Thesis for the Degree of Licentiate of Philosophy

**Modelling Hereditary Diseases Using Galton-Watson Processes**

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Abstract

In this thesis we consider two applications of Galton-Watson branching processes. The first application concerns the estimation of the age of a disease-causing founder mutation of autosomal dominant inheritance. A Galton-Watson branching model is proposed, resulting in an estimate of the mutation age. Also, the problem of combining estimates based on several markers is discussed. Simulations illustrate the various methods and the mutation age estimate is applied to real data. The subject of homozygosity mapping, a method using differences in marker lengths to find the disease locus, is our second application.

Keywords: Age estimation - Galton-Watson process - composite likelihood - homozygosity mapping - BRCA1
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CHAPTER 1

Introduction

One of the matters discussed in this work is the problem of estimating the time since a disease-causing mutation arose in a population. The model proposed for solving this problem is a multitype Galton-Watson branching process, earlier described by (Pankratz, 1998).

We consider an autosomal dominant founder-mutation causing a rare disease, and assume that the disease-gene location is known. In Chapter 3, a Galton-Watson branching process model is constructed, describing the evolution of some genetic marker in the disease-carriers along the family tree. An estimate of the mutation age is derived using the method of moments and, further, a couple of simulation examples illustrate the method.

In the literature, other methods for estimating the age of a founder mutation are proposed. One of them can be found in (Risch et al., 1995). This method is based on the probability that a disease-mutation-bearing chromosome does not carry a founder marker allele, the frequency of that allele among normal individuals \( p_n \) and the recombination frequency \( \theta \) between the marker and the disease locus. It is used in several earlier age estimations in rare diseases among others in Finnish populations (Moisio et al., 1996) and among Ashkenazi Jews (Risch et al., 1995).

A model using haplotypes is introduced in Chapter 4, resulting in formulas containing large matrices. As early as in the two-marker case, the formulas become cumbersome. There seems to be no simple formula describing the marker allele transitions among disease-carriers.
for $t$ generations. This method seems to be a dead end, but can be used in various computational investigations.

The problem of combining several age estimates, one for each marker, is mentioned. The simplest way of combining estimates, and the one used in the simulation examples to follow, is to use the mean estimate. It is also possible, and probably better, to use weights, appropriately chosen. A simulation study is included to illustrate some results.

Another way of finding a combined estimate using several markers at the same time is the method of composite likelihood. The estimate is obtained by adding log likelihood functions and finding the maximum. An example comparing the method of moments estimate and the composite likelihood estimate is included. As is also the conclusion in (Pankratz, 1998), this method does not yield great results in estimating the age of a disease.

In chapter 5, data from a mammary cancer study, conducted in the western part of Sweden, is analysed using the herein described method.

Eighteen families with a specific cancer-causing mutation have been collected and genotyped for thirteen polymorphic markers. The haplotypes are found using the GENEHUNTER software. From family data, 18–20 disease-allele carrying individuals are collected as a sample of the disease population, and 31–38 healthy relatives are used as the normal population sample. The distances from the genetic linkage map used in the study are translated to recombination frequencies using the Haldane map function. Because of some constraints on our estimate, all markers do not yield an estimate. The mean value of our 10 estimates results in an age estimate of 128 generations.

One simulation of 20 and another of 50 generations have been performed, using marker distances and marker allele frequencies obtained in family data. The resulting estimates were 72.1858 and 90.0115 generations respectively. Thus, the estimate obtained above is most likely much larger than the true value.

In chapter 6, mutations are introduced in the earlier G-W model. The microsatellite markers are assumed to follow the Stepwise Mutation Model. Again a formula for the transitions of marker alleles in $t$ generations is deduced.

The concept of homozygosity mapping is then introduced, being
our second Galton-Watson application. Our goal here is to localise a disease gene. Shortly described, homozygosity mapping works by using differences in marker allele lengths to compare normal and disease populations to find the most probable location for the disease gene. Again, we consider a disease caused by a founder mutation, but with the distinction that we are dealing with a recessive disease. Thus, affected individuals are carrying two disease alleles, inherited one from each parent.

The concept used is that due to homozygosity by descent, individuals carrying the disease allele are homozygous in the area of the chromosome surrounding the disease locus in much larger extent than expected (cf. (Kruglyak et al., 1995) and (Lander and Botstein, 1987)).
CHAPTER 2

Basic Genetics

2.1 Introduction

The genetic blueprint of every living organism is found in the nucleotides, stacked in chromosomes which are gathered in the nucleus of each cell as the entire genome of that organism. The nucleotides are paired together, two by two, forming a string of nucleotides attached to a singularly determined complementary string of nucleotides, building up the chromosome in the form of a ladder.

When an organism has one set of chromosomes, it is said to be a haploid organism, and if it has two sets of chromosomes, i.e. pairs of chromosomes, it is called diploid.

The process that produces gametes (a gamete is a haploid cell that fuses with a corresponding haploid cell from the opposite sex to form a new individual) is called meiosis (see Figure 2.1). During this process, certain crossovers may occur, where the sister chromosomes exchange parts. If an odd number of crossovers have occurred between two loci, we call that a recombination event.

The chromosomes carry genetic information in the protein-coding regions called the genes. Non-coding regions are not totally information free, since certain polymorphic sequences can be used as genetic markers. This matter is further explained in the next section. By the term polymorphic, we mean that in the population, several phenotypic forms (alleles) exist at a certain site (locus). As guides to the genome, genetic maps are constructed, containing markers and distances. The unit used is mostly cM, where the distance of 1 cM
between two loci corresponds to the recombination probability between them of 0.01 (i.e. one product of meiosis out of 100 will in average be recombinant).

*Genotype* is what we call the specific allelic arrangement of one or several genes and the *phenotype* is the detectable outward manifestation of a specific genotype.

A *recessive* trait will only be visible in an individual carrying two copies of that trait, i.e. inherited from both parents. If only one copy of the disease allele is enough to express the trait it is said to be *dominant*. *Autosomal dominant* is when the trait is not located on the sex chromosomes, X and Y.

For more detailed information, we refer to existing literature. Two sources used in this introduction are (Griffiths *et al.*, 1996) and (Liu, 1998).

2.2 Markers and Microsatellites

Genetic markers are DNA subsequences that exist in several polymorphic forms in individuals within a species. Several kinds of markers have been detected and are used for various reasons. One of these are further explained in the next subsection.

A large number of certain molecular markers have been collected to
be able to describe and find our way around in the genome. These are detected by probes: one short sequence finding the beginning of the marker and another to find the ending, which 'cut out' our marker of interest and show us the contents, simply explained. These markers, being evolutionary neutral, have a high level of heterozygosity for some type of neutral DNA variation (i.e. polymorphic).

