Macronuclear development in ciliates, with a focus on nuclear architecture

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ABSTRACT

Ciliates are defined by the presence of dimorphic nuclei as they have both a somatic macronucleus and germline micronucleus within each individual cell. The size and structure of both germline micronuclei and somatic macronuclei varies tremendously among ciliates. Except just after conjugation (i.e. the nuclear exchange in sexual cycle), the germline micronucleus is transcriptionally-inactive and contains canonical chromosomes that will be inherited between generations. In contrast, the transcriptionally-active macronucleus contains chromosomes that vary in size in different classes of ciliates, with some lineages having extensively-fragmented gene-sized somatic chromosomes while others contain longer multigene chromosomes. Here, we describe the variation in somatic macronuclear architecture in lineages sampled across the ciliate tree of life, specifically focusing on lineages with extensively fragmented chromosomes (e.g. the classes Phyllopharyngea and Spirotrichea). Further, we synthesize information from the literature on the development of ciliate macronuclei, focusing on changes in nuclear architecture throughout life cycles. These data highlight the tremendous diversity among ciliate nuclear cycles, extend our understanding of patterns of genome evolution, and provide insight into different germline and somatic nuclear features (e.g. nuclear structure and development) among eukaryotes.

INTRODUCTION

Ciliates are a diverse clade of eukaryotes that are more than 1 billion years old (Parfrey et al. 2011). While some clades of ciliates are more extensively researched than others (e.g. the model ciliates *Tetrahymena, Paramecium, Oxtyricha*), many lineages remain underexplored. Unicellular ciliates are recognized by their key characteristics: hair-like cilia surrounding their body in at least one life history stage, and most importantly for this review, their nuclear dualism as each cell has at least one polyploid (i.e through endoreplication) somatic macronucleus and diploid germline micronucleus. While somatic macronuclei engage with different kinds of functions related to metabolism, cell growth, and division, the germline micronuclei are transcriptionally inactive during the non-developmental period. However, the micronucleus plays a significant role in the formation of a new macronucleus after conjugation, a form of sexual reproduction, during which the macronucleus and micronucleus are generated from a zygotic nucleus (Ammermann et al. 1974; Lipps and Eder 1996; Wancura et al. 2017, McGrath et al. 2006; Morgens et al. 2013; Chalker 2013; Maurer-Alcaláet al. 2018). Unusual nuclear processes such as DNA elimination and extensive fragmentation contribute to ciliate genome diversity. Here we highlight some of the diversity of nuclear events in ciliates, focusing on chromosomal rearrangements and other DNA processing.

THE SEXUAL CONJUGATION STAGE OF THE CILIATE

There is considerable variation among ciliates, including in the number and structure of nuclei. Much of the ciliate tree of life has been resolved through studies of genes and, more recently, whole genomes, leading to a major division between the Intramacronucleata (i.e. most classes of ciliates including the Oligohymenophorea, Spirotrichea, and Phyllopharyngea) and the Postciliodesmatophora (the classes Karyorelictea and Heterotrichea). Ciliates in both major clades vary tremendously in nuclear numbers: some have only one (e.g. *Tetrahymena* (Oligohymenophorea) *Monoeuplotes* (Spirotrichea)) or two (e.g. *Loxodes* (Karyorelictea) and *Paramecium* (Oligohymenophorea; Fig. 2b)) micronuclei. One ciliate species, *Urostyla grandis*, can have up to 20 micronuclei (Prescott 1994; Mcgrath et al. 2006) while the suctorian ciliate, *Acineta tuberosa* has a single oval shaped macronucleus with a minimum of two micronuclei (Fig. 2a). The number and shape of macronuclei also varies: *Stylonychia mytilus* and some *Euplotidium* species (both Spirotrichea) contain two macronuclei, with *Euplotidium* having an elongated macronucleus (Fig. 2e) and *Stylonychia mytilus,* a more traditional globular macronucleus (Fig. 2d). *Bursaria truncatella* and *Spathidium spatula* also contain elongated and winding macronuclei with multiple micronuclei (Fig. 2f, g). *Dileptus anser* (Fig. 2c) is unusual in that it contains several dispersed macronuclei, totaling up to 500 nodules (Yudin and Uspenskaya 2007). Lastly, *Spirostomum* sp. (Fig. 2i) is an example of a ciliate with a moniliform macronucleus- “beads on string” and many small micronuclei (Kudo 1954).

