

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Special Issue: *Genetic Novelty and Genomic Variation by RNA Networks and Viruses*

REVIEW

Evolutionary origins and impacts of genome architecture in ciliates

Xyrus X. Maurer-Alcalá and Mariusz Nowacki

Institute of Cell Biology, University of Bern, Bern, Switzerland

Address for correspondence: Mariusz Nowacki, Institute of Cell Biology, University of Bern, Baltzerstrasse 4, 3012 Bern, Switzerland. mariusz.nowacki@izb.unibe.ch

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.,

doi: 10.1111/nyas.14108

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

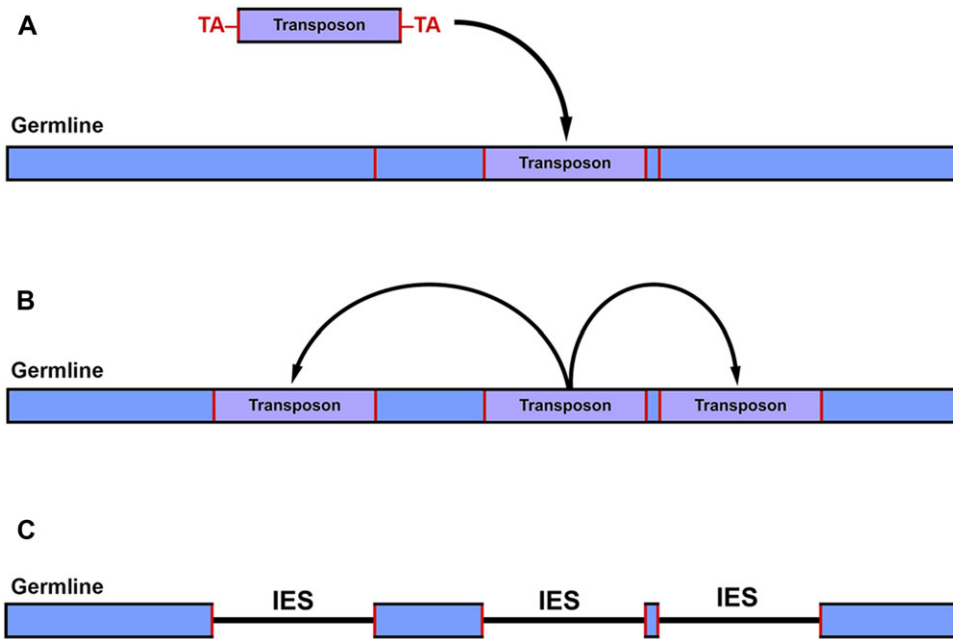


Figure 1. Origin of germ-line genome architecture in ciliates. (A) Invasion of a transposable element into the germ-line genome at a target insertion site (red boundaries, germ-line). (B) Over time, it proliferates (bloom) throughout the germ-line genome. (C) These TEs decay over time into internally 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

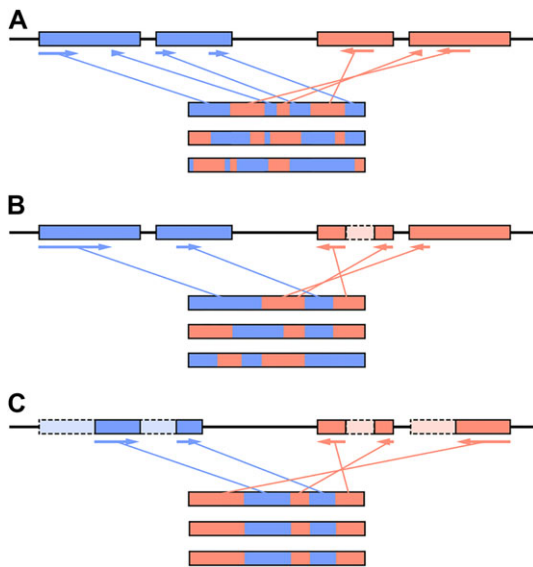


Figure 2. Example of the origin of a scrambled germ-line locus. (A) Following the duplication of a germ-line locus (top), functional proteins/chromosomes (three examples below) can be assembled from a myriad of combinations of the duplicate MDSs (blue and red). Arrows indicate the 5'-3' orientation in 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