2.2.1 Microsatellites

As mentioned earlier, DNA contains a vast amount of information in regions coding for proteins but there is also a huge amount of pieces that seem to carry no information at all. Among these are short strings of DNA called microsatellites or simple sequence repeats (SSR). As the name suggests, they consist of several repeats of short, simple sequences. As a rule, a microsatellite is a short sequence of 1 to 6 basepairs repeated after each other up to 100 times (Liu, 1998).

The most common kind of microsatellites is repetitive DNA based on dinucleotide repeats, like the CA-repeat. This microsatellite consists of the two nucleotides C and A repeated \( n \) times, and is sometimes denoted by \( (CA)_n \).

Even though these DNA pieces seem to be nonsense, we can use them as markers when trying to make something out of the enormous amount of information contained in the genome. The reason why these pieces of DNA are useful is that the number of repeats differ from one chromosome to another. We inherit one set of chromosomes from each parent and thereby also one marker from each. It is feasible to visually see the difference in length of markers using appropriate methods, such as the polymerase chain reaction, PCR. The microsatellites are useful when, among other things, we want to reconstruct phylogenetic relationships, for forensic science, parentage testing and as it seems, in population genetics (cf. Harding et al., 1993)).

2.3 Mutation Mechanisms

2.3.1 IAM - Infinite alleles model

A motivation for using this model is the following. Consider a gene coding for a protein with 300 aminoacids - it has the length of 900 nucleotides. We thereby have \( 4^{900} \approx 10^{542} \) possible ways of placing
one of four nucleotides at each position. That is, we have such large
number of possible combinations that the probability that the same
combination will occur more than once is negligible. Thus, when a
new mutation occurs, a new allele is created that did not exist in the
population earlier.

This model is called the \textit{infinite alleles model}, since there are in-
finitely many states to which an allele can mutate and every mutation
state is equally probable irrespective of the state of the allele. Since
we have infinitely many possibilities and the probability of mutating
to an already existing number of repeats is tiny, we have few states
represented in the population and every mutation will be unique.

What makes this model less useful, though, is that the difference
in the number of repeats between two markers gives us information
about how far back in time they have a common ancestor allele. This
information is not taken into consideration by this model (Goldstein
\textit{et al.}, 1995). When a mutation occurs, we get a new mutant that is
related to the ‘original’ allele, with respect to the number of repeats.
Mutation tends to change the size of the allele very little, by a few
repeats more or less. These two facts are the reasons why we need a
model better suited for microsatellites.

\subsection{SMM - Stepwise mutation model}

This model can be interpreted in two slightly different ways.

\textbf{Strict stepwise mutation model}

This model states that when a microsatellite loci mutates, it always
changes size with one repeat more or less, i.e., an allele with \( i \) re-
peats mutates to \( i - 1 \) or \( i + 1 \) repeats with the same probability \( \theta/2 \)
respectively.

The probabilities for adding and deleting one repeat does not nec-
essarily need to be the same. This model can also be generalised to
permit other variants of mutations.

\textbf{Stepwise mutation model}

The more general model allows other changes in the number of repeats
than the strict stepwise mutation model. It is thus consistent with
2.4 Genetic Linkage

Figure 2.2: The transition probabilities in the SMM model

the distribution of alleles at microsatellite loci, (Valdes et al., 1993), but at the same time also a bit more difficult to implement.

Mutations resulting in a state already represented in the population are the most common ones. In the SMM, there are fewer possible alleles than in the IAM for the same population size and mutation degree. Since our alleles consist of tandemly repeated sequences, this model is more appropriate than the IAM. Also, this model does not predict as high levels of heterozygosity as the IAM (cf. Harding et al., 1993).

2.4 Genetic Linkage

Genetic linkage is what we call the association of genes located on the same chromosome. For genes on the same chromosome, the segregation ratios for the genotypes depart from the Mendelian independent assortment ratios. If the position of a marker is close to the disease locus, resulting in a low probability of crossover at meiosis, we say that we have linkage between them. Thus, linked to the disease locus are some markers. Being linked implies that they are inherited together with the disease allele most of the time, but recombinations and mutation events occasionally break this inheritance pattern.
CHAPTER 3

Estimating the Age of a Disease using Galton-Watson Processes

3.1 Introduction

We consider an autosomal dominant disease which arose in a normal, homogeneous population by a genetic mutation in the DNA of an individual, (from now on called the founder), some unknown time ago. This disease has seemingly no negative effect on the fertility (at least until the carrier has reached a certain age) so the disease-causing mutation has spread to the offspring due to normal segregation for generations. The propagation of the disease is conveniently modelled using a Galton-Watson branching process (further explained in 3.2.1). As of today, we know the disease did not die out, so the branching process considered is supercritical.

On the same chromosome as the disease locus we have several linked genetic markers.

In this chapter, we only have interest in one polymorphic microsatellite marker at a time, chosen at a suitable, known distance from the disease locus.

We assume that the disease-causing mutation is relatively recent, so the population of individuals carrying normal alleles is considerably larger than the disease allele carrying population.

Our interest herein lies in finding an estimate of the time since the disease allele appeared in the population. We will later in this chapter
propose an estimate, found both using the method of moments and the maximum likelihood method, for the number of generations since the founder mutation arose in the population. This estimate is based on information gained from the marker allele frequencies.

3.2 The Model

We fix a suitable genetic marker linked to the disease locus. The marker considered is a polymorphic microsatellite whose position in relation to the disease locus is exactly known.

We need to know the true marker allele frequencies in the normal population, assumed being stable and homogeneous.

The marker allele will not always be inherited together with the disease allele due to recombination events. In the case of cross-over, we presume the new marker allele randomly chosen according to the allelic distribution in the normal population due to panmixia (i.e. random mating in a population).

Our disease should not be too old. The size of the disease population need to be considerably smaller than the normal population, i.e., the disease is not so widely spread in the population. We can then assume that the probability of two carrier chromosomes meeting is negligible. We also disregard mutation events since the disease appeared relatively recent in time and the normal allele distribution is thought to be stable.

Time is measured in number of generations. The fact that the generation span can differ a lot in different branches of the family tree is not taken into consideration.

We use the Galton-Watson branching process to describe the spread of the disease through time, tracing the disease chromosome labelled by the marker allele.