Though nuclear events and the nature of both somatic macronuclei and germline micronuclei vary among ciliates, some aspects of their life cycles are relatively conserved. For example, following conjugation – a process that includes the exchange of haploid nuclei between cells of different mating types – both macronuclei and micronuclei develop from a zygotic nucleus (Fig. 1). Macronulear development in ciliates also share common features including DNA elimination (i.e. removal of mostly non-protein coding including both intergenic and internally excised sequences) and subsequent chromosome fragmentation (which occurs extensively in the classes Phyllopharyngea, Spirotrichea, Armophorea, and perhaps Litostomatea), and then amplification of the remaining chromosomes. We describe these

processes in greater detail below.

Sex in ciliates occurs through conjugation, during which their micronucleus undergoes meiosis to produce four haploid micronuclei (Fig.1a). Three of the four meiotic products are degraded and then the remaining haploid nucleus divides by mitosis to produce two gametic nuclei (Fig.1b; reviewed in Prescott 1994; Jahn and Klobutcher 2002; McGrath et al. 2006). At this point, one of these haploid nuclei is exchanged between conjugating cells (Fig.1c) after which karyogamy, the fusion of nuclei, forms two identical zygotic nuclei in the parental cytoplasm (Fig.1d). These nuclei undergo mitosis to produce two identical nuclei, one of which will differentiate into a new somatic macronucleus and the other becomes a germline micronucleus (Fig.1e; Raikov 1982; Prescott 1994; McGrath et al. 2006). During macronuclear development, the parental macronucleus degrades and is replaced by the developing macronucleus (Fig.1f), leaving the individual cell with a new macronucleus and micronucleus (Fig.1g).

Macronuclear development consists of three parts: fragmentation of zygotic nuclear chromosomes, elimination of germline-specific sequences, and amplification of the remaining genetic material (details in Fig. 3; Prescott 1994; Morgens et al. 2013; Katz and Kovner 2010; Jiang et al. 2019). Ciliates with extensive fragmentation in their macronuclei contain a large number (e.g. 100,000s or more) of chromosomes that often contain only a single gene (Fig. 4; Meyer and Garnier 2002; Morgens et al. 2013). However, mechanisms of this unusual processing in ciliates have yet to be further elucidated, except within some well-studied genera (e.g. *Paramecium, Tetrahymena,* and *Oxytricha* etc.; Kruger et al. 1982; Brownell et al. 1996; Lozupone et al. 2001; Swart et al. 2016). The past decade has revealed the role of epigenetics in shaping developing macronuclei as 'scan RNAs' (scnRNA) transcribed from the micronuclear genome during the early stages of sexual reproduction play a role during the macronuclear development in ciliates. The scan RNAs, which are exported through from the micronucleus and then through both the parental and new macronucleus, provide a mechanism to distinguish between sequences that will be kept or removed during macronuclear development (Mochizuki and Gorovsky 2004a; Mochizuki and Gorovsky 2004b; Karrer 2012; Rzeszutek et al. 2020; Mochizuki et al. 2002; Allen and Nowacki 2020).

MACRONUCLEAR INSIGHTS ACROSS CILIATE CLASSES

The nature of polyploid macronuclei varies between ciliate species due to differences in the amount of DNA eliminated, fragmentation and amplification during macronuclear development (Jahn and Klobutcher 2002; Jönssen et al. 2009; Chalker et al., 2013). The classes including the Spirotrichea, Armophorea, and Phyllopharyngea are marked by extensive fragmentation following a transient stage with "giant" chromosomes in their somatic macronuclei (Fig. 3; Fig. 4; Ammermann 1974; Zufall and Katz 2007; Gao et al. 2015). Below, we exemplify this diversity within a few classes, focusing on model genera (e.g. *Tetrahymena, Paramecium, Oxytricha)* as appropriate.

