC), which becomes transcriptionally active only during sex, and a highly polyploid and transcriptionally active somatic macronuclear genome (MAC) that supports the cell. Complex, epigenetically guided processing underlies the development of new somatic nuclei from a zygotic nucleus.^{18–24} This involves the elimination of germline-limited DNA (e.g., TEs, centromeres, germline-limited genes, and internally eliminated sequences (IESs)) and the assembly of functional somatic regions (i.e.,

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macronuclear-destined sequences (MDS)).^{18,25–30} Although details differ across ciliate lineages, the delineation during development between somatic MDSs and the germline-limited IESs, which separate MDSs, involves RNA-guided mechanisms that resemble epigenetic responses to TE invasion/control in other eukaryotes.^{17,19–24}

Here, we describe how the atypical genome architectures in ciliates, coupled with a predominantly asexual life cycle punctuated by rare sexual events (similar to yeasts and other protists), provide them with an immense evolutionary potential and the means for rapid adaptation. This is largely due to the evolutionary impacts of ancient genome conflict with TEs, which are well known to provide the basis for evolutionary innovation in other eukaryotes.¹⁷ The general exploration of the interrelations between ciliate genome architecture, programmed genome rearrangements, and their life history (i.e., asexual growth with infrequent sexual events) will draw more attention to the role of genome architecture in evolution.

TEs and germ-line genome architecture in ciliates

Due to mechanistic similarities between the developmentally regulated genome rearrangements in ciliates and transposon regulation in other taxa, Klobutcher and Herrick³¹ proposed that evolution of nuclear dualism in ciliates was an evolutionary response to TE invasion, providing the means to purge them from the somatic genome. While the somatic genomes of most ciliates studied to date are effectively free of TEs, the germ-line genomes are enriched with repetitive regions/sequences that interrupt gene-coding sequences (an exception being the model ciliate *Tetrahymena thermophila*, where these regions are predominantly intergenic or intronic) and need to be excised during development of a new somatic genome.^{25–33} While the evolutionary origin of these repetitive regions is unclear given their vast diversity within a single germ-line genome, many harbor signatures of once functional TEs (i.e., repetitive boundary sequences or small terminal inverted repeats, such as TA; Fig. 1C).^{25,27,28,31–33} For example, Tec elements (transposons from the Tc1/Mariner family) in *Euplotes* are present in great abundance, at >10,000 copies and ~20–25% of the estimated germ-line genome size.³² These highly abundant

transposons are flanked by direct TA repeats, as are the majority of *Euplotes* IESs.^{31–33} This observation, coupled with the presence of several documented TEs interrupting coding sequences (which are accurately eliminated during development), implies a common excision mechanism targeting both TEs and IESs. As Klobutcher and Herrick³¹ describe, one explanation for the evolution and widespread distribution of IESs in ciliate germlines may derive from a period of replicative transposon transposition (bloom; Fig. 1B), followed by their inactivation and subsequent degeneration into IESs, where only the terminal sequences necessary for excision (pointer sequences) have remained (decay; Fig. 1C). More recently, a survey of IESs from the complete *T. thermophila* germ-line genome has found that ~42% of the IESs (comprising 10.9 Mbp of the 150 Mbp germ-line genome) are putative TEs and their decayed remnants.²⁷ While all IESs identified among diverse ciliate lineages, spanning ~1 GYA,³⁴ are demarcated by direct repeats at their MDS–IES boundaries,^{25–30,33} these data suggest that TEs have played a role in the origin of some of the IESs found in ciliate germ-line genomes.

