A mathematical model of kinetoplastid mitochondrial gene scrambling advantage

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Abstract

We model and discuss advantages of pan-editing, the complex way of expressing mitochondrial genes in kinetoplastids. The rapid spread and preservation of pan-editing seems to be due to its concomitant fragment dispersal. Such dispersal prevents losing temporarily non expressed mitochondrial genes upon intense intraspecific competition, by linking non expressed fragments to parts which are still needed. We mathematically modelled protection against gene loss, due to the absence of selection, by this kind of fragment association. This gives ranges of values for parameters like scrambling extent, population size, and number of generations still retaining full genomes despite limited selection. Values obtained seem consistent with those observed. We find a quasi-linear correlation between dispersal and number of generations after which populations lose genes, showing that pan-editing can be selected to effectively limit gene loss under relaxed selective pressure.

1 Introduction

Gene fragmentation and dispersal of fragments are found in various organisms ranging from Euglenozoa (found in kinetoplastids, euglenoids, and diplonemids) to Alveolates (found in apicomplexa, ciliates, and dinoflagellates). See (Benne et al., 1986; Gillespie et al., 1999; Marande and Burger, 2007; Kamikawa et al., 2007; Nowacki et al., 2008; Walker, 2007; Spencer and Gray, 2011). Gene fragmentation occurs when the RNA is not made from a single precursor, derived directly from the genome, but it is reconstituted from different RNA sources. This can also happen without dispersal, i.e. spreading the genes for these RNA parts over the genome, intermingled with other genes.

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RNA editing, first used to refer to RNA alteration processes in trypanosomatids, entailing extreme mitochondrial (mt) gene fragment dissemination, was discovered in 1986 (Benne et al., 1986). Trypanosomatids are parasitic unicellular organisms belonging to the kinetoplastid order. Kinetoplastids are characterized by a 'kinetoplast', the strongly staining location of all the mt DNA molecules within their single mitochondrion. Kinetoplastid RNA editing refers to post-transcriptional sequence alteration via insertion and deletion of uridylate residues at specific sites of mt RNAs.

Trypanosomal mt RNAs are encoded by two types of mt DNA molecules: several thousand small minicircles and a few dozen maxicircles (Scott, 1995; Simpson and Thiemann, 1995; Simpson et al., 2000; Madison-Antenucci et al., 2002). Maxicircles contain ribosomal RNA genes and genes mostly encoding subunits of respiratory chain complexes. Many subunit genes need editing upon transcription. Both kind of circles encode guide (g) RNAs necessary for editing of maxicircle encoded RNAs (Blum et al., 1990).

Very extensive editing is illustrated by the *Trypanosoma brucei* cox3 gene. Cox3 is easily found (e.g. by looking at homologies) in *Crithidia fasciculata* and *Leishmania tarentolae*: the transcript undergoes limited editing (e.g. in C. fasciculata only 32 Us are inserted and 2 Us deleted at 14 sites).

The *T. brucei* cox3 'gene' was identified later: its transcript has to be edited overall (Feagin *et al.*, 1988). While the G, A and C nucleotide sequence is maxicircle derived, the U nucleotide sequence is generated by editing, using minicircle encoded gRNAs, with 547 Us inserted and 41 Us deleted at 223 sites. The transcript derived from the 'GAC sequence' (called the 'cryptogene' (Simpson and Shaw, 1989)) has less than half the edited size.

In *T. brucei*, most of the mt encoded proteins come from such cryptogenes (9 out of 17, with 5 unedited and 3 partially edited transcripts remaining). The extensive editing of cryptogene transcripts is known as pan-editing (Simpson and Shaw, 1989; Sturm and Simpson, 1990). The precise molecular mechanisms of editing, using sequential basepairing between gRNAs and mRNA, are described in (Schnaufer *et al.*, 2003) and references therein (Madison-Antenucci *et al.*, 2002; Blum *et al.*, 1990; Feagin *et al.*, 1988; Simpson and Shaw, 1989). The consequence of pan-editing is that information necessary for production of (some) proteins is spread and mixed over the entire mt DNA.

Here, we propose a mathematical model to support the concept (Speijer, 2006; Speijer, 2007) that such gene fragment dispersal can function as a biological adaptation preventing gene loss caused by intense intraspecific competition, due to selection on growth rate in limited spaces (e.g. a host). The process lowers the chance that rapidly growing deletion mutants replace all the more complex, ecologically versatile, organisms, as large deletions will contain linked 'active' segments. It thus works to 'preserve by association'. To put it another way: mt gene scattering prevents the translation of short-term advantages into long-term disaster.

2 Modelling the effects of mitochondrial gene fragment dispersal in changing environments

Our model describes the 'life cycle' of the mt genetic material of a trypanosomatid. In the absence of functional selection we do not distinguish between competition at the levels of individual mt genomes (e.g. maxicircles) and whole organisms. At the molecular level small circles outcompete larger circles by faster replication while at the level of the organism, organisms carrying less mt DNA can also grow faster. At both levels mt deletions will be selected then: they even reinforce each other. We need to include the following parameters to determine the relationship between some of them:

- 1. Population size (s_p) , 10^{10} individuals;
- 2. Deletion size (l);
- 3. Probability of a deletion (p), 10^{-6} ;
- 4. Level of fragmentation/editing (k);
- 5. Number of generations in each environment (in the case of parasites 'host');
- 6. Replication advantage of smaller DNA (r(x));
- 7. Confidence parameter (d) that governs the level of approximation in our second simulation.

As mitochondrial DNA polymerases are much more error-prone than their nuclear counterparts (see (Larsson, 2010) and references therein), a value of 10^{-6} for the deletion probability is not unrealistic. We first model the process of replication and deletion in its most general form, and then simplify it, to enable us to simulate for realistic values of our parameters. In all our simulations, we assume there are two environments, A and B, e.g. A, tsetse fly and B, human host. A sequence 'a' of base pairs of length n_a is necessary for survival in A and a sequence 'b' of length n_b is needed in B, but not in A. For simplicity we excluded sequences needed in both (violet segments in Figure 1). Total length of mt DNA will therefore be $n = n_a + n_b$ base pairs. We chose n_a to represent 65% and n_b 35% of total mt DNA (~ 23000 basepairs). We assume the parasite to be in environment A.

