Protist, Vol. 166, 224–233, May 2015
http://www.elsevier.de/protis
Published online date 12 March 2015

Protist

ORIGINAL PAPER

Amitotic Chromosome Loss Predicts Distinct Patterns of Senescence and Non-Senescence in Ciliates

David W. Morgens[1], and **Andre R.O. Cavalcanti**[2]

Biology Department, Pomona College, 175 W 6th Street, Claremont, CA 91711, USA

Submitted August 27, 2014; Accepted March 2, 2015
Monitoring Editor: Joseph Frankel

Over time and repeated asexual divisions, many ciliate species display the characteristics of senescence, reduced fecundity and increased mortality. Their only path to recovery is sexual conjugation or autogamy. While more traditional models of cellular aging have been proposed, one of the most accepted explanations relies on the faulty mechanism by which ciliates duplicate their somatic nucleus, a process referred to as amitosis. Amitosis involves the random segregation of chromosomes with no consideration for homology. Over subsequent divisions, chromosome copy numbers will fluctuate until an entire chromosome is lost, resulting in death. Via simulations of this process, we find that senescence and death via chromosome loss is not the only possible result of amitosis. Random chromosome loss is less damaging to populations than previously thought, and strict adherence to the model predicts that *Paramecium tetraurelia* would not senesce. A combination of the reciprocal nature of amitosis and lethal selection against low-copy number chromosomes is responsible for this startling prediction. Additionally, our results provide an alternate explanation to recent evidence for selection on chromosome copy number in *Tetrahymena thermophila* and peculiar patterns of senescence in *Tetrahymena pyriformis*.

Key words: Amitosis; ciliates; clonal aging.

Introduction

Ciliates are unicellular eukaryotes characterized by the presence of cilia and nuclear dimorphism. Each ciliate cell possesses two distinct types of nuclei, a transcriptionally silent germline micronucleus (MIC) and a transcriptionally active somatic macronucleus (MAC). Ciliate cells alternate between sexual conjugation and periods of asexual division, referred to as vegetative growth.

The genomic organization varies widely between the MIC and the MAC and between different ciliate species (Prescott 1994). The MIC resembles the nucleus of other eukaryotes – diploid and containing several large chromosomes, while the MAC is highly polyploid with an expanded number of shorter chromosomes. During vegetative growth the MIC divides through mitosis, and the MAC divides through an alternative and poorly understood process known as amitosis. Amitosis involves the random assortment of individual chromosomes

[1]Present Address: Department of Genetics, Stanford University, 300 Pasteur Drive, Stanford, CA 94305, USA
[2]Corresponding author; fax +1-909-621-8878
e-mail andrecaval@gmail.com (A.R.O. Cavalcanti).

http://dx.doi.org/10.1016/j.protis.2015.03.002

to the daughter cells, without consideration for homology. Thus after a round of amitosis, the copy number of a given chromosome in the MAC may vary between the daughter cells, and such variation can be amplified by successive amitotic divisions. Amitosis only occurs during asexual division; during sexual conjugation, the MAC is destroyed, and a new one is created from the MIC, potentially correcting fluctuations in chromosome copy numbers (Prescott 1994).

In this stochastic model of amitosis, all copies of a certain chromosome can be inherited by a single daughter cell, leaving the sister cell without any copies of the genes contained in the chromosome. The loss of an entire chromosome in this manner would likely be lethal. The highly polyploid nature of the MAC can reduce the chance of such an occurrence, as it is less likely that every copy of a given chromosome will be lost for higher copy numbers (Duerr et al. 2004; Kimura 1957). Even if the loss of a given chromosome is not lethal, analysis can be limited to only those chromosomes whose loss is lethal, e.g. containing an essential gene.

Ploidy in the MAC varies widely in ciliates: While *Tetrahymena thermophila* contains 45 copies of each macronuclear chromosome (Eisen et al. 2006), in ciliates of the class Spirotrichea individual macronuclear chromosomes are present in about 1,000 to 15,000 copies depending on the species (Klobutcher and Herrick 1997; Riley and Katz 2001), with *Oxytricha trifallax*, perhaps the most well characterized species in this class, having ~1,900 copies of each macronuclear chromosome (Swart et al. 2013). This extreme organization is present in other ciliate lineages as well and may have evolved to avoid the harmful consequences of amitosis (Morgens et al. 2013; Riley and Katz 2001).

While the concept of aging in a single-celled organism may be foreign, clonal senescence is a well-studied phenomenon among organisms that engage in both asexual and sexual reproduction (Pedersen 1995). In many ciliate species, if lineages are propagated asexually, they will senesce, resulting in both decreased rates of growth and the eventual collapse of the lineage (Adl and Berger 2000; Bell 1988; Brito et al. 2010; Duerr et al. 2004; Komori et al. 2004; Sonneborn 1954; Sung et al. 2012). Senescence is widespread in ciliates, but it is important to note that some species of ciliates do not seem to senesce, for example *Tetrahymena pyriformis* has been maintained asexually since the 1920s (Nanney 1974).

Senescence has been shown in *Paramecium tetraurelia* to result from changes in the MAC. Injection of the MAC of a young cell into an old cell prolonged the proliferation of the recipient cell, while the injection of a MAC of an old cell into a young cell led to premature aging of the recipient cell (Aufderheide 1986). Similar studies transplanting cytoplasmic material failed to detect any change in the life span of the recipient cells (Aufderheide 1984). This effect of the MAC on aging is commonly attributed to the loss of chromosomes via amitosis (Bell 1988; Duerr et al. 2004; Kimura 1957). Here we will focus exclusively on the consequences of this hypothesis, where chromosome copy imbalances or low copy number have no fitness effect, and ciliates die only when a chromosome is lost.

These assumptions have been explored analytically over fifty years ago by Kimura (1957). He derived an equation predicting the probability that a cell will not completely lose any chromosome and thus will survive, given a number of successive amitotic generations:

$$P = \left(1 - \exp\left(-\frac{4X}{G+4}\right)\right)^N$$

where X is the number of copies of each chromosome, G is the number of generations, and N is the number of different chromosome types. The derivation of the equation is based on diffusion processes and models a specific type of experiment where a single ciliate is allowed to divide, and only one daughter is chosen randomly and isolated to become the parent cell in the next generation. The process continues until the currently selected daughter cell dies, which Kimura equated to chromosome loss in the model. The equation was subsequently validated by Duerr et al. (2004) using simulations.

Kimura's model does not take into account many population level effects that may be relevant. One such possibility suggests that there are fitness effects associated with fluctuating or low copy numbers of chromosomes, and selection could act to slow or even annul the effects of amitosis on senescence (Bell 1988; Birchler and Veitia 2007; Brito et al. 2010; Spring et al. 2013). Recent experimental evidence in populations of ciliates demonstrated that senescence was sensitive to effective population size, supporting a role of selection (Brito et al. 2010). An additional possibility concerns the reciprocal nature of amitosis, namely that when one daughter cell does not inherit a specific chromosome, the sister cell must inherit it. As a population, this may make ciliates more robust to amitosis than Kimura's equation would predict, even without invoking selection on copy numbers.