Phyllopharyngean ciliates (e.g. *Chilodonella*) contain extensively fragmented genomes and during their nuclear development, DNA-rich and poor stages appear, producing a heteromeric macronucleus (Fig. 3; Fig. 4; Raikov 1996; Maurer-Alcalá and Katz 2016). Macronuclear development in most other Phyllopharyngea remain to be studied with high-powered microscopes and/or genome-scaled data, but the suctoria (e.g. *Acineta* Fig. 2a) are noteworthy in that they reproduce by budding daughter cells that take a micronucleus and a piece of the parental macronucleus (Raikov 1996). The phyllopharyngean ciliate, *Chilodonella*

uncinata contains a large number of scrambled genes and numerous gene families are generated through the alternative processing of germline-specific sequences during macronuclear development (Katz and Kovner 2010; Gao et al. 2014; Gao et al. 2015; Maurer-Alcalá et al. 2018a).

Macronuclei in the class Spirotrichea undergo complex genome rearrangements as the macronucleus develops from a mitotic product of the zygotic nucleus, and the genera *Oxytricha, Stylonychia* and *Euplotes* have all been the focus of numerous studies in past decades (e.g. Rautian 2010; Swart et al. 2013; Yerlici and Landweber 2014; Rzeszutek et al. 2020). Following conjugation in the spirotrich ciliate, *Stylonychia mytilus*, there are three different stages during macronuclear development: initial amplification, a DNA poor stage, and final amplification (Fig. 3). After about 40 hours, DNA content reaches ~2000N, and this highly-polyploid stage is followed by a substantial decline in DNA content leading to the DNA poor stage (Fig. 3; for review Ammermann 1971; Ammermann et al. 1974). Following fragmentation of the somatic chromosomes, another amplification stage occurs again to achieve the final somatic structure (Fig 3). In spirotrichean ciliates (e.g. *Euplotes*, *Oxytricha*, *Stylonychia*) a large portion, around 80-98%, of the sequences in the zygotic nucleus are eliminated in generating the somatic macronucleus (Nowacki et al. 2011; Fang et al. 2012; Swart and Nowacki 2015; Pilling et al. 2017). Another unusual genome feature, gene scrambling (i.e. the non-canonical ordering of protein coding sequences in germline genomes), was first identified in the sprioritrich *Stylonychia* (Prescott and Greslin 1992) and has now been well-studied in several species within Spirotrichea (e.g. Landweber et al. 2000; Möllenbeck et al. 2008).

Macronuclear development in the class Oligohymenophorea has been extensively reviewed elsewhere given the importance of the model ciliates, *Paramecium* and *Tetrahymena* (Prescott 1994; Rzeszutek et al. 2020; Hamilton 2016; Soares 2019; Lepère 2008; Chalker 2008; Fass 2011; Ruehle et al. 2016; Aury 2006; Cheng 2020). In *Paramecium*, for example, macronuclear development includes several rounds of endoreplication to produce up to 800 chromosome copies (Sperling 2011), while *Tetrahymena thermophila* is reported to produce around 181 macronuclear chromosomes, each of which has 45 copies (Eisen 2006; Sheng et al 2020). After chromosome fragmentation, removal of germline-specific sequences and the amplification occurs. Ciliates in the genera *Tetrahymena* and *Paramecium* generate circular and oval shaped macronuclei, respectively (Sperling 2011; Berger 1973; Ruehle et al. 2016; Rzeszutek et al. 2020; Xiong 2019; Gorovsky 1973).

Ciliates in the class Heterotrichea are tremendously diverse with unusual macronuclear development, though ciliates in this class are less-well studied. Nuclear division in heterotrich ciliates relies on extranuclear microtubules, while all other ciliates that have macronuclei capable of diversion use intranuclear microtubules. Heterotrich macronuclei undergo a series of changes in their morphology during development, and contain very small introns (e.g. *Stentor*; Terra 1983; Raikov 1994; Lynn 2008; Shazib et al. 2014; Slabodnick et al. 2017). A recent experimental study by Wancura et al. 2017 reported the ploidy level of macronuclei of the heterotrich ciliate, *Blepharisma* using fluorescent microscopy to be ~1,027 N (Fig. 4; Miyake 1991; Kovaleva 1997; Riley and Katz 2001; Yan 2016).