The level of mt DNA fragmentation for an individual parasite is simply modelled as follows. The fragmentation and spreading that pan-editing of RNA entails, intertwines subsequences of a and b modelled by the sequence $a_1b_1a_2b_2...a_kb_k$. We assume the DNA and hence this sequence to be circular, so that b_k connects with a_1 , and we call k-1 the level of fragmentation. Initially, length of subsequence a_i is n_a/k and length of subsequence b_i is n_b/k . We model replication rounds, in which for every round, with probability p, deletion of DNA with random length l, at a random position, may occur. Any deletion in an 'a'

sequence is lethal. Surviving individuals replicate such, that the smaller their total DNA, the faster they replicate. This advantage (given as an increased representation in the next round) is modelled by a function r(x). For example, a complete deletion of 35% (n_b) of the DNA will translate in a 3/2 stronger representation in the next generation.

The process is modelled using a Markov chain approach, with simplifying assumptions described in the appendix. We study the value $t_{\text{max}}(k)$ monitoring the expected number of generations until no individuals are left with 'b' of length n_b . Any individual, missing part of b, can not survive in the 'next' environment (B) anymore. The increase of $t_{\text{max}}(k)$ gives us the added protection that additional fragmentation (k) brings. At level k we have $(n_k/k+1)k$ many states in our Markov chain (see appendix). Realistically, n_b is set around 8000 base pairs and k can be over 200, making the Markov chain too big to handle.

By exploiting symmetries and simplifying our model we approximate our Markov chain, obtaining a manageable model that we can simulate for realistic values of n_b and k. An additional feature of this approximation is parameter d, the confidence level, which allows us to smoothly interpolate between the simplified and the original Markov chain. Varying d suggests that already for small values we obtain accurate approximations of the original process. See appendix and Figure 2.

3 Results obtained by modelling the effects of increasing mitochondrial gene fragment dispersal

In a first simulation (see Figure 1B) we decided to compare our highly simplified genome with and without a fragmented gene (k=2) under partially relaxed selection (when only part of the genome is needed) with concomitant strong (intraspecific) competition. We simulated a modest (e.g. in the bloodstream much less of the mt genome is needed) situation in which most (2/3) of the mt DNA is still necessary for survival (environment A: 'tsetse'). Starting with 10^{10} individuals, we look at the number of generations needed to reduce the amount of individual organisms that have retained 'ecological flexibility' (i.e. they still contain all genetic information to be able to make the switch to another part of the life cycle) to 1. Without any fragmentation: after 108 generations no organism has the possibility to switch to the next stage of the life cycle. With 1 split: no such individual is left after 156 generations (values obtained starting with 108 individuals: 96 and 146 respectively; with 10^{12} individuals: 119 and 169). See the table below:

		Population size	
k	10^{8}	10^{10}	10^{12}
1 (no split)	96	108	119
2 (one split)	146	156	169

The allowed generation time under periods of partially relaxed selective pressure has been extended by more than 40%. We next altered the 'simple' simulation by using a fixed deletion size for gene fragments (instead of allowing the full range of sizes of our previous model) while at the same time making it more informative by varying the amount of gene fragmentation (k, see Figure 1C). This (full Markov chain) simulation gave a surprising result: we obtained a direct, quasi-linear correlation between the degree of dispersal/fragmentation (k) and the number of generations after which a complete population loses ecological competence (see Figure 2). In other words, the function $t_{\text{max}}(k)$ has an almost linear growth rate.

4 Comparing the model to values observed

A triatomine bug (the insect vector used by $Trypanosoma\ cruzi$) will contain on average 105 parasites (Kollien and Schaub, 1998); while a bite with the tsetse fly will transmit $0-40,000\ (mean, \sim 3,000)\ T.\ brucei$ infective metacyclics, giving rise to over 10^{10} parasites in a Vervet monkey ($Chlorocebus\ pygerythrus$) at peak infection ((Thuita $et\ al., 2008$) and references therein). We chose populations of 10^8 , 10^{10} and 10^{12} parasites in our simple modelling (see the table) and 10^{10} for our full Markov chain approach. Under the parameter values of our model the size of the population does not seem of major importance (cf the table).

We chose to model $\sim 65\%$ of the total mt DNA in use, and $\sim 35\%$ free from functional selection (for trypanosomatids in mammalian hosts, much less mt DNA seems essential). Doubling time in mammals can be 4.5 hours if unimpeded by immune responses, while infections can be sustained for months. Thus, our model should look at effects over many generations. In our basic approach we compare only two instances ('no split' and 'only one split'), but allow all possible deletion sizes. In the full Markov chain approach we use an approximation (depending on d) allowing us to model random deletions of varying sizes.

Because we have modelled optimal dissemination (always strictly intermingling constitutively used and conditionally used regions), we tentatively infer that the fragmentation values of Figure 2 are relevant below 150. Recall that the *complete T. brucei* required set of gRNAs for *all* lifecycles is about 150 (Hong and Simpson, 2003).

Surprisingly, only 77 rapid passages (at least 600 generations) in mice of tsetse fly infective *T. brucei* already gave rise to a homogeneous population of parasites that could no longer infect the insect and did not display mt (oligomycin sensitive) ATPase activity anymore (Hajduk and Vickerman, 1981), demonstrating rapid takeover by mt DNA mutants. These mutants in the end indeed *divided more rapidly* than the wildtype in blood but did not contain large scale mt deletions. This could of course be explained by the fact that certain parts of the present-day highly dispersed mt genome are still necessary and thus protect against large scale deletions under these circumstances, as described by our model of selection for linkage.