3.2.1 Galton-Watson

Let $M_1, M_2, \ldots, M_k$ denote the $k$ marker alleles. Let further $r$ be the recombination frequency between the loci of the disease gene and the marker and $Z_t$ is the number of individuals in generation $t$. The
3.2 The Model

$Z_t$ variables are based on earlier events as

$$Z_{t+1} = \sum_{j=1}^{Z_t} X_{jt},$$

where $X_{jt}$ denotes the number of offspring of individual $j$ in generation $t$. To emphasise the multitype nature of this process we take

$$Z_t(i) = \# \text{ individuals carrying allele } i \text{ in generation } t, \ i \in \{1, 2, \ldots, k\}.$$

We assume that the reproduction law is such that $E[X_{jt}] = 1 + \lambda$ for all $i$ and $t$ and that the process is supercritical, i.e. that $\lambda > 0$. Moreover we assume that the $X_{jt}$'s are independent.

Denote $M = (m_{ij})_{i,j}$ as the matrix consisting of

$$m_{ij} = E[\# \text{ offspring of type } j \text{ from a type } i \text{ individual}].$$

Now it is easily shown that

$$m_{ij} = \begin{cases} (1 + \lambda)rp_{nj} & i \neq j \\ (1 + \lambda)(1 - r) + rp_{nj} & i = j \end{cases}$$

where $p_{ni}$ is the proportion of individuals carrying marker allele $i$ in the normal population. If the marker allele is changed, it is due to recombination. When instead the progeny inherits the same marker allele it can be for one out of two reasons: either no recombination has occurred or a recombination occurred giving back the same marker allele, randomly chosen according the normal allele distribution.

Let $Z_t = [Z_t(1), Z_t(2), \ldots, Z_t(k)]$, where $Z_t(i)$ is as above. A well known result for Galton-Watson processes (cf. (Jagers, 1975)) allows us to write

$$E[Z_t | Z_0] = Z_0 M^t$$

where $M^t$ stands for the matrix $(m_{ij}^{(t)})_{i,j}$ where $m_{ij}^{(t)}$ is the expected number of progeny of type $j$ born to an individual of type $i$ $t$ generations later.

Define the founder generation as

$$Z_0 = [1, 0, \ldots, 0]$$

We are interested in the first row of the matrix $M^t$, that is $Z_0 M^t$. The reason being that we assume that the probable founder allele is placed in the first position. First, we need to find a formula describing $M^t$. This is done in the next section.
3.2.2 Finding $M^t$

It is not difficult to see that in this case

$$M = (1 + \lambda)[(1 - r)I + rP]^t$$

(recall the formula for $m_{ij}$). As earlier, $1 + \lambda$ is the expectation of the offspring distribution. The $P$-matrix gives the transition probabilities between the different alleles

$$P = \begin{pmatrix}
    p_{n1} & p_{n2} & \cdots & p_{nk} \\
    \vdots & \vdots & & \vdots \\
    p_{n1} & p_{n2} & \cdots & p_{nk}
\end{pmatrix}$$

where $p_{nj}$ is the proportion of individuals with allele $j$ in the normal population. Since it is assumed that the population in which the disease appeared is stable and homogeneous, $p_{nj}$ is a fixed quantity from generation to generation. The corresponding quantities in the disease population, $p_d$ are defined as the proportion of individuals with allele $j$ in the disease population in the present time, but the true frequencies are unknown. As an estimate we use the observed allele proportions.

If we expand the model, adding the possibility of mutations, we get another kind of matrix describing transitions from one allele to another. This case will be discussed in a chapter 6.

We now have the following marker allele distribution for the offspring:

Recall that

$$M = E[\# \text{ offspring with marker allele } j \text{ when the founder had allele } i]$$

where $i, j \in \{1, 2, \ldots, k\}$ and notice that

$$\frac{M^t}{(1 + \lambda)^t} = [(1 - r)I + rP]^t$$

$$= r^tP^t + \sum_{i=1}^{t-1} \binom{t}{i}((1 - r)I^i(rP)^{t-i}) + (1 - r)^tI^t$$

$$= r^tP^t + P \sum_{i=1}^{t-1} \binom{t}{i}(1 - r)^i(rP)^{t-i} + (1 - r)^tI^t$$

$$= r^tP + (1 - r^t - (1 - r)^t)P + (1 - r)^tI =$$

$$= (1 - r)^tI + (1 - (1 - r)^t)P$$
where we use that \( \sum_{i=0}^{t} \binom{t}{i} (1 - r)^{t-i} = 1 \) and that \( P^t = P \).

We now make a simple but crucial approximation:

\[
\frac{M}{1 + \lambda} \simeq \left( P(\text{offspring has marker } j, \text{ founder had marker } i) \right)_{ij}.
\]

From this we get

\[
\frac{M^t}{(1 + \lambda)^t} \simeq \left( P(\text{offspring has marker } j \text{ after } t \text{ generations}) \right)_{ij}
\]

which can be obtained by replacing the actual empirical ratios by the ratios of expectations.

### 3.3 The Estimate

First assume that we have a sample of \( n_d \) individuals from generation \( t \) carrying the disease allele. Let \( n_{dj} \) be the number of individuals carrying marker allele \( j \) in the sample. We can then use that information to estimate \( p_{dj} \), namely as \( \hat{p}_{dj} = \frac{n_{dj}}{n_d} \).

We will now show how to find the same estimate

\[
\hat{t} = \frac{\ln(\frac{\hat{p}_{d1}-\hat{p}_{n1}}{1-\hat{p}_{n1}})}{\ln(1 - r)}
\]

using two different methods: The method of moments and the maximum likelihood method.

#### 3.3.1 Method of Moments Estimate

Using the formula for \( t \) generations \( (1 - r)^t I + (1 - (1 - r)^t)P \) obtained earlier, we have two cases:

- \( i = 1 \): Using the first row of the above matrix, corresponding to the founder allele, we get \( (1 - r)^t + (1 - (1 - r)^t)p_{n1} = p_{n1} + (1 - p_{n1})(1 - r)^t \) and thus
  \[
p_{d1} = p_{n1} + (1 - p_{n1})(1 - r)^t
\]

where \( p_{d1} \) is replaced by its estimate \( \hat{p}_{d1} \). The equation is solved for the unknown variable \( t \), as
\[ \hat{t} = \frac{\ln(\hat{p}_{di}/p_{ni})}{\ln(1-r)} \]

Note that we require that \( \hat{p}_{di} > p_{n1} \).

\( i \neq 1 \): Here we use the \( i^{th} \) row in the matrix above:

\[ p_{di} = p_{ni} - p_{ni}(1-r)^t \]

which also gives us an estimate of \( t \) as:

\[ \hat{t} = \frac{\ln(p_{ni}/p_{ni})}{\ln(1-r)} \]

In this case we have the requirement of \( p_{ni} > \hat{p}_{di} \).