While TEs are tightly linked with the evolution of ciliate germ-line genome architecture, they have also become an indispensable player in programmed DNA elimination. In *Paramecium tetraurelia* and *T. thermophila*, domesticated PiggyBac transposases (e.g., PiggyMAC or TPB encoded in the *Paramecium* and *Tetrahymena* somatic genomes, respectively) perform the bulk excision of germline-limited DNA, including TEs, from the developing somatic genome.^{35–39} Silencing *P. tetraurelia*'s PiggyMac transposase during development ultimately results in the retention of most of the ~45,000 IESs, resulting in a nonfunctional somatic genome.²⁵ Although taming transposons appears to be required for the massive DNA elimination observed in ciliates, the degree of domestication (i.e., recruitment into the somatic genome versus limited to the germline) is variable. For example, the transposases of thousands of presumably active germline-limited telomere-bearing elements (TBEs, part of the Tc1/Mariner family) facilitate the developmentally regulated genome rearrangements during *Oxytricha trifallax* development.⁴⁰ Silencing these germline-limited TBEs in *O. trifallax* during development hampers the genome rearrangement process, resulting in the accumulation

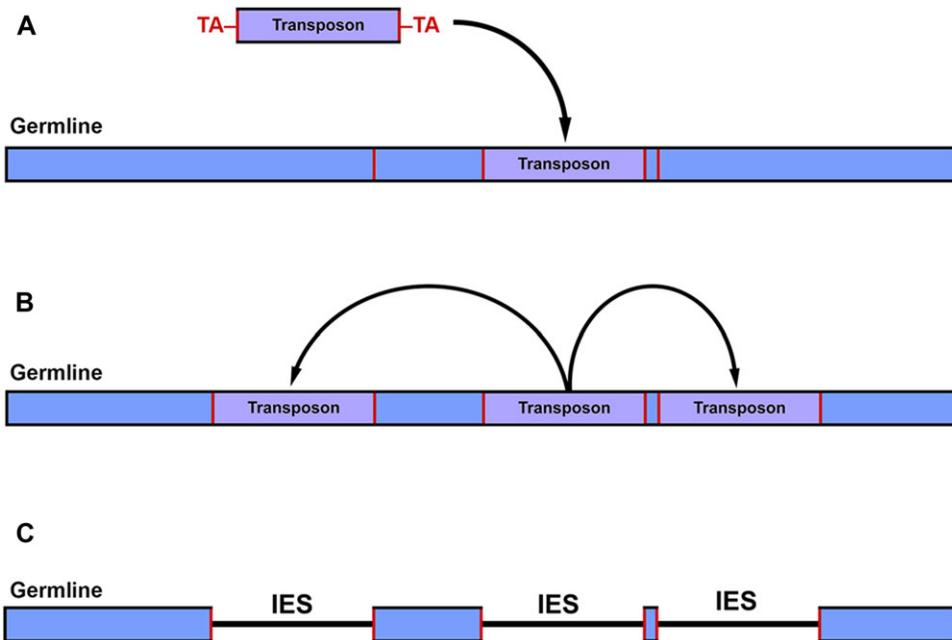


Figure 1. Origin of germ-line genome architecture in ciliates. (A) Invasion of a transposable element into the germ-line genome at a target insertion site (red boundaries, germline). (B) Over time, it proliferates (bloom) throughout the germ-line genome. (C) These TEs decay over time into internally eliminated sequences (IESs), ultimately generating the traditional nonscrambled genome architecture. The original insertion target sequences (red) remain as pointer sequences that guide MDS organization in many ciliates (e.g., *Paramecium* and *Euplotes*).

of quasi-germline chromosomes (misarranged, atypically large, harboring IESs) in the new somatic nucleus.⁴⁰ Their absence from the somatic genome suggests that these germline-limited transposases are not domesticated to the same degree as in *Paramecium* and *Tetrahymena*. Interestingly, a germline-limited *PiggyBac* transposase has been identified in *Tetrahymena* and is required for precise excision of germline-limited DNA, whereas the somatic *PiggyBac*, which is responsible for the bulk of IES excision, does so at variable boundaries.⁴¹ These data from ciliates are yet another example of how TE proteins, regardless of their domestication status, have often been co-opted into numerous pathways as adaptations to a variety of evolutionary conflicts spanning the tree of life.^{37,41–43}