Takeover by deletion mutants could thus have been even faster, as mt replication times are possibly limiting when doubling every 4.5 hrs. This also means (cf. Figure 2) that at a fragmentation level of between ~ 10 and 20, deletion mutants would contribute about as much to depletion of the wt population as all other inactivating mutations (> 600 generations). For $T.\ brucei$ we would expect a fragmentation level of > 20 and << 150, which seems realistic. However, all $T.\ brucei$ parameter values are very difficult to estimate in real life, because:

- 1. It is not known precisely how little mt DNA is essential in the mammalian host,
- 2. Population sizes and generation time estimates are complicated by waves of parasitemia, reflecting the immune response to alternating variant surface glycoprotein expression. Very limited mt function and repeating population bottlenecks could explain why *T. brucei* has the most extensive editing observed (see below).

A further conclusion, shedding light on another point of contention (Landweber, 2007; Speijer, 2008): physical linkage of all mt genes is not essential for the model to work. This aspect is important when considering the cox1 gene in the parasitic diplonemid *Diplonema* papillatum apparently split up in \sim 250-bp fragments, located on individual unlinked DNA circles (Marande et al., 2005) and the existence of minicircles with only one gRNA in certain trypanosomatids (though often physically linked).

As the modelling makes clear, it is only reducing the chance of large deletions giving replication advantages which is essential. Losing a substantial fraction of -one gRNA encoding- minicircles (a 'large' deletion) will still compromise viability directly, regardless of *physical* linkage. Networks themselves did probably evolve to make takeover by (deletion) mutants even less likely (Borst, 1991).

5 Evolution of RNA editing: gene fragment dispersal to counter gene loss?

We model fragment dissemination as advantageous to parasites (Speijer, 2006; Speijer, 2007), but others defend 'neutral' models (such as 'Constructive Neutral Evolution' (Covello and Gray, 1993; Gray et al., 2010)); see (Lukes et al., 2009; Speijer, 2010; Lukes et al., 2011; Flegontov et al., 2011; Speijer, 2011). Parasitism is not essential for our model to function: any free-living ancestor in periodically changing environments adapting its mt function could favour mt gene fragmentation when encountering strong intraspecific competition.

Quickly changing environments could also be at the basis of the glycosome (Hannaert et al., 2003; Gualdron-Lopez et al., 2012). Rapidly changing oxygen levels would lead to either aerobiosis with mt respiration or anaerobiosis with glycosomal activity for energy generation in non-parasitic ancestors. For these ancestors, the lack of selective pressure

on the mt genome has been invoked as the raison d'etre for RNA editing before (Cavalier-Smith, 1997).

The fact that novel kinetoplastids and diplonemids have been found in anoxic deepsea basins recently, make such an ancestor more likely (Lara et al., 2009; Edgcomb et al., 2011). Indeed, despite corrections to the kinetoplastid phylogenetic tree (Moreira et al., 2004; Katz et al., 2012), pan-editing is still seen as ancestral, originating 500 to 700 million years ago (Lukes et al., 1994; Fernandes et al., 1993). Kinetoplastids are part of the Euglenozoa (with diplonemids and euglenoids). Interestingly, another form of mt gene fragmentation occurs in the diplonemid D. papillatum (Marande et al., 2005; Marande and Burger, 2007), as described above.

Transcript editing has become less extensive in more recently evolved species (e.g. compare cox3 editing in L. tarentolae and C. fasciculata with its pan-editing in T. brucei). Editing loss results from reverse transcription of (almost completely) edited RNA followed by homologous recombination of the cDNA with mt DNA. The 5' and 3' homology requirements of the cDNA would sometimes result in a need for 5' editing of the 'new' transcript encoded by the mt DNA upon recombination. This is actually seen in cox3 and cytb in L. tarentolae and C. fasciculata (Landweber, 1992).

Another nice example of recent editing domain length reduction during cryptogene evolution is found in the ND8 gene of three related insect trypanosomatids, again strongly suggestive of correlations between life cycle complexity and editing extent (see below; Gerasimov et al., 2012). Pan-editing entails a lot of extra complexity and energetic costs, so losing it is indeed what one would indeed expect, as soon as reduction of life cycle complexity allows it.

6 Pan-editing counters gene loss

In 1993 Covello and Gray (Covello and Gray, 1993) introduced a general model for the evolution of different RNA editing forms. RNA editing activity is first acquired by (a combination of) pre-existing enzymes. Mutations at 'editable' nucleotide positions in the genome occur next. Later on, editing becomes essential after fixation by a chance process in which an altered form replaces the original without a selective advantage. In the case of kinetoplastid editing this model could in principle explain the emergence of a few 'limited' editing instances, but it is very hard to envisage how it explains the rapid acquisition of multiple instances of pan-editing with hundreds of gRNAs.

This model does not identify selective pressure(s) responsible for an *active* increase of editing potential. This pressure was postulated to reside in the fact that gene scattering could protect against loss of temporarily non expressed mt genes during periods of intense intraspecific competition (Speijer, 2006), by warding off large deletion mutants outcompeting wildtype kinetoplastids (see above and Figure 1A). Thus, ecological flexibility is retained, allowing kinetoplastids to occupy highly diverse (parasitic) niches and undergo

extensive speciation (cf. the repeated evolution of *T. brucei* strains that can not infect their insect vectors anymore (Lun *et al.*, 2010)).

Pan-editing seems an effective way of making large deletions improbable. It necessitates presence of *all* cognate small gRNAs (containing information for the U sequence) to express a cryptogene (encoding the 'GAC sequence'). These 'gene fragments' colonize mt DNA so that every random large scale deletion will now contain some information still under selective pressure (Speijer, 2006; Speijer, 2007). The following observations fit our model.