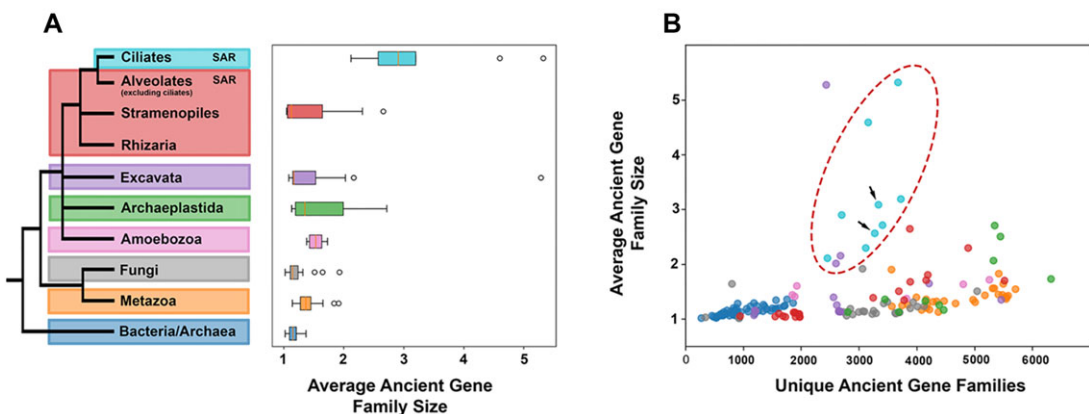


Figure 3. (A) Ciliate genomes harbor fewer, but larger gene families than other eukaryotes. This trend holds true across most ciliate taxa (B, red ellipse), including parasitic ciliates (e.g., *Ichthyophthirius multifiliis* and *Pseudocohnilembus persalinus*), which possess a substantially reduced proteome. (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

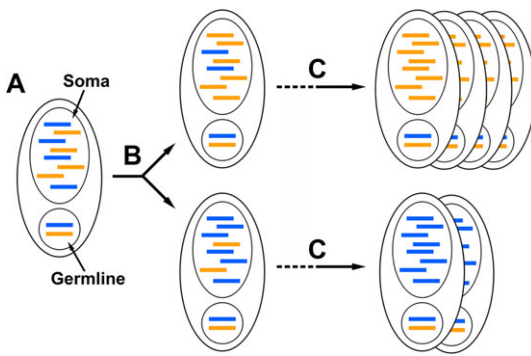


Figure 4. Phenotypic assortment through amitosis enhances the impact of selection. (A) Two alleles, one slightly deleterious (blue), are present in roughly equal copy number in the somatic nucleus. (B) After the first amitotic division, the alleles are separated randomly, resulting in nonidentical daughter 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.