Origins of ciliate scrambled germ-line genome architecture

Descriptions of ciliate germ-line genome architecture fall into two categories: a nonscrambled organization, with consecutive somatic MDSs separated by germline-limited IESs in the same

orientation (the most obvious result of the TE invasion–bloom–decay hypothesis; Fig. 1),³¹ and scrambled, where some MDSs are in nonconsecutive order and/or encoded on opposing DNA strands (Fig. 2).^{26,29,30,44–49} While nonscrambled germ-line organization is common across the ciliate phylogeny, emerging evidence from poorly sampled lineages suggests that scrambled germ-line loci may be more common than previously expected.³⁰

A proposed model for the origin of scrambled germ-line loci involves an initial duplication event that generates long stretches of identical DNA, irrespective of orientation.⁴⁸ From these duplicated loci, combinatorial rearrangements can take place during development (guided by the large pool of redundant pointer sequences), generating identical somatic sequences (Fig. 2A).^{43–49} Over time, decay/divergence of redundant pointers and/or identical coding regions could become fixed, with negligible impacts on fitness (Fig. 2B and C). Alternative processing has been suggested to be the intermediate stage between duplication and fixation of a single orientation of MDSs,^{45–49} where

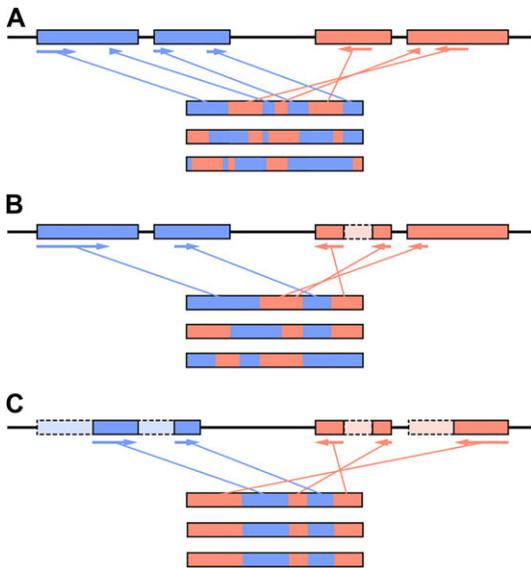


Figure 2. Example of the origin of a scrambled germ-line locus. (A) Following the duplication of a germ-line locus (top), functional proteins/chromosomes (three examples below) can be assembled from a myriad of combinations of the duplicate MDSs (blue and red). Arrows indicate the 5'-3' orientation in the germline and represent the portions of the MDSs that are assembled into the top-most chromosome/gene. (B) Eventually some portions of MDSs decay (red, dashed box), forcing the alternative processing of the germ-line loci to still produce functional chromosomes. (C) Eventually, the decay becomes complete, resulting in a single combination of the remaining functional MDS portions in a scrambled orientation.

numerous paralogous genes/chromosomes can be formed from duplicated germ-line loci. However, given enough time, these highly diverged regions can be targeted for elimination if absent from the parental genome. For example, in *Oxytricha* piRNAs, two types of RNAs are involved in forming a faithful reproduction of the old parental genome. Small piRNAs protect MDSs from elimination, which presumably then use RNA copies of whole chromosomes that guide accurate rearrangement of these protected MDSs, regardless their orientation in the germline.^{19,21,23,50}

Generation of diversity through genome rearrangements

Despite the critical importance of accurate complex genome reorganization in the development of a new somatic genome in ciliates, the process itself remains susceptible to heritable changes (errors) linked with epigenetic processes and environmental conditions. For example, in *P. tetraurelia*, mating-type determination involves the retention of a single 195 bp IES at the mating-type locus, where the IES is retained in MT-E (IES⁺) and absent in MT-O (IES⁻).⁵¹⁻⁵⁵ When growing under optimal conditions, spontaneous switches in mating type from MT-E to MT-O occur in ~1/3000 cells, whereas the opposite is much rarer, <1/50,000 cells.⁵⁵ Environmental conditions (e.g., temperature differences) can strongly alter the above patterns of mating-type