- 1. Especially transcripts encoding gene products which are crucial in all life cycle stages should be prone to pan-editing (such as ATP6 and RPS12, encoding components of the *T. brucei* mt F1Fo ATPase and ribosome respectively). It is exactly because they are always required that fragmenting their genes with concomitant spread of cognate gRNA genes in mt DNA leads to efficient 'mt DNA integrity checkpoints'.
- 2. The role of gRNAs as such mt DNA integrity checkpoints can also be performed by evenly distributed tRNA genes. Compare e.g. tRNA genes in human mt DNA (Anderson et al., 1981) and E. coli's multiple rRNA operons distributed in such fashion (Blattner et al., 1997). However, exceptionally, all trypanosomatid mt tRNA genes are absent, mt tRNAs coming from the cytoplasm (Hancock and Hajduk, 1990).
- 3. There seems to be a clear correlation between life cycle complexity and amount of pan-editing, *i.e.* gene fragment dissemination (see above and *e.g.* (Gerasimov *et al.*, 2012). *T. brucei*, with its highly complex life cycle, still encodes a COX III cryptogene, much reduced in length (see above), so its loss in the mammalian host would be less advantageous. Much more importantly, its dispersed gRNA segments protect parts (encoding NADH dehydrogenase subunits) of the genome not used in the fly (Speijer, 2006; Speijer, 2007). Thus, gene fragmentation implicit in mt RNA editing can be seen as an instance of a general tendency to 'evenly' distribute genetic information over the genome. In this way large scale deletion of pieces released from selection becomes impossible, no longer threatening ecological flexibility.
- 4. Our modelling demonstrates that the number of generations a population retains individuals with a full mt genome complement increases quasi-linearly with increases in RNA editing (i.e. with its concomittant gene scrambling). It thus allows positive selection of small, incremental increases of editing potential under the appropriate circumstances (e.g. less frequent environment-host exchange).

Constructive Neutral Evolution, ignoring 'evolvability' aspects tries to explain panediting as resulting from genetic drift and population bottlenecks only, but our model explains both its rapid spread and current patterns. These patterns correlate not only with a history of population bottlenecks, but specifically with alternating selection pressures on mt DNA (Speijer, 2006; Gray et al., 2010; Lukes et al., 2011; Flegontov et al., 2011; Speijer, 2011).

7 Kinetoplastid mitochondrial genome evolution

Every time kinetoplastids readjust to different environments, founder effects can occur. The observed rapid speciation and development of unexpected, 'weird' biochemical properties could then be a natural result. Such founder effects might explain acquisition of (limited) RNA mt editing/gene fragmentation in Euglenozoa (Walker, 2007) in the first place. The kind of selection described here would then quickly give pan-editing in lineages with alternating mitochondrial demands.

Present-day kinetoplastids show an enormous diversity in mt DNA: maxicircles of up to 200 kb, gRNA encoding minicircles of 1-3 kb with only one to several gRNA genes, and 200 kb circles possibly encoding hundreds (Simpson et al., 2000; Maslov and Simpson, 1994; Blom et al., 2000). Minicircles clearly evolved later, possibly in response to coding capacity demands of pan-editing. This increased mt DNA size and led to networks combating minicircle loss (Borst, 1991). As mentioned, over time editing becomes less extensive in some kinetoplastids (Landweber, 1992; Gerasimov et al., 2012), reflecting the complexity and overall burden of the elaborate editing system.

8 Conclusion

We have modelled mt gene scrambling, matching it with observations regarding the frequency of pan-editing and the ecology and population sizes of trypanosomatids in which it occurs. Important modelling parameters include: the population size, the deletion size (as a percentage of total genome size), the probability of a deletion in each new generation and the replication advantage value for a deletion. The outcome for every simulation was the number of generations after which no viable individual was left. We took the T. brucei lifecycle to deduce fixed values, only varying population size and amount of gene fragmentation. Population size did not seem a major determinant. Our output correlated extent of fragmentation/dispersal and increase in number of generations retaining ecological competence, which turned out to be a quasi-linear function. The 'fragmentation value' of T. brucei mt DNA seems to be in the range suppressing large scale deletion population takeover. To prevent such takeover, condemning parasites to die with their host, a gene architecture of tremendous complexity evolved. It links survival of genes released from selection pressure to survival of genes still under such control. Thus, while fragmentation reduces efficiency it seems to enhance long term ecological success. We are currently looking at gene fragmentation of *Plasmodium falciparum* (Feagin et al., 2012) and ciliates (Nowacki et al., 2011) in light of this mechanism, with only the malaria parasite possibly fitting the mould.

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References

- [1] Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J., Staden, R., and Young, I. G. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457-465.
- [2] Benne, R., van den Burg, J., Brakenhoff, J. P., Sloof, P., Van Boom, J. H., and Tromp, M. C. 1986. Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. Cell 46:819-826.
- [3] Blattner, F. R., Plunkett, G., III, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. 1997. The complete genome sequence of Escherichia coli K-12. Science 277:1453-1462.
- [4] Blom, D., de, H. A., van den Burg, J., van den Berg, M., Sloof, P., Jirku, M., Lukeš, J., and Benne, R. 2000. Mitochondrial minicircles in the free-living bodonid Bodo saltans contain two gRNA gene cassettes and are not found in large networks. RNA. 6:121-135.
- [5] Blum, B., Bakalara, N., and Simpson, L. 1990. A model for RNA editing in kinetoplastid mitochondria: "guide" RNA molecules transcribed from maxicircle DNA provide the edited information. Cell 60:189-198.
- [6] Borst, P. 1991. Why kinetoplast DNA networks? Trends Genet. 7:139-141.
- [7] Cavalier-Smith, T. 1997. Cell and genome coevolution: facultative anaerobiosis, glycosomes and kinetoplastan RNA editing. Trends Genet. 13:6-9.
- [8] Covello, P. S., and Gray, M. W. 1993. On the evolution of RNA editing. Trends Genet. 9:265-268.
- [9] Edgcomb, V. P., Orsi, W., Breiner, H. W., Stock, A., Filker, S., Yakimov, M. M., and Stoeck, T. 2011. Novel active kinetoplastids associated with hypersaline anoxic basins in the Eastern Mediterranean deep-sea. Deep Sea Research Part I: Oceanographic Research Papers 58:1040-1048.

