Models for the evolution of microsatellites

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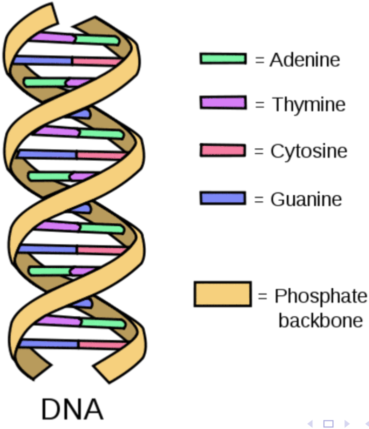
February 14, 2019

DNA Basics

- DNA is a molecule found in all life.
- Typically appears as a double helix structure.
- Made up of nucleotides



Neucleotides

- We consider 4 distinct nucleotides:
  1. A - Adenine.
  2. T - Thymine.
  3. C - Cytosine.
  4. G - Guanine.

- The genetic code is written in the language defined by these nucleobases.
- A piece of code may be regarded as a string of nucleobases
- e.g. ATCCATATG

Base Pairing

- Nucleobases on one strand chemically bond with those on the other
- Each bonds with precisely one other:
  1. A and T bond.
  2. C and G bond.

- Base pairing leads to a natural complementary relationship between strings of code.
- E.g. ATCCATATG has TAGGTATAC as its complement

Mutation

- Two strands of double helix separate
- Each strand's complimentary sequence is generated and bonds to it.
- There exists a possibility for errors to occur.
- Most errors are corrected, some lead to a change in the code.
- We refer to uncorrected errors as mutations.

Selection

- Mutations may give rise to new alleles.
- Often, this will make an organism more or less fit.
- Many microsatellites are *not* subject to selection, which allows for demographic information to be faithfully preserved.

Microsatellites

- Repeats of a short motif, e.g. AT repeated 6 times:

$$\boxed{A}\boxed{T}\boxed{A}\boxed{T}\boxed{A}\boxed{T}\boxed{A}\boxed{T}\boxed{A}\boxed{T}\boxed{A}\boxed{T}$$

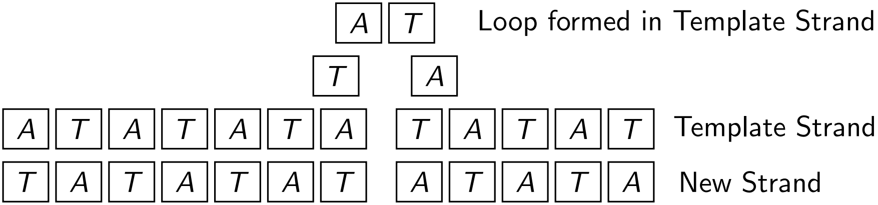
- Usually, think of microsatellites as repeat units:

$$\boxed{AT}\;\boxed{AT}\;\boxed{AT}\;\boxed{AT}\;\boxed{AT}\;\boxed{AT}$$

Slipped-strand mispairing

Contraction

During replication, a loop may form in the template strand leading to a decrease in the number of repeats in the new strand.



A T Loop formed in Template Strand

T A

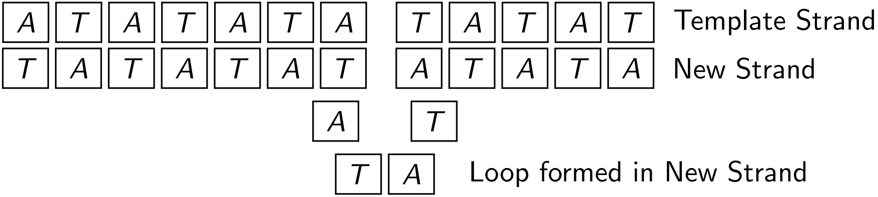
A T A T A T A T A T A T Template Strand

T A T A T A T A T A T A New Strand

Slipped-strand mispairing

Expansion

Alternatively, a loop may form in the new strand, leading to an increase in repeat number relative to the template.