As we see here, we get an estimate of \( t \) also in the case where the individual does not carry the original marker allele. This case will not be further studied in this work.

### 3.3.2 Maximum Likelihood Estimate

The same estimate as above can also be derived from the Maximum Likelihood method. One condition for using this method is that the individuals are independent of each other. In reality, however, we have individuals who are related, e.g. siblings, cousins, second cousins etc, and hence not independent. When sampling from the last generation, we will get individuals who are likely to be less related, and we get approximately independence, which should suffice.

As earlier, let

\[ \pi_j = \begin{cases} 
\frac{p_{ni} + (1-p_{ni})(1-r)^t}{p_{ni} - p_{ni}(1-r)^t} & i = 1 \\
\frac{p_{ni} - p_{ni}(1-r)^t}{p_{ni} - p_{ni}(1-r)^t} & i \neq 1 
\end{cases} \]

The likelihood function is

\[ L(n_d, \ldots, n_d; t) = \prod_{j=1}^{k} \pi_{nj}^{n_{dj}} = (p_{n1} + (1-p_{n1})(1-r)^t)^{n_{d1}} \cdot \prod_{j=2}^{k} (p_{nj} - p_{nj}(1-r)^t)^{n_{dj}} \]
and the log likelihood is

\[ l = \ln L = n_{d1} \cdot \ln(p_{n1} + (1 - p_{n1})(1 - r)^t) + \sum_{j=2}^{k} n_{dj} \cdot \ln(p_{nj} - p_{nj}(1 - r)^t) = \]

\[ n_{d1} \cdot \ln(p_{n1} + (1 - p_{n1})(1 - r)^t) + \sum_{j=2}^{k} n_{dj} \cdot \ln(p_{nj}(1 - (1 - r)^t)) \]

Maximum is obtained by solving the equation

\[ 0 = \frac{\delta \ln L}{\delta t} = \]

\[ \frac{n_{d1}(1 - p_{n1})(1 - r)^t \ln(1 - r)}{p_{n1} + (1 - p_{n1})(1 - r)^t} - \sum_{j=2}^{k} \frac{n_{dj}(1 - r)^t \ln(1 - r)}{1 - (1 - r)^t} = \]

\[ \frac{n_{d1}(1 - p_{n1})}{p_{n1} + (1 - p_{n1})(1 - r)^t} - \frac{\sum_{j=2}^{k} n_{dj}}{1 - (1 - r)^t} \]

where \( \sum_{j=2}^{k} n_{dj} = n_d - n_{d1} \). Thus, we have that

\[ \frac{n_{d1}(1 - p_{n1})}{p_{n1}(1 - p_{n1})(1 - r)^t} = \frac{n - n_{d1}}{1 - (1 - r)^t} \]

Solving this equation for \( t \) (in several steps) yields

\[ n_{d1}(1 - p_{n1})(1 - (1 - r)^t) = (n_d - n_{d1})(p_{n1} + (1 - p_{n1})(1 - r)^t) \]

and then

\[ n_{d1} - n_{d1}(1 - r)^t + n_{d1}p_{n1}(1 - r)^t = \]

\[ np_{n1} + n(1 - p_{n1})(1 - r)^t - n_{d1}(1 - p_{n1})(1 - r)^t. \]

Now, divide by \( n_d \) on both sides and use that \( \hat{p}_{d1} = n_{d1}/n_d \),

\[ \hat{p}_{d1} - p_{n1}(1 - r)^t + \hat{p}_{d1}p_{n1}(1 - r)^t = \]

\[ p_{n1} + (1 - p_{n1})(1 - r)^t - \hat{p}_{d1}(1 - p_{n1})(1 - r)^t \]

Notice that \( \hat{p}_{d1}(1 - p_{n1})(1 - r)^t = (\hat{p}_{d1} - \hat{p}_{d1}p_{n1})(1 - r)^t \), which is a term on the left side of the equation. Dividing on both sides yields

\[ \hat{p}_{d1} = p_{n1} + (1 - p_{n1})(1 - r)^t \]
which can be solved for \( t \), resulting in the ML-estimate

\[
\hat{t} = \frac{\ln\left(\frac{\hat{p}_{d1} - \hat{p}_{n1}}{1 - \hat{p}_{n1}}\right)}{\ln(1 - r)}
\]

Notice that this estimate uses the distance between \( p_{d1} \) and \( p_{n1} \) in an intuitively natural manner. It also has a certain resemblance to the so-called \( p_{ascens} \) as is described in (Weir, 1996). To investigate the behaviour of the estimate we have performed a simulation study.

### 3.4 Preliminary Simulations

The simulation source code was written in MATLAB. Some details can be found in Appendix A.

We start with the first generation with one founder individual carrying the marker allele named 1. The subsequent generation will consist of a certain number of disease-allele carrying individuals (obtained by a formula described below), some with other marker alleles but most with the same as the founder. If a recombination changes a marker allele from the founder allele, it is possible to retrieve it by recombination since this allele also exists in the normal population. The family tree is then built up, generation by generation, consisting only of disease allele carriers, where individuals are represented by their marker allele.

As described in the previous section and using the same notation, we use the Galton-Watson branching process resulting in the following model:

Take \( Z_t(i) = \# \) individuals in generation \( t \) carrying marker allele \( i \). As before, the marker allele carried by the founder is named allele 1 and the other alleles are numbered 2, \ldots, \( k \). The founder generation can be written as

\[
Z_0(1) = 1, \; Z_0(2) = 0, \; Z_0(3) = 0, \ldots, \; Z_0(k) = 0
\]

or in vector form

\[
Z_0 = [1, 0, \ldots, 0].
\]

The \((t + 1)^{th}\) generation is generated according to the formula (cf. (Kaplan and Weir, 1997))

\[
Z_{t+1}(i) \sim \text{Po} i \left((1 + \lambda) \left[ (1 - r) Z_t(i) + r p_{ni} Z_{\text{tot}}(t) \right] \right)
\]
where \( i = 1, \ldots, k, \lambda > 0 \) is the population growth factor in a supercritical Galton-Watson branching process, \( r \) is the recombination frequency between the marker and disease loci, \( p_{hi} \) is, as described earlier, the frequency of marker allele \( i \) in the normal population and

\[
Z_{hi}(t) = \sum_{j=1}^{k} Z(t(j)) = \# \text{ of individuals in generation } t.
\]

The expected number of children of each individual is \( 1 + \lambda \) as earlier.