- [10] Feagin, J. E., Abraham, J. M., and Stuart, K. 1988. Extensive editing of the cytochrome c oxidase III transcript in Trypanosoma brucei. Cell 53:413-422.
- [11] Fernandes, A. P., Nelson, K., and Beverley, S. M. 1993. Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: perspectives on the age and origins of parasitism. Proc. Natl. Acad. Sci. U. S. A 90:11608-11612.
- [12] Flegontov, P., Gray, M. W., Burger, G., and Lukeš, J. 2011. Gene fragmentation: a key to mitochondrial genome evolution in Euglenozoa? Curr. Genet. 57:225-232.
- [13] Gerasimov, E. S., Kostygov, A. Y., Yan, S., and Kolesnikov, A. A. 2012. From cryptogene to gene? ND8 editing domain reduction in insect trypanosomatids. Eur. J. Protistol. 48:185-193.
- [14] Gillespie, D. E., Salazar, N. A., Rehkopf, D. H., and Feagin, J. E. 1999. The fragmented mitochondrial ribosomal RNAs of Plasmodium falciparum have short A tails. Nucleic Acids Res. 27:2416-2422.
- [15] Gray, M. W., Lukeš, J., Archibald, J. M., Keeling, P. J., and Doolittle, W. F. 2010. Cell biology. Irremediable complexity? Science 330:920-921.
- [16] Gualdron-Lopez, M., Brennand, A., Hannaert, V., Quinones, W., Caceres, A. J., Bringaud, F., Concepcion, J. L., and Michels, P. A. 2012. When, how and why glycolysis became compartmentalised in the Kinetoplastea. A new look at an ancient organelle. Int. J. Parasitol. 42:1-20.
- [17] Hajduk, S. L., and Vickerman, K. 1981. Absence of detectable alteration in the kinetoplast DNA of a Trypanosoma brucei clone following loss of ability to infect the insect vector (Glossina morsitans). Mol. Biochem. Parasitol. 4:17-28.
- [18] Hancock, K., and Hajduk, S. L. 1990. The mitochondrial tRNAs of Trypanosoma brucei are nuclear encoded. J. Biol. Chem. 265:19208-19215.
- [19] Hannaert, V., Bringaud, F., Opperdoes, F. R., and Michels, P. A. 2003. Evolution of energy metabolism and its compartmentation in Kinetoplastida. Kinetoplastid. Biol. Dis. 2:11.
- [20] Hong, M., and Simpson, L. 2003. Genomic organization of Trypanosoma brucei kinetoplast DNA minicircles. Protist. 154:265-279.
- [21] Kamikawa, R., Inagaki, Y., and Sako, Y. 2007. Fragmentation of mitochondrial large subunit rRNA in the dinoflagellate Alexandrium catenella and the evolution of rRNA structure in alveolate mitochondria. Protist. 158:239-245.

- [22] Katz, L. A., Grant, J. R., Parfrey, L. W., and Burleigh, J. G. 2012. Turning the crown upside down: gene tree parsimony roots the eukaryotic tree of life. Syst. Biol. 61:653-660.
- [23] Kollien, A. H., and Schaub, G. A. 1998. Trypanosoma cruzi in the rectum of the bug Triatoma infestans: effects of blood ingestion by the starved vector. Am. J. Trop. Med. Hyg. 59:166-170.
- [24] Landweber, L. F. 1992. The evolution of RNA editing in kinetoplastid protozoa. Biosystems 28:41-45.
- [25] Landweber, L. F. 2007. Genetics. Why genomes in pieces? Science 318:405-407.
- [26] Lara, E., Moreira, D., Vereshchaka, A., and Lopez-Garcia, P. 2009. Pan-oceanic distribution of new highly diverse clades of deep-sea diplonemids. Environ. Microbiol. 11:47-55.
- [27] Larsson, N. G. 2010. Somatic mitochondrial DNA mutations in mammalian aging. Annu. Rev. Biochem. 79:683-706.
- [28] Lukeš, J., Archibald, J. M., Keeling, P. J., Doolittle, W. F., and Gray, M. W. 2011. How a neutral evolutionary ratchet can build cellular complexity. Iubmb Life 63:528-537.
- [29] Lukeš, J., Arts, G. J., van den Burg, J., de, H. A., Opperdoes, F., Sloof, P., and Benne, R. 1994. Novel pattern of editing regions in mitochondrial transcripts of the cryptobiid Trypanoplasma borreli. EMBO J. 13:5086-5098.
- [30] Lukeš, J., Leander, B. S., and Keeling, P. J. 2009. Cascades of convergent evolution: the corresponding evolutionary histories of euglenozoans and dinoflagellates. Proc. Natl. Acad. Sci. U. S. A 106 Suppl 1:9963-9970.
- [31] Lun, Z. R., Lai, D. H., Li, F. J., Lukeš, J., and Ayala, F. J. 2010. Trypanosoma brucei: two steps to spread out from Africa. Trends Parasitol. 26:424-427.
- [32] Madison-Antenucci, S., Grams, J., and Hajduk, S. L. 2002. Editing machines: the complexities of trypanosome RNA editing. Cell 108:435-438.
- [33] Marande, W., and Burger, G. 2007. Mitochondrial DNA as a genomic jigsaw puzzle. Science 318:415.
- [34] Marande, W., Lukeš, J., and Burger, G. 2005. Unique mitochondrial genome structure in diplonemids, the sister group of kinetoplastids. Eukaryot. Cell 4:1137-1146.