Developmental programming in the class Karyorelictea is unusual as these ciliates lack the ability to divide their macronuclei (Kovaleva and Raikov 1978; Raikov and Karadzhan 1985; Raikov 1994; Katz 2001; Yan et al. 2017). Most ciliates in this class contain two macronuclei (either spherical or elliptical in shape) and a single micronucleus (mostly circular in shape), or four macronuclear nodules and two micronuclei (Raikov 1982; Lynn 2008; Andreoli

et al. 2009; Yan et al. 2017). Like in other ciliates, macronuclear development in the Karyorelictea proceeds from the zygotic nucleus, but instead of macronuclei dividing during cell division, a new macronucleus must be generated each generation from a mitotic product of the micronucleus. Early research on Karyorelictea suggested there was limited endoreplication of the genome leading to these lineages being described as 'paradiploid'. (Raikov 1957 and 1982). However, recent molecular analyses, based on quantitative PCR, indicate at least some genetic material in the macronucleus is differentially amplified (Maurer-Alcalá et al. 2018b). Distinct to karyorelectids, macronuclei also appear to age as evidenced by changes in morphology and associated DNA content; it is unclear whether these older macronuclei eventually degrade to be replaced by newly-generated macronuclei (Fig. 1; Fig. 4; Raikov 1994; Yan et al. 2017).

Interpreting features of macronuclear development across the ciliate tree of life highlights the variability within this >1 billion-year-old clade (Fig. 4). For example, giant chromosomes, extensive fragmentation and gene scrambling co-occur in the non-sister classes Spirotrichea, Phyllopharyngea and Armophorea (Pyne 1978; Ammermann 1987; Juranek et al. 2005; Postberg et al. 2008); at least some chromosomes are extensively-fragmented within the less well-studied class Litostomatea (Maurer-Alcalá et al. 2018b; Balbiani 1890). Though most ciliates have homomeric macronuclei (i.e. DNA evenly spread, 'homo' Fig. 4), some members of the class Phyllopharyngea have heteromeric macronuclei with DNA poor centers ('hetero'; Fig. 4). Other exceptions include the appearance of a 'replication band' during DNA synthesis in some members of the class Spirotrichea, and the inability of macronuclei to divide in the class Karyorelictea (Fig. 4).

CONCLUSION

Nuclear number, size and structure varies tremendously across the cilite tree of life (Fig. 4), as do the mechanisms underlying the development of somatic macronuclei. Synthesis of the data presented here highlights the plasticity in these features among ciliates and points to areas for future study (i.e. investigation of the presence of gene scrambling and level of genome amplification among the many poorly-studied ciliates classes). Ciliates serve as models for studies of genome evolution, and thus discussion of their nuclear features furthers our understanding of evolution across the eukaryotic tree of life.