### 3.4.1 Simulation 1

The first simulation is of a population of 20 generations and the population growth rate was set as 1.50. That is, each individual will get the expected number of 1.50 disease carrying children. The recombination probability between the marker and disease loci is 0.03. We fix the number of marker alleles in the population to be 5. The simulation was iterated 2000 times, but all simulations where the disease population died out before the 20\(^{th}\) generation were removed, here resulting in 1077 full iterations.

Using the previously described estimate for each iteration, we get the age distribution in Figure 3.1 below, with a mean value of 18.9809 and standard deviation 15.3634.

![Figure 3.1: Distribution of sample estimates](image-url)
3.4.2 Simulation 2

The second simulation is also of a population of 75 generations, but to illustrate the importance on the choice of marker locus, we here illustrate the case when the distance between the marker and disease loci is smaller than in the previous simulation. The recombination probability is set to 0.01. The other parameters are the same as in the first simulation example. Here we got the estimated number of generations 62.9196.

To get a feeling for the robustness of the estimate especially when based on a small number of individuals, 1000 samples of size 20 was collected. Individuals, here represented by their marker alleles, are chosen with a probability obtained from the observed allele frequencies in the last generation of the simulation. 1000 samples of size 20 from the last generation resulted in the mean estimate 65.5629 with the standard deviation 28.1057.

![Figure 3.2: Estimates](image)

As is evident in the Figure 3.2, we here got an underestimation of the number of generations. The probable cause could be that the disease is somewhat older and the marker locus is chosen a bit too closely to the disease locus. Many recombinations have had time to occur, and the allele distribution has already begun to resemble the one from the normal population.
CHAPTER 4

Haplotype and Composite Likelihood

4.1 Introduction

In this chapter, we consider the same situation as in the previous chapter except for the fact that we allow for more than one marker. Our starting point is that we know how to estimate the age of a disease using every marker separately. The question now is how such estimates could be combined.

In what follows we will consider three possible ways to do this:

1. Studying the haplotypes, using multi-type Galton Watson processes

2. Using weighted averages

3. Using composite likelihood

4.2 Haplotype

Rather than using both sister chromosomes in the diploid individuals, we concentrate on the sister chromosome carrying the dominant disease. Thereby we get a useful simplification of the model: Shortly explained, we trace the disease carrying chromosome throughout the generations.
Let us first examine the case with two markers and a disease locus, as illustrated in the Figure 4.1 below.

![Figure 4.1: Two markers](image)

As can be seen, there are five possible haplotypes resulting from a disease-allele carrying individual. The fifth case, not shown in Figure 4.1, is the case where the recombination results in an individual who did not inherit the disease allele and our interest lies only in individuals carrying the disease.

i) The first case seems trivial since the haplotype is identical to the original. Considering that recombinations can have given back the same marker/markers as before, there are four different possibilities to acquire this result.

ii) One of the markers seem to be unchanged. Thus, case ii implies a recombination between the two markers, disregarding the possibility that the recombination gave in return the same allele on one of the two markers. Thus, there might instead have been a recombination between disease locus and the first marker, leaving the first marker unchanged by chance.

iii) The third case can occur either by a recombination between the disease locus and the first marker where the new piece is carrying the same allele at marker 2 as the founder, or by two recombinations, exchanging the piece between the disease locus and the second marker.

iv) To obtain case iv with two entirely different markers but still the disease locus, the recombination has to have occurred between the disease locus and the first marker.
For simplicity, let us use the notation described in Figure 4.2 below, when describing the possible transitions. The aim of the following is to show how complicated things become in this relatively simple case with only two markers. The results arrived at can however be used as the basis of various computational investigations.

![Diagram](image)

Figure 4.2: The notation

Transitions from the marker allele pair \((i, j)\) to the four different haplotypes are shown below with the corresponding probabilities.

\[
\begin{align*}
ij &: (1 - r_1)(1 - r_2) \quad \text{no recombination} \\
(1 - r_1)r_2p_{ij} &: \text{rec. between } M_1 & M_2 \\
r_1r_2p_e &: \text{rec. between } D & M_1 \text{ and } M_1 & M_2 \\
r_1(1 - r_2)p_{ij} &: \text{rec. between } D & M_1 \\
ib &: (1 - r_1)r_2p_{ib} \quad \text{rec. between } M_1 & M_2 \\
r_1(1 - r_2)p_{ib} &: \text{rec. between } D & M_1 \\
a_j &: r_1r_2p_{a} \quad \text{rec. between } D & M_1 \text{ and } M_1 & M_2 \\
r_1(1 - r_2)p_{aj} &: \text{rec. between } D & M_1 \\
ab &: r_1(1 - r_2)p_{ab} \quad \text{rec. between } D & M_1
\end{align*}
\]

Thus, we have the following four terms: \((1 - r_1)(1 - r_2)\), \((1 - r_1)r_2\), \(r_1(1 - r_2)\) and \(r_1r_2\). Also note that \(p_i = \sum_j p_{ij}\) and \(p_j = \sum_i p_{ij}\).

The expectation matrix \(M\) will get the following appearance:
Recall that the elements \( m_{ij,ab} \) of \( M \) can be seen as the transitions from allele \( i \) to \( a \) in the first marker locus and from allele \( j \) to \( b \) in the second. Here, \( i, a = 1, 2, \ldots, n_1 \) and \( j, b = 1, 2, \ldots, n_2 \). These transitions are further described in

\[
m_{ij,ab} = \begin{cases} 
(1 - r_1)(1 - r_2) & (a,b) = (i,j) \\
(1 - r_1)r_2 p_{ab} & a \neq i \\
r_1(1 - r_2)p_{ab} & (a,b) \neq (i,j) \\
r_1r_2 p_{a} & b \neq j
\end{cases}
\]

The allele frequencies in the normal population are assembled in the \( P \) matrix:

\[
P = \begin{pmatrix}
p_{11} & \cdots & p_{1n_2} & p_{21} & \cdots & p_{2n_2} & \cdots & p_{n_11} & \cdots & p_{n_1n_2} \\
\vdots & & \vdots & \vdots & & \vdots & & \vdots & & \vdots \\
p_{11} & \cdots & p_{1n_2} & p_{21} & \cdots & p_{2n_2} & \cdots & p_{n_11} & \cdots & p_{n_1n_2} \\
\end{pmatrix}
\]

The dimensions of the matrices \( M \) and \( P \) are \( n_1n_2 \times n_1n_2 \).