A T A T A T A   T A T A T   Template Strand

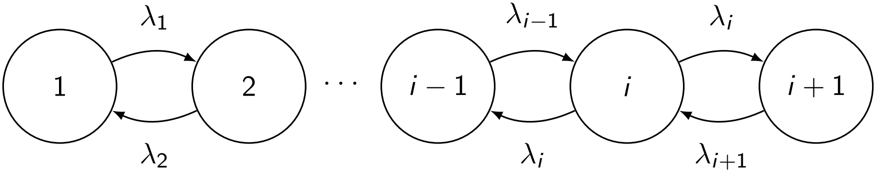
T A T A T A T   A T A T A   New Strand

A   T

T A   Loop formed in New Strand

Models for repeat number

- e.g. a symmetric random walk:



- The main factors accounted for are:
  - Length dependence of mutation rate.
  - Bias towards contraction or expansion.
  - Size of the mutation events.

Point mutation

- Microsatellites also susceptible to point mutations.

$$\boxed{AT}\ \boxed{AT}\ \boxed{AT}\ \boxed{AC}\ \boxed{AT}\ \boxed{AT}$$

- How to deal with this?

Point mutation

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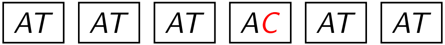
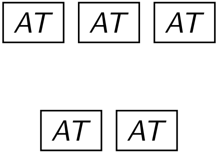


AT AT AT AC AT AT

- How to deal with this?
- One way is to model point mutation as splitting a single microsatellite into two smaller ones.

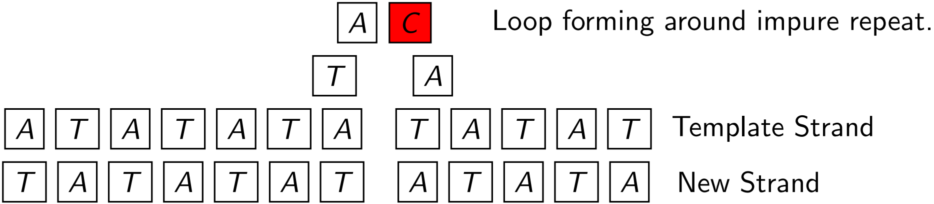


AT AT AT

AT AT

Some problems

- These models lose useful information, and may invalidate IID assumption.



A C Loop forming around impure repeat.

T A

A T A T A T A T A T A T Template Strand

T A T A T A T A T A T A New Strand

Our model

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- $i_{\text{min}}, i_{\text{max}}$ and j^i_{max} are all absorbing states, although taking $i_{\text{max}} = \infty$ is natural.
- Generator $\mathbf{Q} = [q_{(i,j).(k,l)}]$ with

$$q_{(i,j)(k,l)} = \begin{cases} r_s(i,j)\beta(i) & \text{for } k = i+1, l = j \\ r_s(i,j)(1-\beta(i))H(j-l, iL, j, L) & \text{for } k = i-1, j-L \leq l \\ r_m(i,j) & \text{for } k = i, l = j+1 \\ r_p(i,j) & \text{for } k = i, l = j-1. \end{cases} \tag{1}$$

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$$r_s(i,j) = (u_0 + u_1(i-1))c^j, \tag{2}$$

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$$r_p(i,j) = \frac{1}{3}dj \tag{5}$$

A short aside on whole genome derived sequence data...

Ultimately, we want to fit our model to some data from real microsatellite sequences. In order to obtain data with information about the number of interruptions in the repeat sequence, we use whole-genome data.

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- Detection component does some statistics to find (a sample of) candidate microsatellites in the genome.
- Analysis component determines (among other things) the repeat motif and measures how well the observed sequence matches a theoretical sequence of the same length consisting of perfect copies of the repeat sequence.