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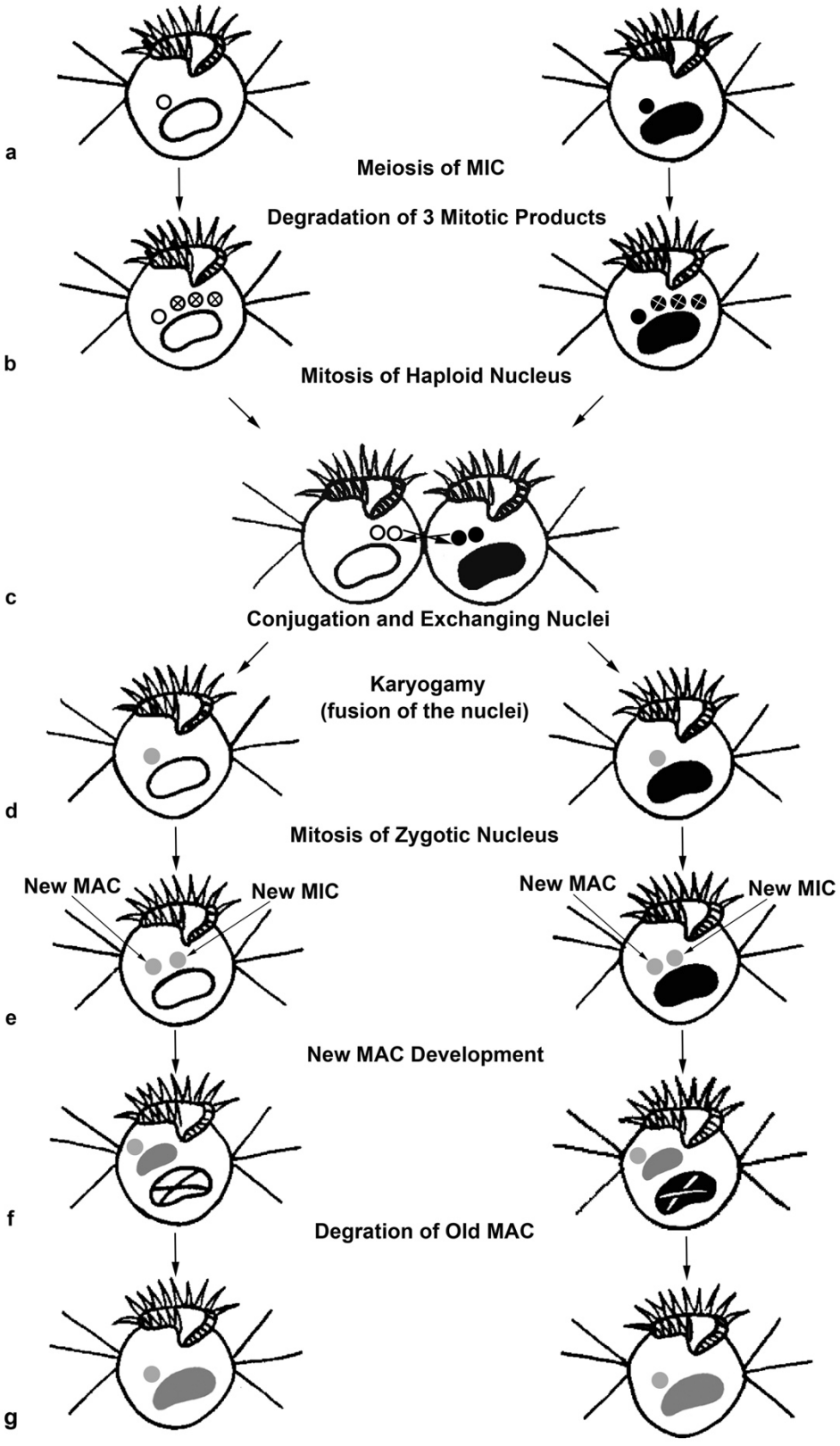
FIGURE LEGENDS

Figure 1. Typified ciliate life cycle and the nuclear events using *Halteria* morphospecies- in preparation for the conjugation process (a), the micronucleus undergoes meiosis cell division. As a result, four gametic nuclei are formed (haploid), three of which degrade while one remains to generate two micronuclei by the mitosis cell division (b). Remaining gametic nucleus is exchanged by each individual to produce a zygotic nucleus (c). Finally, zygotic nuclei generate a mature macronuclei and micronuclei in the individual cells via mitotic division, the new nucleus develops and old nucleus degrades, and becomes a mature individual (d-g). **MAC.** Macronuclei, **MIC.** Micronuclei. Note: the overall size of *Halteria* species varies between 10-30 μm.

Figure 2. Polyploid macronuclear structure in different ciliate groups (a - i). **a.** *Acineta tuberosa*; **b.** *Paramecium caudatum*; **c.** *Dileptus anser*; **d.** *Stylonychia mytilus*; **e.** *Euplotidium* sp.; **f.** *Bursaria truncatella*; **g.** *Spathidium spatula*; **h.** *Didinium nasutum*; **i.** *Spirostomum* sp.. Scale bars- a = 15 μm; b,g = 70 μm; c,h = 100 μm; d = 100 μm; e,f = 70; i = 150 μm (reviewed in Raikov 1982).