- [35] Maslov, D. A., and Simpson, L. 1994. RNA editing and mitochondrial genomic organization in the cryptobiid kinetoplastid protozoan Trypanoplasma borreli. Mol. Cell Biol. 14:8174-8182.
- [36] Moreira, D., Lopez-Garcia, P., and Vickerman, K. 2004. An updated view of kinetoplastid phylogeny using environmental sequences and a closer outgroup: proposal for a new classification of the class Kinetoplastea. Int. J. Syst. Evol. Microbiol. 54:1861-1875.
- [37] Nowacki, M., Vijayan, V., Zhou, Y., Schotanus, K., Doak, T. G., and Landweber, L. F. 2008. RNA-mediated epigenetic programming of a genome-rearrangement pathway. Nature 451:153-158.
- [38] Schnaufer, A., Ernst, N. L., Palazzo, S. S., O'Rear, J., Salavati, R., and Stuart, K. 2003. Separate insertion and deletion subcomplexes of the Trypanosoma brucei RNA editing complex. Mol. Cell 12:307-319.
- [39] Scott, J. 1995. A place in the world for RNA editing. Cell 81:833-836.
- [40] Simpson, L., and Shaw, J. 1989. RNA editing and the mitochondrial cryptogenes of kinetoplastid protozoa. Cell 57:355-366.
- [41] Simpson, L., and Thiemann, O. H. 1995. Sense from nonsense: RNA editing in mito-chondria of kinetoplastid protozoa and slime molds. Cell 81:837-840.
- [42] Simpson, L., Thiemann, O. H., Savill, N. J., Alfonzo, J. D., and Maslov, D. A. 2000. Evolution of RNA editing in trypanosome mitochondria. Proc. Natl. Acad. Sci. U. S. A 97:6986-6993.
- [43] Speijer, D. 2006. Is kinetoplastid pan-editing the result of an evolutionary balancing act? Iubmb Life 58:91-96.
- [44] Speijer, D. 2007. Evolutionary aspects of RNA editing. pp. 199-227. in: H.U.Göringer (Ed.), RNA editing, Springer-Verlag, Springer-Verlag, pp. 199-227.
- [45] Speijer, D. 2008. Making sense of scrambled genomes. Science 319:901.
- [46] Speijer, D. 2010. Constructive neutral evolution cannot explain current kinetoplastid panediting patterns. Proceedings of the National Academy of Sciences of the United States of America 107:E25.
- [47] Speijer, D. 2011. Does constructive neutral evolution play an important role in the origin of cellular complexity?: Making sense of the origins and uses of biological complexity. Bioessays 33:344-349.

- [48] Spencer, D. F., and Gray, M. W. 2011. Ribosomal RNA genes in Euglena gracilis mitochondrial DNA: fragmented genes in a seemingly fragmented genome. Mol. Genet. Genomics 285:19-31.
- [49] Sturm, N. R., and Simpson, L. 1990. Kinetoplast DNA minicircles encode guide RNAs for editing of cytochrome oxidase subunit III mRNA. Cell 61:879-884.
- [50] Thuita, J. K., Kagira, J. M., Mwangangi, D., Matovu, E., Turner, C. M., and Masiga, D. 2008. Trypanosoma brucei rhodesiense transmitted by a single tsetse fly bite in vervet monkeys as a model of human African trypanosomiasis. PLoS. Negl. Trop. Dis. 2:e238.
- [51] Walker, G. 2007. Meeting Report: 16th Meeting of the International Society for Evolutionary Protistology; Wroclaw, Poland, August 2-5, 2006 (ISEP XVI). Protist. 158:5-19.

A Model

We want to mathematically model the life cycle of genetic material of a parasite. We need to model the following parameters:

- 1. Population size (s_n) ;
- 2. Deletion size;
- 3. Probability of deletion (p);
- 4. Level of fragmentation/editing (k);
- 5. Number of generations in each host;
- 6. Replication advantage of smaller DNA (r(x));
- 7. Stop criterion: number of parasites that still have their full DNA and will be able to survive in a different host.

The model will enable us to determine the relationship between some of these parameters.

We will first model the process of replication and deletion in its most general form and then simplify it to enable us to study/simulate the model more accurately. We assume there are two environments, in which the parasite lives. We call these A and B. A could be when the parasite, for example a trypanosome, occupies a tsetse fly and B when it is in the human host. The level of fragmentation of the mitochondrial DNA of an individual parasite is modeled as follows. Each parasite has a sequence a base pairs of length n_a necessary to survive in environment A and b of length n_b needed in environment B. The total length of it's mitochondrial DNA will therefore be $n = n_a + n_b$ base pairs. For the remaining we assume that the parasite is in environment A.

The process of editing induces that the base-pairs of a and b are intertwined modeled as follows by the sequence $a_1b_1a_2b_2...a_kb_k$. We assume that the DNA and hence this sequence is circular, so that b_k connects with a_1 , and we call k the amount of fragmentation. Moreover we assume initially that the length of each subsequence is the same: a_i is n_a/k and b_i is n_b/k .

A.1 Deletion

During each replication cycle deletion of DNA may occur. We will model this by setting p < 1 the probability that a deletion occurs. When a deletion occurs we model this by picking uniformly at random a point $1 \le \text{position} \le n$ on the cyclic DNA and cut out a uniformly random chosen length $1 \le l \le n$. Two things may now happen. Either the resulting smaller circle of DNA misses part of its DNA that is essential for its current

environment– for example in environment A all DNA in the parts a_i are needed and any deletion in such part will kill the parasite– or the smaller DNA still has all the a_i parts intact and the deletion only removed a part of the B- type DNA. The resulting DNA of a surviving individual is the sequence $a'_1b'_1 \ldots a'_kb'_k$ where for each $a'_i = a_i = n_a/k$ and $b'_i \leq b_i$; note that b'_i may become 0.

A.2 Replication

Next, the surviving individuals replicate in such a way that the smaller their total DNA the faster they replicate, which translates into more descendants (replication advantage). We use a function r(x) that models the replication advantage. Let $s(x) = \sum_{i=1}^{k} b_i$ be the total size of the *B*-part (number of *B* base-pairs) of sequence $x = a_1b_1 \dots a_kb_k$. Let \max_r be the maximum multiplicative replication advantage, individuals who lost all their *B*-DNA replicate with a factor \max_r (e.g. 3), and set \min_r to be the minimum replication factor, for individuals who still have their whole DNA intact (e.g. 2).

$$r(x) = \max_{r} -s(x) * (\max_{r} - \min_{r})/n_b$$
(1)

Note that the function r(x) linearly interpolates between \max_r and \min_r , depending on the size of the DNA.

A.3 Simulation

We will need to model both the process of deletion and that of replication. We start by describing the first.