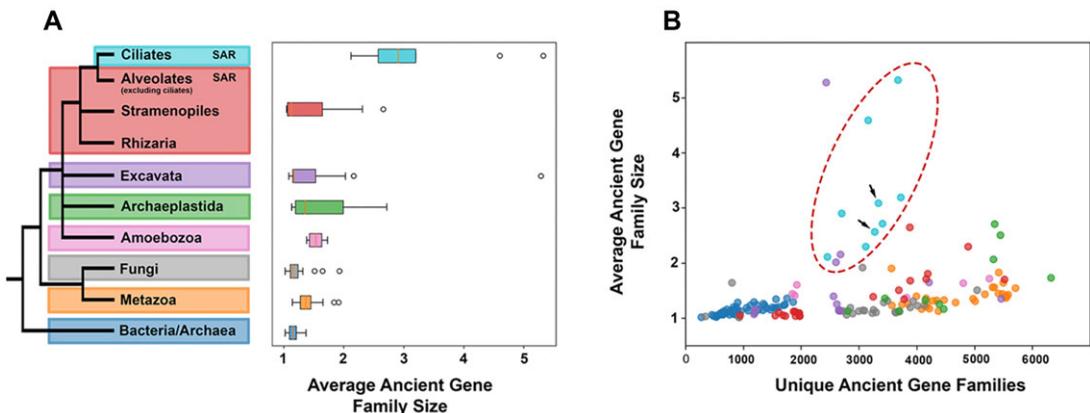


Figure 3. (A) Ciliate genomes harbor fewer, but larger gene families than other eukaryotes. This trend holds true across most ciliate taxa (B, red ellipse), including parasitic ciliates (e.g., *Ichthyophthirius multifiliis* and *Pseudocohnilembus persalinus*), which possess a substantially reduced proteome. (B) The ciliates with scrambled germ-line genomes (black arrows, *Stylonychia lemnae* and *Oxytricha trifallax*; lower left and upper right, respectively), possess comparable paralog diversity in ancient gene families to ciliates lacking genome scrambling, despite evidence for scrambling-associated gene family expansion. These gene families are more likely to be lineage-specific or are too divergent for gene family binning and as such do not show up on this plot.

inheritance. Interestingly, when exposed to reduced temperatures (13 °C), MT-O individuals predominantly maintain their mating type; however, the frequency of spontaneous switches from MT-O to MT-E, the difference being the retention of a single specific IES in MT-E (IES⁺), increases dramatically with increasing temperature.^{53,54} More generally in *Paramecium*, the protein machinery involved in the developmentally regulated genome rearrangements are themselves error prone, as the accidental elimination of portions of MDSs has been noted to occur and nearly 1/10th of IESs are inaccurately excised or incompletely excised.⁵⁶ However, the severity of the impact of IES retention in *Paramecium* is likely offset by its rather great ploidy (~800N).⁵⁷

Although the frequency of aberrant structural variation events during development in *Paramecium* may be relatively common, they rarely appear to be fixed in the population of chromosomes. For example, for a *Paramecium* to express the surface antigens found only in the MT-E (IES⁺) rather than MT-O (IES⁻) cells, enough copies of the mating-type locus retaining the IES must be present; otherwise, the cell remains MT-O.^{53,54} This suggests that aberrant excision events and/or retained IESs, even when present at low abundance, can impact a cell's fitness by either reducing the number of functional copies of a gene (i.e., through frameshifting or the introduction of premature stop codons), or through the insertion of novel sequences that alter the protein structure itself or its regulatory regions.