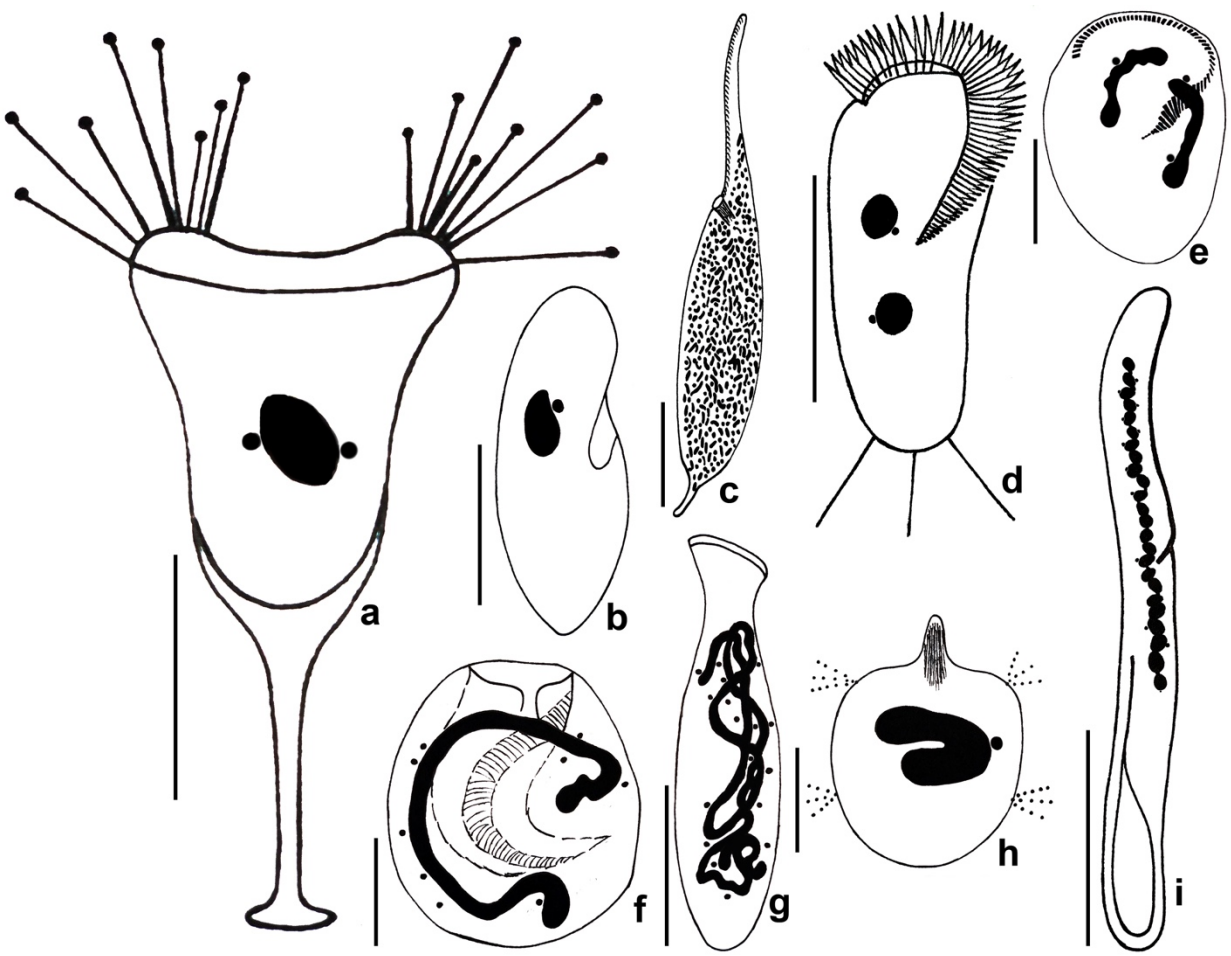
Figure 3. DNA content depicted during macronucleus development in spirotrich ciliates, reused from Ammermann et al. 1974.