A.3.1 Deletion

We use a Markov chain to model our process, though the entries will not be probabilities since they can be larger than 1. A Markov chain is a graph G(E, N), where N is the set of nodes corresponding to all the possible states of the parasite being alive: the sequences $a_1b'_1 \ldots a_kb'_k$, with $b'_i \leq b_i$. There is a directed edge from node x to node y in G when sequence y can be obtained from sequence x via a single deletion. The edges of G are labeled with the probability that these deletions occur. There are also edges (self loops) from each node x to itself, with label (1-p). These self loops represent the probability that no deletion occurs.

We next construct a matrix D out of our Markov chain G(E, N) of size |N| * |N| as follows. The entry D(x, y), is equal to the label of edge (x, y), and 0 when (x, y) is not an edge in G. The starting state of our process corresponds to all the individuals that have all their DNA still present. This corresponds to the unit vector v_0 , with $v_0(1) = 1$ and $v_0(i) = 0$ for $2 < i \le |N|$. In other words we fix the first entry of our vector to correspond to the state where all the DNA is still present. Likewise entry x corresponds to the fraction of individuals in state x.

A.3.2 Replication

In order to model the replication process we define the diagonal matrix R(x,x) = r(x). Multiplication with R corresponds to replicating state x with replication factor r(x). A single deletion step followed by a replication step is now simply the matrix M = RD.

The vector $v' = Mv_0$ corresponds to our population after a deletion and replication step of our process. Note that the vector v' does not have L_1 norm 1 anymore¹. We now need to take into account the boundary conditions induced by the maximum population size of the parasites as follows. We would like to view v_0 and v' as the probability distributions over the state space. Initially all the probability mass is on the full DNA state and progressively this mass flows to other states. We can then interpret the multiplication of $v_t(i)$ with the size of the maximum population s_p , $s_pv_t(i)$ as the expected number of individuals that have DNA corresponding to state i after t generations. This means that we have to renormalize our vector: $v_1 = v'/|v'|_1$ in order to make it a probability. This completes one full generation step of our process, and in general:

$$v_t = \frac{Mv_{t-1}}{|Mv_{t-1}|_1}$$

Since M is a linear map, we may renormalize at the end, so that:

$$v_t = \frac{M^t v_0}{|M^t v_0|_1}$$

Note that implicit in M is the value of k, the fragmentation level, which we have omitted in our notation so far for simplicity. Note that when k grows so does the size of the Markov chain. Keeping track of this parameter we define for each k:

$$v_{t,k} = \frac{M_k^t v_{0,k}}{|M_k^t v_{0,k}|_1}$$

Let $t_{max}(k)$ be the maximum t such that $s_p v_{t,k}(1) \ge 1$. The value $t_{max}(k) + 1$ tells us the expected number of generations until there are no individuals left that have their full DNA, at a fragmentation level k. We are interested in the growth rate of this function $t_{max}(k)$.

B Reduction of the State Space

It is easy to see that at fragmentation level k we have $(n_b/k+1)^k$ many states in our Markov chain. Typically the total size of the mitochondrial DNA of type B will be around 10,000 base pairs and k can be over 200. This means that for these values the Markov chain becomes way too big to handle. We will first exploit some symmetries in our problem and then show how we can approximate the Markov chain in order to obtain a more manageable formulation that we can simulate.

¹The L_1 norm of v, $|v|_1 = \sum_{i=1}^{|N|} |v(i)|$.

B.1 Symmetries

Since we are only interested in evaluating the first entry of $v_{t,k}$, we do not need the information of the states $a_1b'_1 \dots a_kb'_k$ with $b'_i \leq b_i$, we only need to keep track of how many blocks we have of size $0 \leq s \leq n_b/k$. For example the starting state corresponds to the following tuple with $n_b/k+1$ entries $(k,0,\ldots,0)$, the first entry indicating how many blocks we have of size n_b/k , the second how many of size one less and the last how many of size 0. Following this convention, the fully depleted state becomes $(0,\ldots,k)$. So these are precisely the states $(c_1,\ldots,c_{n_n/k+1})$ such that $\sum_{i=1}^{n_b/k+1}c_i=k$. This corresponds exactly to the number of multi-sets of cardinality k with elements taken from a finite set of cardinality $n_b/k+1$. This multi-set coefficient is equal to $\binom{(n_b/k)+k}{k}$ and can be bound from below by $((n_b/k+k)/k)^k$. This second representation is significantly smaller than our initial set-up, but still too large for the range of parameters we are interested in. We therefore have to simplify our process further.

B.2 Simplification

We modeled a deletion of DNA, by randomly picking a position pos in the (circular) DNA and then remove a piece starting at pos of random length. Individuals survived this deletion whenever only DNA from within a B-block i was deleted. We now simplify this as follows. Fix a parameter d, $1 \le d < n_b$, which we will call the *confidence level*. We will only keep track for each block i whether it has size $a*n_b/(k*d)$, with $0 \le a \le d$. Whenever a random deletion left us with a block size

$$a * n_b/(k * d) \le b'_i < (a+1) * n_b/(k * d),$$

we set the block size $b'_i = a * n_b/(k * d)$, while keeping the probability of this event the same ². We thus give slightly more probability to deleting larger parts within block *i*. We will see later that this change is not very significant.

For example, setting d = 1, models that whenever a deletion falls within block i, we completely remove block i (i.e. it will have size 0). On the other hand for $d = n_b/k$ we get back our old process. Confidence level d thus allows us to interpolate smoothly between the simplified process and the original process.

For confidence level d, the states of our Markov chain will be d+1-tuples (c_1, \ldots, c_{d+1}) such that $\sum_{i=1}^{d+1} c_i = k$. The first entry indicates the number of blocks that have size $n_b/k = (n_b/k * d) * d$, the second entry describes the number of blocks that have size $(n_b/k * d) * (d-1)$, and the last entry the number of blocks that have size $0 = (n_b/k * d) * 0$.

The number of states we have in our Markov chain, with fragmentation k at confidence level d is equal to the number of multi-sets of cardinality k out of a finite set of cardinality d+1, which is $\binom{k+d}{k}$.