We need a formula describing the \( M \) matrix. This is done a bit easier by first rewriting the form of the matrices \( M \) and \( P \). Let

\[
M = \begin{pmatrix}
M_{11} & M_{12} & \cdots & M_{1n_1} \\
M_{21} & M_{22} & \cdots & M_{2n_1} \\
\vdots & \vdots & \ddots & \vdots \\
M_{n_11} & M_{n_12} & \cdots & M_{n_1n_1}
\end{pmatrix}
\]
where $M_{ij}$ is an $n_2 \times n_2$ matrix. Thus, the mutation matrix $M$ is decomposed into several matrices $M_{ij}$, where

$$ M_{ij} = \begin{pmatrix} 
  m_{i1,j1} & m_{i1,j2} & \cdots & m_{i1,jn_2} \\
  m_{i2,j1} & m_{i2,j2} & \cdots & m_{i2,jn_2} \\
  \vdots & \vdots & \ddots & \vdots \\
  m_{in_2,j1} & m_{in_2,j2} & \cdots & m_{in_2,jn_2} 
\end{pmatrix} $$

In the same way, the $P$ matrix is split up in elements $P_i$ with the same dimension $n_2 \times n_2$

$$ P_i = \begin{pmatrix} 
  p_{i1} & p_{i2} & \cdots & p_{in_2} \\
  \vdots & \vdots & \ddots & \vdots \\
  p_{i1} & p_{i2} & \cdots & p_{in_2} 
\end{pmatrix} $$

giving us the new form of $P$ as

$$ P = \begin{pmatrix} 
  P_1 & P_2 & \cdots & P_{n_1} \\
  \vdots & \vdots & \ddots & \vdots \\
  P_1 & P_2 & \cdots & P_{n_1} 
\end{pmatrix} $$

In the formula for $M$ we also need the following matrix

$$ P.* = \begin{pmatrix} 
  p_{11} & p_{22} & \cdots & p_{n_2} \\
  \vdots & \vdots & \ddots & \vdots \\
  p_{11} & p_{22} & \cdots & p_{n_2} 
\end{pmatrix} $$

And so, the mutation matrix consists of smaller matrices of the following form

$$ M_{ij} = \begin{cases} 
  (1 - r_1)(1 - r_2)I_{n_2 \times n_2} + (1 - r_1)r_2 P.* & i = j \\
  r_1r_2 p_{ij} I_{n_2 \times n_2} + r_1(1 - r_2)P_i & i \neq j 
\end{cases} $$

where as usual, $I_n$ denotes the $n \times n$ identity matrix. The problem starts when trying to see what happens in $t$ generations. There seem to be no simple formula for describing $M^t$. 


4.3 Combining Estimates

With many markers we can use the formula

\[ \hat{t} = \frac{\ln\left( \frac{r(1-r^2)}{1-r} \right)}{\ln(1 - r)} \]

to obtain an estimate of \( t \) for each marker. A simple way to combine these is to take the average. Note, however, that if a marker is situated too close to the disease locus, few recombinations will occur and thus the age estimate will become nonsense. If instead the distance is too large, several recombinations have had time to occur and the time estimate will be less accurate. That is why the different estimations should be weighted.

One way of obtaining a combined estimate of the time \( t \) using weights, is to set \( \hat{t} = \sum_i w_i \hat{t}_i \). The \( w_i \)'s should be chosen so that \( \sum_i w_i = 1 \). One criteria of choosing the weights is to require the variance \( \text{Var}(\hat{t}) \) to be minimised subject to the condition \( \sum_i w_i = 1 \).

In the simulations to follow, the mean of the estimates is used as combined estimate. If the markers are chosen at nice symmetrical distances, we should get an acceptable estimate.

4.3.1 Simulation example - combination of estimates

The computer language used in this example to implement this method is JAVA. Further descriptions of this application can be found in the Appendix B. As long as either the number of generations or the probability of having two children is kept low, it runs painlessly.

The simulation is repeated 100 times. Having the pairwise map distance of 1 cM, we fix the number of alleles for each of the eight markers to 5. The probability of having one offspring is 0.85 and we continue the simulation for 50 generations.

We get the following distribution:
The simulation resulted in the mean estimate 52.9184 and the standard deviation 23.5796.
4.4 Composite likelihood

To be able to use information from several markers at the same time, the method of composite likelihood is convenient. An estimate is obtained by combining information from several (possibly dependent) sources by adding together conditional or marginal log likelihoods (cf. (Devlin et al., 1996) and (Pankratz, 1998)).

Let $L_i(t)$ be the likelihood function for marker $i$. Then, $l_i(t)$ is the log likelihood function (marginal or conditional). These are combined as the composite likelihood function according to

$$CL(t) = \sum_{i=1}^{k} l_i(t).$$

The value of the parameter $t$ that maximises the composite likelihood function solves the equation

$$\frac{\delta CL(t)}{\delta t} = \sum_{i=1}^{k} \frac{\delta l_i(t)}{\delta t} = 0.$$

The terms $S_i(t) = \frac{\delta l_i(t)}{\delta t}$ can also be called the (composite) score functions.

Another way to obtain the most probably value of $t$, is to find the
maximum of the $CL(t)$ function,

$$CL(t) = \sum_{i=1}^{k} l_i(t),$$

where the log likelihood functions $l_i(t)$s are found using calculations similar to those in Chapter 3.3.2.

We can use what we already know, and find a somewhat more simplified equation to maximise:

$$l_i(t) = n_i^{(i)} \ln(p_{ni}^{(i)} + (1 - p_{ni}^{(i)})(1 - r^{(i)})^t) + \sum_{j=2}^{k} n_j^{(i)} \ln(p_{nj}^{(i)} (1 - (1 - r^{(i)})^t)) =$$

$$n_i^{(i)} \ln(p_{ni}^{(i)} + (1 - p_{ni}^{(i)})(1 - r^{(i)})^t) + \sum_{j=2}^{k} n_j^{(i)} \ln(1 - (1 - r^{(i)})^t)$$

independent of $t$

Thus, maximum of $CL(t)$ is obtained at the same parameter value of $t$ as in the somewhat simpler

$$l_i(t) = n_i^{(i)} \ln(p_{ni}^{(i)} + (n - n_i^{(i)})(1 - p_{ni}^{(i)})(1 - r^{(i)})^t) + \ln(1 - (1 - r^{(i)})^t).$$

The Figure 4.3 illustrates the composite likelihood estimate of the age of a disease, where data is taken from a simulation of two markers where the first marker is chosen at the distance of 1 cM from the disease gene and the second marker is located 1 cM from the first marker. Both markers have 5 alleles each. 50 generations are simulated and the result of the method of moment estimate of 886 iterations are shown in the Figure 4.4. The data used in the above composite likelihood example is chosen from the simulation iteration for which the method of moment yielded an age estimate of 50.1053 generations. It is possible to get several composite likelihood estimates using the simulation data, but we use only this example to illustrate that the composite likelihood method seem to give rather low estimates of the mutation age. The same result is obtained in (Pankratz, 1998).
Figure 4.3: The maximum is the composite likelihood estimate of $t$

Figure 4.4: Estimates using method of moments
CHAPTER 5

The example of a BRCA1 mutation

5.1 Background

In this chapter, the model presented in the previous chapters is applied to real data to find an age estimate of a founder mutation found on the west coast of Sweden resulting in mammary cancer.