Although IESs can alter pre-existing protein structure/expression, genes from scrambled germ-line loci are a particular source of genomic diversity/novelty (Fig. 3). For example, in *Chilodonella uncinata* there are at least four divergent β -tubulin genes that are generated from the alternative processing of three scrambled germ-line loci.^{45,46} Genes from scrambled germ-line loci comprise surprisingly great proportions of *C. uncinata*'s largest gene families; however, these large gene families are also fairly new (lacking homologs in other taxa).²⁹ The expansion of novel (i.e., lineage-specific) gene families through gene scrambling is common among taxa with substantial germ-line scrambling (e.g., *O. trifallax* and its relatives; Figs. 2 and 3B).⁴⁸ In these taxa, small RNAs from the parental genome aid in demarcating portions of the germ-line genome that ought to be retained,^{21,23} as in *Paramecium* and *Tetrahymena*, but full chromosomes (i.e., long tem-

plate RNAs) are transcribed to ensure the correct rearrangement order.^{19,20,50}

Potential mistakes that occur during unscrambling (resembling alternative processing) ultimately diversify the population of chromosomes. Once present, these alternatively arranged chromosomes can undergo functional divergence (i.e., subfunctionalization or neofunctionalization). Analyses of alternatively processed paralogs from both *Chilodonella* and *Oxytricha* show strong purifying selection acting upon shared MDSs, whereas those paralog-specific sequences can be incredibly divergent.⁴⁵⁻⁴⁹ While some of these paralogs may be nonfunctional, RNA-seq analyses of different time points show little overlap of expression between alternatively processed paralogs.⁴⁸ These data clearly demonstrate how genome unscrambling can generate novel genetic diversity upon which selection can act. So long as these alternatively processed chromosomes do not negatively impact the cell's fitness, these rearranged chromosomes provide the template for unscrambling the respective germ-line loci during the next sexual event.^{19,20,50}

Although not all IESs require small RNAs to identify and guide their excision (i.e., nonmaternally controlled IESs), the parental genome's influence through RNA over the developing somatic genome appears common across diverse ciliates. For example, experimental deletions in the parental somatic genome in *Paramecium* correspond to changes in the pool of scanning RNAs that aid in delineating germline from soma, ultimately resulting in the inheritance of the same deletion in the developing somatic genome.⁵⁸ Similarly, experiments hindering the production of these small RNAs in *Paramecium* and *Tetrahymena* result in the retention of large portions of the germline-limited DNA in the developing genome.^{59,60} This transnuclear crosstalk through RNA intermediates helps the parental soma to further shape the next generation's somatic genome.

Accelerated protein evolution and amitosis

Asexuality dominates the majority of a ciliate's life cycle, while sexual events (and the mutagenic potential of genome rearrangements) are brief points. During many rounds of asexual division between sexual events, the diploid germline divides through conventional mitosis, steadily

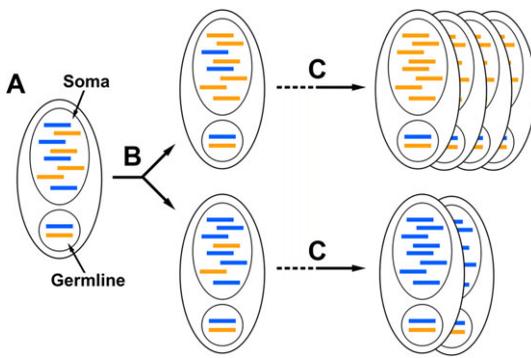


Figure 4. Phenotypic assortment through amitosis enhances the impact of selection. (A) Two alleles, one slightly deleterious (blue), are present in roughly equal copy number in the somatic nucleus. (B) After the first amitotic division, the alleles are separated randomly, resulting in nonidentical daughter cells/nuclei. (C) Over many amitotic divisions, an incredibly fit lineage emerges (top), dominating the population of cells, whereas cells predominantly possessing the slightly deleterious allele divide more slowly, becoming an increasingly smaller proportion of the population of cells (bottom).

accumulating mutations.⁶¹ Although most of the mutations that arise in the germline are likely deleterious, they remain hidden from selection, providing the time needed for compensatory mutations to arise with minimal impact on fitness.⁶¹ Eventually, these mutations will be exposed to selection in the somatic genome after sex. However, during these abundant asexual divisions, the polyploid somatic genome undergoes amitosis (ciliates in the class Karyorelictea being the exception),^{62–65} which separates masses of chromosomes in the absence of mitotic spindles, resulting in the unequal partitioning of DNA (i.e., aneuploidy).^{18,62}