²Strictly speaking we should write $\lceil a * n_b/(k*d) \rceil$, we approximate this and assume that n_b is divisible by d*k.

B.3 Transition Probabilities

Here we will make precise the transition probabilities between any pair of states in our Markov chain. Given any state $x = (c_1, \ldots, c_{d+1})$ such that $\sum_{i=1}^{d+1} c_i = k$. The transition from x to x, *i.e.* no deletion occurred, is labeled with (1-p)*r(x), where r(x) is taken as in equation 1 with s(x) the size function adapted for these simplified states:

$$s(x) = n_a + \sum_{i=0}^{d} c_{i+1} \frac{n_b(d-i)}{k*d}$$

Transition from $x = (c_1, \ldots, c_{d+1})$ to $x' = (c'_1, \ldots, c'_{d+1})$ are only possible if there is an i < j such that $c_i = c'_i + 1$ and $c_j = c'_j - 1$, and for all the other indices i' the states are the same: $c_{i'} = c'_{i'}$. This guarantees that exactly one B-block of size corresponding to $i : n_b(d+1-i)/(k*d)$ transforms, by means of a deletion, to a block of size corresponding to $j : n_b(d+1-j)/(k*d)$. The probability that this transition occurs turns out to be:

$$\frac{c_i * m(j,k,d)}{s(x)^2} * p \tag{2}$$

Where m(j, k, d) is the number of ways one can transform a block of length corresponding to i, i.e of length $n_b * (d+1-i)/(k*d)$, to a block of length corresponding to j, using the rule of rounding down described in section B.2. Note that this number only depends on j, k, and d and not on i. For example if i = 1 and j = 2 this corresponds to the number of ways one can delete a sequence of length 1 up-to $n_b/(k*d)$ in a sequence of length n_b/k , which is equal to

$$n_b/k + (n_b/k - 1) + \ldots + (n_b/k - n_b/(k*d) + 1).$$

In general this becomes

$$m(j,k,d) = \sum_{\substack{i' = \frac{n_b*(d-1(j-1))}{k*d} \\ k*d}}^{\frac{n_b*(d-1(j-1))}{k*d}} i'$$

In equation 2 we divide by $s(x)^2$ because each possible deletion has probability $s(x)^2$ to occur at a fixed position and is of a fixed length. Finally we multiply in equation 2 with p, the probability that a deletion occurs.

C Results

We started by simulating our process with a confidence level of 1. This gives a state space of size k + 1 with the corresponding simulation matrix of size $(k + 1)^2$. We ran the simulation with the following parameters: $p = 10^{-6}$, total size of the mitochondrial DNA is

 $26*(10^3)$, with 34% part being of type B. We set the maximum population size $s_p = 10^{10}$. The replication advantage was computed as in equation 1 with $\max_r = 3$ and $\min_r = 2$. The function $t_{\max}(k)$ when plotted for values of k ranging from 1 to 200 shows a nearly perfect line, indicating that the advantage of fragmentation is almost linear. We were able to show rigorously that in the simple case of d = 1 the fragmentation advantage can never be more than linear, that is, we were able to show a linear upper bound on the function $t_{\max}(k)$. This was done by studying the spectrum of a simplified 2×2 Markov chain.

Next we simulated the same process with increasing confidence values d. These results show that in each case, for these parameters, we get almost straight lines each one with a slightly steeper slope. However for successive values of d the *increase* of the slope appears to be halving each time. This suggests that already for a small value of d we have a reasonably good approximation of our original Markov chain.

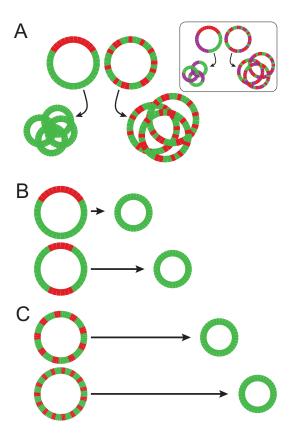


Figure 1: Schematic representation of gene scrambling in organisms alternating between life cycles with different selective pressures and strong intraspecific competition (A) and of modelling used in this study (B,C). Inset: kinetoplastids are represented by multicoloured mt DNA circles with 2 or 3 classes of genes: 'green' genes required in one life cycle (e.g. mammalian blood for T. brucei), 'red' genes required in another (e.g. Tsetse fly) and 'violet' genes required in both. Only presence/absence of selective pressure is important in modelling. Full figure: red (absence of selective pressure) and green only. Arrow lengths (not to scale) represent number of generations upon which the population loses ecological competence ($t_{\text{max}}(t)$ of our model). (A) Red genes disappear during many generations of strong competition. Gene scrambling will slow down this process. (B) Simulations of instances of scrambling over successive generations. In the first simulation we deduce how many generations more the population is allowed by a split before losing ecological flexibility (represented by the smaller green circle), using many possible deletions. (C) In the second simulation we deduce how many generations more retain full flexibility by stepwise scrambling increase, using complete segment deletions only.

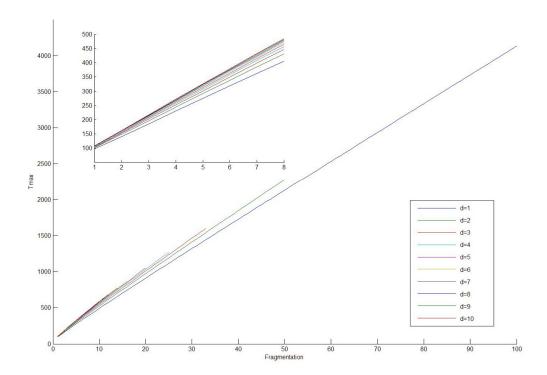


Figure 2: Quasi-linear correlation between amount of fragments (x-axis, parameter k of our model) and maximum number of generations upon which the population loses ecological competence (y-axis; $t_{\text{max}}(k)$ of our model). Inset: close-up of fragmentation levels 1-8; inset on the right: colour code for different confidence levels (d). The higher d, the better the approximation (see appendix).