The study consists of 18 apparently unrelated families, where at least one member in each family carries a specific mutation in the BRCA1 gene (called 3171ins5). All families seem to have their geographical origin in the western part of Sweden.

Thirteen polymorphic markers have been genotyped for all available family members and by using the software GENEHUNTER ((Kruglyak et al., 1996) and (Kruglyak and Lander, 1998)) the haplotypes were derived.

To estimate the time $t$ since the appearance of the mutation, an estimate found using the method of moments, based on the theory of Galton-Watson branching processes is used:

$$
\hat{t} = \frac{\ln(\hat{p}_{a1} - p_{a1})}{\ln(1 - r)}.
$$

Time is measured in the number of generations since the appearance of the mutation in the population.

For every marker, the founder allele frequency in the sample can
be estimated by the observed allele frequency, \( \hat{p}_{dl} = \frac{n_{dl}}{n_d} \), where \( n_{dl} \) is the number of individuals carrying both the disease allele and the founder allele and \( n_d \) is the sample size.

We assume that the normal population is stable, i.e. that the allele frequencies \( p_{ni} \), are unchanged from generation to generation. Here, \( i \in \{1, \ldots, k\} \) and \( k \) is the number of alleles at that certain locus in the population. As estimates of the normal allele frequencies, the observed frequencies are used.

Here, the allele frequencies were based on the haplotypes of 18 – 20 disease-allele carrying individuals and 31 – 38 individuals assumed normal (healthy family members not carrying the disease mutation). The locations of the markers in relation to the disease locus is shown in the linkage map in Figure 5.1.

The recombination frequency \( r \) is found using the Haldane map function (see (Haldane, 1919)), translating map distances to recombination frequencies:

\[
r_{AB} = \frac{1}{2}(1 - e^{-2d_{AB}/100})
\]

where \( d_{AB} \) is the distance between two loci \( A \) and \( B \), measured in cM and hence \( r_{AB} \) is the recombination frequency.

The criteria for choosing founder allele is the most common allele among the families with confirmed disease-associated allele.

5.2 Results from family data

Using the methods described in the previous chapters, the following table gives estimates of the age of the mutation, based on the frequencies of the different marker alleles available.

Using the mean of these estimates result in an estimate of 127.7 generations. Assuming that a generation is 25 years long, we conclude that the disease is about 3190 years old. One of the markers yield an unreasonable result, probably out of one of the reasons stated below. Removing that particular marker, gives us an estimate of 85.4 generations, implying an age of 2135 years. When removing all markers situated at a distance less than 1 cM, we get the estimate 60.3 generations, or 1508 years.
5.2 Results from family data

<table>
<thead>
<tr>
<th>Marker</th>
<th>Age estimate</th>
<th>Founder allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>D17s1872</td>
<td>33.88</td>
<td>133</td>
</tr>
<tr>
<td>D17s946</td>
<td>61.55</td>
<td>126</td>
</tr>
<tr>
<td>D17s250</td>
<td>118.24</td>
<td>156</td>
</tr>
<tr>
<td>D17s800</td>
<td>99.47</td>
<td>173</td>
</tr>
<tr>
<td>D17s1299</td>
<td>62.83</td>
<td>192</td>
</tr>
<tr>
<td>D17s846</td>
<td>223.37</td>
<td>234</td>
</tr>
<tr>
<td>D17s1321</td>
<td>508.08</td>
<td>144</td>
</tr>
<tr>
<td>D17s855</td>
<td>-</td>
<td>144</td>
</tr>
<tr>
<td>D17s1325</td>
<td>122.92</td>
<td>195</td>
</tr>
<tr>
<td>D17s902</td>
<td>29.56</td>
<td>152</td>
</tr>
<tr>
<td>D17s588</td>
<td>16.74</td>
<td>154</td>
</tr>
<tr>
<td>D17s790</td>
<td>-</td>
<td>187</td>
</tr>
<tr>
<td>D17s787</td>
<td>-</td>
<td>137</td>
</tr>
</tbody>
</table>

Comments:

Our estimate can only be used when \( \hat{p}_{dl} > p_{n1} \), i.e. when the founder allele is more common in the disease population than among the normal population. Thus, the markers D17s855, D17s790 and D17s787 will not provide any information.

The markers located at a distance less than 1 cM from the disease locus result in estimates much larger than expected. This effect can be out of several reasons, some of them mentioned in the sources of error section below.

To get a feeling of the estimate variance, bootstrapping should be performed. This will be done in a coming article by (Bergman et al., 2000).

There are several sources of error:

*Founder allele:* The choice of founder allele is naturally of importance, since the difference between allele frequencies in the two populations is the foundation of the estimate.

*Genotyping:* Genotyping errors caused in the laboratory will result in errors in the observed allele frequencies and hence give us a skew estimate.
Haplotyping errors caused by the software GENEHUNTER will result in errors in the observed allele frequencies and hence give us a skew estimate.

Recombination frequency: If a marker is set at a location closer to the disease locus than it is in reality, we will get a larger estimate, implying an older mutation. The estimate is very sensitive for changes in this parameter.
Figure 5.1: The linkage map: chromosome 17q21
The example of a BRCA1 mutation
CHAPTER 6

Mutations and Homozygosity Mapping

6.1 Introduction

Loosely speaking, homozygosity mapping is concerned with locating the locus of the disease gene for a recessive disease. Since an affected individual is necessarily homozygous for the disease, we expect such an individual to be homozygous also for markers surrounding the disease gene.

The method of homozygosity mapping is generally used for mapping rare recessive traits in children of consanguineous marriages. The disease is more probable to arise in inbred children due to the phenomenon homozygosity by descent in the adjacent region around the disease locus.

Homozygosity mapping is known to be convenient to use for mapping recessive diseases using data based on a very small number of families with few affected offspring in each, since data from unrelated individuals can be combined (cf. (Lander and Botstein, 1987)).