This absence of controlled segregation (i.e., a metaphase plate) results in unique daughter nuclei that are not only initially distinct in ploidy, but potentially in genomic content as well. For example, during the development of a new somatic genome, two alleles (i.e., an exposed germ-line mutation now exposed in the soma, retained IES, or a unique alternatively processed gene or chromosome) arise at roughly equal copy numbers, one of which is deleterious, in the somatic genome (Fig. 4A). Even after the first amitotic division, the proportion of the deleterious allele would be different between daughter cells, due to the random segregation of bulk DNA (Fig. 4B). The daughter nucleus and cell with the lower proportion of the deleterious allele

would be favored by selection. Over time and many amitotic divisions, the random assortment of alleles will result in asexual cell lineages with increasingly fewer copies of this disadvantageous allele. These cells will outgrow most other cells, ultimately comprising greater proportions of the population (Fig. 4C).

The efficiency of phenotypic assortment through amitosis (and the efficacy of selection) is strongly tied to the structure of the somatic genome. For instance, in *Chilodonella* and *Oxytricha*, the somatic nuclei harbor thousands of unique gene-sized chromosomes, with each chromosome at independent copy numbers ranging from several hundred to nearly a million copies.^{66–69} The fate of every gene in these genomes is independent of the others. Selection can favor those nuclei harboring fewer deleterious mutations/arrangements, which could be lost over time without impacting other genes/chromosomes. By contrast, in *Paramecium* and *Tetrahymena*, most genes share the large (>50 kbp) chromosomes with ~100–400 other genes, and are at much lower ploidy (~800N in *P. tetraurelia* and ~45N in *T. thermophila*).^{57,70} Complete chromosome loss would be catastrophic and deleterious mutations may be more likely to be retained, albeit at minimal ploidy (e.g., retention of an IES in a subpopulation of chromosomes from MT-E *Paramecia*). The combination of ciliate genome architecture and amitosis are tied to the observed elevated rates of protein evolution.^{46,48,71,72}

The protein-coding genes in those ciliates with extremely processed somatic genomes (i.e., composed of millions of gene-sized chromosomes) exist nearly free of any gene linkage. This organization greatly enhances the rate and efficacy of phenotypic assortment since the evolutionary fates of alleles and chromosomes are unique. Coupled with the ability to alternatively process germ-line loci, these taxa are able to generate highly divergent proteins in relatively short periods of time.^{29,44–49} Even though this trend is strongest in ciliates possessing gene-sized chromosomes, this general trend holds true for most of the ciliate clade, even among highly conserved proteins.^{71,72} The exception to this pattern is members of the ciliate class Karyorelictea, whose MACs are unable to divide and must be generated from a diploid MIC with every cell division. In these taxa, there is evidence for substantially greater

purifying selection acting on orthologous and paralogous genes compared to ciliates able to divide their macronuclei through amitosis (unpublished data). This appears to occur despite germ-line genome architectures similar to other ciliate taxa³⁰ and indicates the ability that amitosis has to enhance patterns of protein evolution.

Conclusion

Genome conflict and the epigenetic process have greatly contributed to the great diversity of observed genome architectures across the tree of life. In ciliates, this has led to a dynamic developmental system, where epigenetic processes have been co-opted into dramatic genome remodeling, including RNA-guided DNA elimination and chromosome copy number control. Errors in these processes provide the means for the rapid development of new genes and alterations in regulatory networks within a few sexual generations. While ciliate genome architectures are the source of novelty, the ability to amitotically divide their somatic genomes facilitates their adaptability through the proliferation or loss of novel mutations. Unfortunately, without more data (both genomic and experimental) from a greater diversity of ciliates and other eukaryotes, it remains difficult to disentangle the roles of amitosis and genome architecture (both somatic and germline) in the context of adaptability and molecular evolution.

Competing interests

The authors declare no competing interests.

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