References

- Wassenegger, M., S. Heimes, L. Riedel & H.L. Sanger. 1994. RNA-directed *de novo* methylation of genomic sequences in plants. *Cell* **76**: 567–576.
- Matzke, M.A., M.F. Mette & A.J.M. Matzke. 2000. Transgene silencing by the host genome defense: implications for the evolution of epigenetic control mechanisms in plants and vertebrates. *Plant Mol. Biol.* **43**: 401–415.
- Slotkin, K.R. & R. Martienssen. 2007. Transposable elements and the epigenetic regulation of the genome. *Nat. Rev. Genet.* **8**: 272.
- Shabalina, S.A. & E.V. Koonin. 2008. Origins and evolution of eukaryotic RNA interference. *Trends Ecol. Evol.* **23**: 578–587.
- Hollister, J.D. & B.S. Gaut. 2009. Epigenetic silencing of transposable elements: a trade-off between reduced transposition and deleterious effects on neighboring gene expression. *Genome Res.* **19**: 1419–1428.
- Matzke, M.A. & R.A. Mosher. 2014. RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. *Nat. Rev. Genet.* **15**: 394.
- Charlesworth, B., P. Sniegowski & W. Stephan. 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**: 215.
- Orgel, L.E. & F.H.C. Crick. 1980. Selfish DNA: the ultimate parasite. *Nature* **284**: 604.
- Levis, R.W. *et al.* 1993. Transposons in place of telomeric repeats at a *Drosophila* telomere. *Cell* **75**: 1083–1093.
- Glöckner, G. & A.J. Heide. 2009. Centromere sequence and dynamics in *Dictyostelium discoideum*. *Nucleic Acids Res.* **37**: 1809–1816.
- Haas, B.J. *et al.* 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* **461**: 393–398.
- Kumari, V. *et al.* 2011. Differential distribution of a SINE element in the *Entamoeba histolytica* and *Entamoeba dispar* genomes: role of the LINE-encoded endonuclease. *BMC Genomics* **12**: 267.
- Vetukuri, R.R. *et al.* 2013. Phenotypic diversification by gene silencing in *Phytophthora* plant pathogens. *Commun. Integr. Biol.* **6**: e25890.
- Molinier, J., G. Ries, C. Zipfel & B. Hohn. 2006. Transgenerational memory of stress in plants. *Nature* **442**: 1046–1049.
- Oliver, K.R. & W.K. Greene. 2009. Transposable elements: powerful facilitators of evolution. *Bioessays* **31**: 703–714.
- Kathiria, P. *et al.* 2010. Tobacco mosaic virus infection results in an increase in recombination frequency and resistance to viral, bacterial, and fungal pathogens in the progeny of infected tobacco plants. *Plant Physiol.* **153**: 1859–1870.
- Maurer-Alcalá, X.X. & L.A. Katz. 2015. An epigenetic toolkit allows for diverse genome architectures in eukaryotes. *Curr. Opin. Genet. Dev.* **35**: 93–99.
- Prescott, D.M. 1994. The DNA of ciliated protozoa. *Microbiol. Rev.* **58**: 233–267.
- Nowacki, M. *et al.* 2008. RNA-mediated epigenetic programming of a genome-rearrangement pathway. *Nature* **451**: 153.
- Jönsson, F., J. Postberg & H.J. Lipps. 2009. The unusual way to make a genetically active nucleus. *DNA Cell Biol.* **28**: 71–78.
- Fang, W. *et al.* 2012. Piwi-interacting RNAs protect DNA against loss during *Oxytricha* genome rearrangement. *Cell* **151**: 1243–1255.
- Bracht, J.R. *et al.* 2013. Genomes on the edge: programmed genome instability in ciliates. *Cell* **152**: 406–416.
- Zahler, A.M. *et al.* 2012. Mating of the stichotrichous ciliate *Oxytricha trifallax* induces production of a class of 27 nt small RNAs derived from the parental macronucleus. *PLoS One* **7**: e42371.
- Allen, S.E. & M. Nowacki. 2017. Necessity is the mother of invention: ciliates, transposons, and transgenerational inheritance. *Trends Genet.* **33**: 197–207.
- Arnaiz, O. *et al.* 2012. The *Paramecium* germline genome provides a niche for intragenic parasitic DNA: evolutionary dynamics of internal eliminated sequences. *PLoS Genet.* **8**: e1002984.