Figure 4. Ciliate groups denoting the presence or absence of extensive fragmentation and large sized chromosomes in the macronucleus (Ammermann 1974; Gustav Fischer 1996; Riley and Katz 2001; Yan et al. 2017; Maurer-Alcalá et al. 2018b). **Homo.** Homomeric, **Hetero.** Heteromeric, **Rep. Band.** Replication Band, **Non-div.** Non-dividing. **?.** Questionable/No Clear Record.


a
Meiosis of MIC
Degradation of 3 Mitotic Products
b
Mitosis of Haploid Nucleus
c
Conjugation and Exchanging Nuclei
Karyogamy
(fusion of the nuclei)
d
Mitosis of Zygotic Nucleus
New MAC
New MIC
New MAC
New MIC
e
New MAC Development
f
Degration of Old MAC
g
Individual Cell with Newly Formed MAC and MIC

482

483



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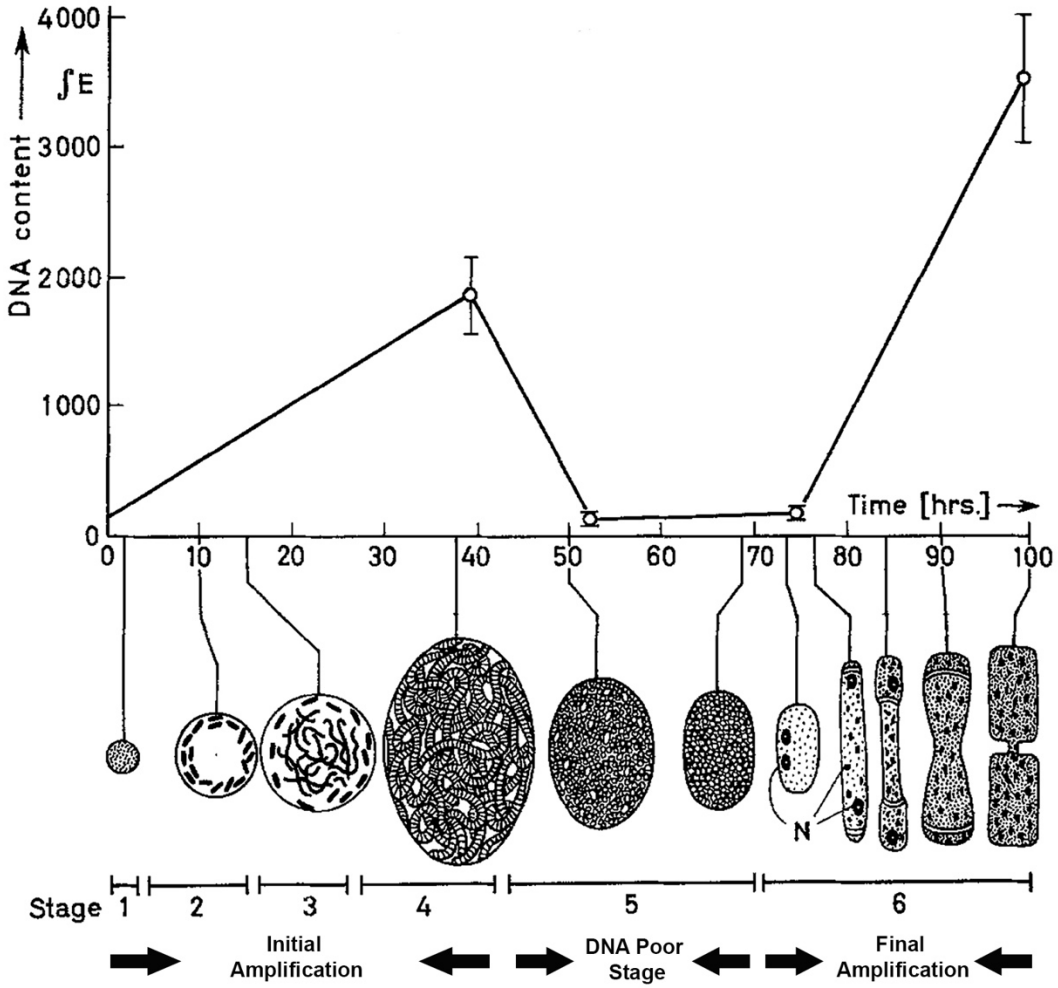
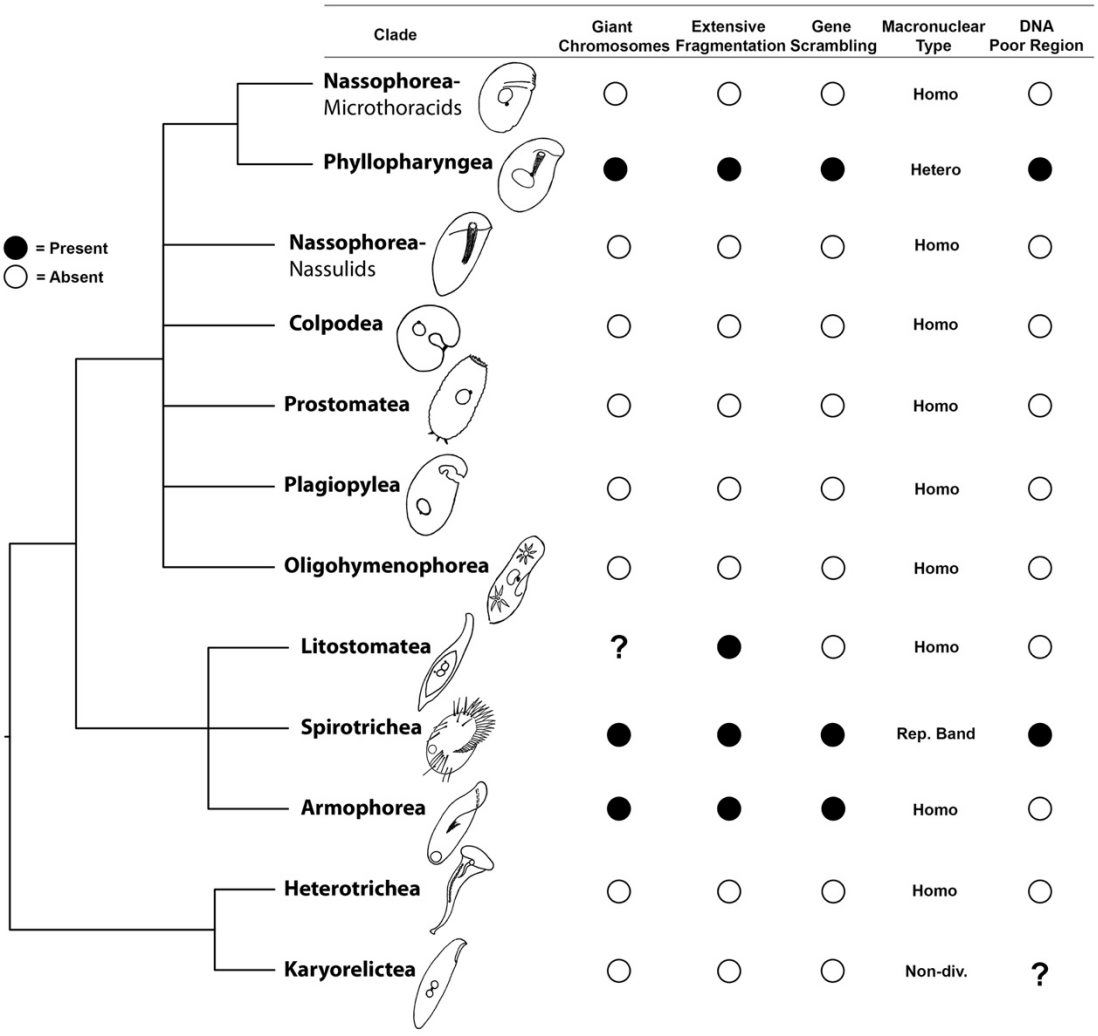

DNA content
4000
∫E
3000
2000
1000
0
Time [hrs.]
0
10
20
30
40
50
60
70
80
90
100
N
Stage 1
2
3
4
5
6
Initial Amplification
DNA Poor Stage
Final Amplification

486
487


Clade
Giant Chromosomes
Extensive Fragmentation
Gene Scrambling
Macronuclear Type
DNA Poor Region
Nassophorea- Microthoracids
Phyllopharyngea
Nassophorea- Nassulids
Colpodea
Prostomatea
Plagiopylea
Oligohymenophorea
Litostomatea
Spirotrichea
Armophorea
Heterotrichea
Karyorelictea
Homo
Hetero
Rep. Band
Non-div.
?
= Present
= Absent

488