Observable sequences

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- We set the 'absorbing boundary' of the model to match the boundary of observability under TRF, so that i_{min} and j^{i}_{max} are determined by the aforementioned criteria.
- We chose i_{max} to be the maximum observed sequence length in each subset of our dataset (which we partitioned by motif-length).

Fitting the model to whole-genome derived sequence data

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We extend the model to the population-level by assuming a Poisson birth process for microsatellites, born with some initial distribution $\underline{\alpha}$.

Fitting the model to whole-genome derived sequence data

We derive the distribution (in terms of the individual-level model) of a microsatellite observed at a time t.

- Ignoring any imperfection in the process of observation, the event that a microsatellite is observed at time t^* is equivalent to the event that it was born before time t^*, and is absorbed after time t^*.
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$$\begin{aligned} f_{T_0}(t^* - t \mid T_0 < t^* < T_a) &= \frac{S(t)}{\int_{t=0}^{T} S(t)dt} \\ &= \frac{\underline{\alpha}_0 e^{\mathbf{Q}^* t} \underline{1}}{\underline{\alpha}_0 (e^{\mathbf{Q}^* t} - \mathbf{I})(\mathbf{Q}^*)^{-1} \underline{1}}. \end{aligned} \tag{6}$$

where S is the survival function and $\mathbf{Q}^*$ is the subgenerator associated with the model.

Fitting the model to whole-genome derived sequence data

Now we can write the probability that a microsatellite observed at time t is in state s as

$$\begin{aligned}
&P(X(t^* - T_0) = s \mid T_0 < t^* < T_a) \\
&= \int_{t=0}^{t^*} P(X(t) = s \mid T_0 = t^* - t < t^* < T_a) f_{T_0}(t^* - t \mid T_0 < t^* < T_a) dt \\
&= \int_{t=0}^{t^*} \left(\frac{[\underline{\alpha}_0 e^{\mathbf{Q}^* t}]_s}{\underline{\alpha}_0 e^{\mathbf{Q}^* t} \underline{1}} \right) \left(\frac{\underline{\alpha}_0 e^{\mathbf{Q}^* t} \underline{1}}{\underline{\alpha}_0 (e^{\mathbf{Q}^* t^*} - \mathbf{I})(\mathbf{Q}^*)^{-1} \underline{1}} \right) dt \\
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\end{aligned}
$$

which we write in vector form as

$$
\underline{\pi}^*(t^*) = \frac{\underline{\alpha}_0 (e^{\mathbf{Q}^* t^*} - \mathbf{I})(\mathbf{Q}^*)^{-1}}{\underline{\alpha}_0 (e^{\mathbf{Q}^* t^*} - \mathbf{I})(\mathbf{Q}^*)^{-1} \underline{1}}, \qquad (8)
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This provides a way to test the validity of the assumption of independence — if the fitted $\underline{\pi}^*(1) \approx \lim_{t^* \to \infty} \underline{\pi}^*(t^*)$, then we can conclude that the empirical distribution is at or near equilibrium.

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However, there is one major problem with this model — we require data from bona-fide microsatellite sequences which includes information about interruptions.

In theory, TRF (or similar software) applied to whole-genome data should provide this. In practice, our data appears to be polluted with non-microsatellite sequences.

More on microsatellites

Fundamentally, the problem is that repetitive structure is not enough for a sequence to be considered a microsatellite. It must also exhibit ‘characteristic microsatellite behaviour‘ — i.e. it should undergo high rates of slipped-strand mispairing.

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We not only need to separate repetitive non-microsatellite sequences from proper microsatellites, but we also need to identify ‘ex-microsatellites‘ — repetitive sequences which were evolving rapidly due to slipped-strand mispairing before becoming highly interrupted.

Acknowledgements

Supervisors

- Dr Małgorzata O'Reilly
- Dr Barbara Holland

- Dr Bennet McComish