26. Chen, X. *et al.* 2014. The architecture of a scrambled genome reveals massive levels of genomic rearrangement during development. *Cell* **158**: 1187–1198.
27. Hamilton, E.P. *et al.* 2016. Structure of the germline genome of *Tetrahymena thermophila* and relationship to the massively rearranged somatic genome. *Elife* **5**: e19090.
28. Guérin, F. *et al.* 2017. Flow cytometry sorting of nuclei enables the first global characterization of *Paramecium* germline DNA and transposable elements. *BMC Genomics* **18**: 327.
29. Maurer-Alcalá, X.X., R. Knight & L.A. Katz. 2018. Exploration of the germline genome of the ciliate *Chilodonella uncinata* through single-cell omics (transcriptomics and genomics). *MBio* **9**: e01836-17.
30. Maurer-Alcalá, X.X. *et al.* 2018. Twisted tales: insights into genome diversity of ciliates using single-cell omics. *Genome Biol. Evol.* **10**: 1927–1938.
31. Klobutcher, L.A. & G. Herrick. 1997. Developmental genome reorganization in ciliated protozoa: the transposon link. *Prog. Nucleic Acid Res. Mol. Biol.* **56**: 1–62.
32. Jaraczewski, J.W., J.S. Frels & C.L. Jahn. 1994. Developmentally regulated, low abundance Tec element transcripts in *Euplotes crassus*—implications for DNA elimination and transposition. *Nucleic Acids Res.* **22**: 4535–4542.
33. Klobutcher, L.A. & G. Herrick. 1995. Consensus inverted terminal repeat sequence of *Paramecium* IESs: resemblance to termini of Tc1-related and *Euplotes* Tec transposons. *Nucleic Acids Res.* **23**: 2006–2013.
34. Parfrey, L.W. *et al.* 2011. Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proc. Natl. Acad. Sci. USA* **108**: 13624–13629.
35. Baudry, C. *et al.* 2009. PiggyMac, a domesticated PiggyBac transposase involved in programmed genome rearrangements in the ciliate *Paramecium tetraurelia*. *Genes Dev.* **23**: 2478–2483.
36. Cheng, C.Y. *et al.* 2010. A domesticated PiggyBac transposase plays key roles in heterochromatin dynamics and DNA cleavage during programmed DNA deletion in *Tetrahymena thermophila*. *Mol. Biol. Cell* **21**: 1753–1762.
37. Dubois, E. 2012. Transposon invasion of the *Paramecium* germline genome countered by a domesticated PiggyBac transposase and the NHEJ pathway. *Int. J. Evol. Biol.* **2012**: 436196.
38. Cheng, C.Y. *et al.* 2016. The PiggyBac transposon-derived genes TPB1 and TPB6 mediate essential transposon-like excision during the developmental rearrangement of key genes in *Tetrahymena thermophila*. *Genes Dev.* **30**: 2724–2736.
39. Bischerour, J. *et al.* 2018. Six domesticated PiggyBac transposases together carry out programmed DNA elimination in *Paramecium*. *Elife* **7**: e37927.
40. Nowacki, M. *et al.* 2009. A functional role for transposases in a large eukaryotic genome. *Science* **324**: 935–938.
41. Feng, L. *et al.* 2017. A germline-limited PiggyBac transposase gene is required for precise excision in *Tetrahymena* genome rearrangement. *Nucleic Acids Res.* **45**: 9481–9502.
42. Krupovic, M. *et al.* Casposons: a new superfamily of self-synthesizing DNA transposons at the origin of prokaryotic CRISPR-Cas immunity. *BMC Biol.* **12**: 36.
43. Jangam, D., C. Feschotte & E. Betrán. 2017. Transposable element domestication as an adaptation to evolutionary conflicts. *Trends Genet.* **33**: 817–831.
44. Prescott, D.M. & A.F. Greslin. 1992. Scrambled actin I gene in the micronucleus of *Oxytricha nova*. *Dev. Genet.* **13**: 66–74.
45. Zufall, R.A. & L.A. Katz. 2007. Micronuclear and macronuclear forms of β -tubulin genes in the ciliate *Chilodonella uncinata* reveal insights into genome processing and protein evolution. *J. Eukaryot. Microbiol.* **54**: 275–282.
46. Katz, L.A. & A.M. Kovner. 2010. Alternative processing of scrambled genes generates protein diversity in the ciliate *Chilodonella uncinata*. *J. Exp. Zool. B Mol. Dev. Evol.* **314**: 480–488.
47. Gao, F., W. Song & L.A. Katz. 2014. Genome structure drives patterns of gene family evolution in ciliates, a case study using *Chilodonella uncinata* (Protista, Ciliophora, Phyllopharyngea). *Evolution* **68**: 2287–2295.
48. Chen, X. *et al.* 2015. Combinatorial DNA rearrangement facilitates the origin of new genes in ciliates. *Genome Biol. Evol.* **7**: 2859–2870.
49. Gao, F., S.W. Roy & L.A. Katz. 2015. Analyses of alternatively processed genes in ciliates provide insights into the origins of scrambled genomes and may provide a mechanism for speciation. *MBio* **6**: e01998-14.
50. Lindblad, K.A. *et al.* 2017. Thousands of RNA-cached copies of whole chromosomes are present in the ciliate *Oxytricha* during development. *RNA* **23**: 1200–1208.
51. Sonneborn, T.M. 1975. The *Paramecium aurelia* complex of fourteen sibling species. *Trans. Am. Microsc. Soc.* **94**: 155–178.
52. Brygoo, Y. 1977. Genetic analysis of mating-type differentiation in *Paramecium tetraurelia*. *Genetics* **87**: 633–653.
53. Meyer, E. & A.M. Keller. 1996. A mendelian mutation affecting mating-type determination also affects developmental genomic rearrangements in *Paramecium tetraurelia*. *Genetics* **143**: 191–202.
54. Singh, D.P. *et al.* 2014. Genome-defence small RNAs exapted for epigenetic mating-type inheritance. *Nature* **509**: 447–452.
55. Orias, E., D.P. Singh & E. Meyer. 2017. Genetics and epigenetics of mating type determination in *Paramecium* and *Tetrahymena*. *Annu. Rev. Microbiol.* **71**: 133–156.
56. Swart, E.C. *et al.* 2014. Genome-wide analysis of genetic and epigenetic control of programmed DNA deletion. *Nucleic Acids Res.* **42**: 8970–8983.
57. Duret, L. *et al.* 2008. Analysis of sequence variability in the macronuclear DNA of *Paramecium tetraurelia*: a somatic view of the germline. *Genome Res.* **18**: 585–596.
58. Garnier, O. *et al.* 2004. RNA-mediated programming of developmental genome rearrangements in *Paramecium tetraurelia*. *Mol. Cell. Biol.* **24**: 7370–7379.
59. Mochizuki, K. & M.A. Gorovsky. 2004. Conjugation-specific small RNAs in *Tetrahymena* have predicted properties of scan (scn) RNAs involved in genome rearrangement. *Genes Dev.* **18**: 2068–2073.
60. Malone, C.D. *et al.* 2005. Germ Line transcripts are processed by a dicer-like protein that is essential for

- developmentally programmed genome rearrangements of *Tetrahymena thermophila*. *Mol. Cell. Biol.* **25**: 9151–9164.
61. Long, H. *et al.* 2013. Accumulation of spontaneous mutations in the ciliate *Tetrahymena thermophila*. *Genetics* **195**: 527–540.
 62. Raikov, I.B. 1982. *The Protozoan Nucleus—Morphology and Evolution*. Springer.
 63. Orias, E. 1991. Evolution of amitosis of the ciliate macronucleus: gain of the capacity to divide. *J. Protozool.* **38**: 217–221.
 64. Katz, L.A. 2001. Evolution of nuclear dualism in ciliates: a reanalysis in light of recent molecular data. *Int. J. Syst. Evol. Microbiol.* **51**: 1587–1592.
 65. Yan, Y. *et al.* 2017. Unusual features of non-dividing somatic macronuclei in the ciliate class Karyorelictea. *Eur. J. Protistol.* **61**: 399–408.
 66. Nowacki, M. *et al.* 2010. RNA-mediated epigenetic regulation of DNA copy number. *Proc. Natl. Acad. Sci. USA* **107**: 22140–22144.
 67. Bellec, L. & L.A. Katz. 2012. Analyses of chromosome copy number and expression level of four genes in the ciliate *Chilodonella uncinata* reveal a complex pattern that suggests epigenetic regulation. *Gene* **504**: 303–308.
 68. Xu, K. *et al.* 2012. Copy number variations of 11 macronuclear chromosomes and their gene expression in *Oxytricha trifallax*. *Gene* **505**: 75–80.
 69. Huang, J. & L.A. Katz. 2014. Nanochromosome copy number does not correlate with RNA levels though patterns are conserved between strains of the ciliate morphospecies *Chilodonella uncinata*. *Protist* **165**: 445–451.
 70. Eisen, J.A. *et al.* 2006. Macronuclear genome sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote. *PLoS Biol.* **4**: e286.
 71. Katz, L.A. *et al.* 2004. Dramatic diversity of ciliate histone H4 genes revealed by comparisons of patterns of substitutions and paralog divergences among eukaryotes. *Mol. Biol. Evol.* **21**: 555–562.
 72. Zufall, R.A. *et al.* Genome architecture drives protein evolution in ciliates. *Mol. Biol. Evol.* **23**: 1681–1687.