Recently (Amos et al., 1999) have proposed the use of homozygosity mapping based on population (as opposed to family) data. In this chapter we propose a Galton-Watson model to describe such data. This model has two new characteristics: We consider the case of a recessive single-locus disease and assume that the disease is old. The second assumption implies that mutations in the marker can no longer
be ignored. For the exact nature of the marker, see below.

The use of Galton-Watson processes as models of recessive traits has been criticised in the literature (Lange and Fan, 1997), but as shown in (Kaplan and Weir, 1997) and (Pankratz, 1998) the results based on such models are quite good.

6.2 The Model

Again, we have the transition matrix $P$ as earlier. We assume a stepwise mutation model (SMM) as described in the Chapter 2 and the mutation matrix $U$,

$$
U = \begin{pmatrix}
1 - v & v & \cdots & 0 \\
0 & 1 - u - v & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
0 & 0 & \cdots & 1 - u
\end{pmatrix}
$$

We now have two cases:

1. Mutation occur before the recombination

$$
M_{mr} = (1 + \lambda)U[(1 - r)I + rP] = (1 + \lambda)[(1 - r)U + rUP] = (1 + \lambda)[(1 - r)U + rP]
$$

2. Recombination occur before the mutation

$$
M_{rm} = (1 + \lambda)[(1 - r)I + rU]U = (1 + \lambda)[(1 - r)U + rPU]
$$

So, do mutations occur before or after the recombination event? Most likely, the mutations should occur in the process of DNA replication and since replication occur before recombination, then mutations should also occur before recombination (cf. (Pankratz, 1998)). I will thereby use $M_{mr}$ as mutation matrix, simply calling it $M$.

Assume that mutation events occur independently of recombination events. The formula $\pi = \pi U$ gives us the stationary distribution of $U$. Here, $\pi$ is the stationary distribution and $U$ is the mutation matrix. Our transition matrix $P$ will thereby get this form:

$$
P = \begin{pmatrix}
\pi \\
\pi \\
\vdots \\
\pi
\end{pmatrix}.$
Since every row of $P$ is the stationary distribution of $U$, the following is valid: $PU = P$, $UP = P$ and $P^2 = P$.

Now, the interesting question was: What does $M^t$ look like? Some long calculations show that

$$\frac{M^t}{(1 + \lambda)^t} = (1 - r)^t U^t + (1 - (1 - r)^t)P$$

where $M = (1 - r)U + rP$. The $i^{th}$ row is obtained as

$$e_i^t = (1 - r)^t U_{ij}^t + (1 - (1 - r)^t)p_{nj}$$

where $e_i = (0, \ldots, 0, 1, 0, \ldots, 0)$, i.e. a 1 at the $i^{th}$ position, 0s otherwise. The reason for our interest in the $i^{th}$ row in this matrix is that we wish to find the distribution of the founder marker allele which we placed in the $i^{th}$ position.

The transitions of the SMM model are described in Figure 6.1. As

\[ \begin{array}{ccc}
    u & v \\
    i-1 \quad i \quad i+1 \\
    1-u-v
    \end{array} \]

Figure 6.1: Transitions

an example of how $U$, $\pi$ etc can look like, we consider an example. Assume now that the evolution of the marker allele follows a SMM model (cf. Chapter 2). Since we assume that a long time has passed since the appearance of the disease, it is plausible to require that the values of the marker have reached stationarity. Using the formula $\pi = \pi U$ we obtain

$$\pi_2 = \frac{v}{u} \pi_1$$
$$\pi_2 = v \pi_1 + (1 - u - v) \pi_2 + u \pi_3$$
$$\Leftrightarrow u \pi_3 = u \pi_2 + v \pi_2 - v \pi_1 = v \cdot \frac{v}{u} \pi_1$$
$$\Leftrightarrow \pi_3 = (\frac{v}{u})^2 \pi_1$$

Thus, $\pi_j = \frac{v}{u} \pi_{j-1} = \frac{v}{u} \cdot (\frac{v}{u})^{j-2} \pi_1 = (\frac{v}{u})^{j-1} \pi_1$. 

Let \( \rho = \frac{u}{v} \) and hence \( \pi_j = \rho^{j-1}\pi_1 \). Since \( \sum_{i=1}^{k} p_{ni} = 1 \), where \( p_{ni} = \pi_1 \rho^{i-1} \), we get that

\[
1 = \sum_{i=1}^{k} p_{ni} = \sum_{i=0}^{k-1} \pi_1 \rho^i = \pi_1 \sum_{i=0}^{k-1} \rho^i = \pi_1 \frac{1 - \rho^k}{1 - \rho} \Rightarrow \pi_1 = \frac{1 - \rho}{1 - \rho^k} = c.
\]

Finally, we get an expression for our transition matrix \( P \)

\[
P = \frac{1}{1 - \rho^k} \begin{pmatrix}
p_{n1} & p_{n2} & \cdots & p_{nk} \\
p_{n1} & p_{n2} & \cdots & p_{nk} \\
p_{n1} & p_{n2} & \cdots & p_{nk}
\end{pmatrix} = \begin{pmatrix}
c & c\rho & \cdots & cp^{k-1} \\
c & c\rho & \cdots & cp^{k-1} \\
c & c\rho & \cdots & cp^{k-1}
\end{pmatrix}.
\]

Recall that \( r \) is the recombination frequency, that is, \((1 - r)^t = P(\text{no recombination in } t \text{ generations})\) and thus \(1 - (1 - r)^t = P(\text{at least one recombination})\). The formula

\[
\frac{M^t}{(1 + \lambda)^t} = (1 - r)^t U^t + (1 - (1 - r)^t) P
\]

admits the following interpretation. It can be viewed as a probability distribution \( f \) consisting of a mixture of two distributions, \( f_1 \) and \( f_2 \), according to

\[
f(x) = \phi f_1(x) + (1 - \phi) f_2(x).
\]

Misusing the notation we can represent the expectation and the variance of \( f(x) \) as

\[
E[f(x)] = \phi E[f_1(x)] + (1 - \phi) E[f_2(x)]
\]

and

\[
\text{Var}(f(x)) = \phi^2 \text{Var}(f_1(x)) + 2\phi(1 - \phi) \text{Cov}(f_1(x), f_2(x)) + (1 - \phi)^2 \text{Var}(f_2(x)).
\]

### 6.3 Homozygosity mapping

The reasoning behind this method is shown in Figure 6.2. Individuals with a recessive disease being homozygous for the disease gene should also be homozygous for markers in the vicinity of that gene. This provides a criteria for mapping the disease genes. Moreover comparing individuals homozygous for the disease with normal
individuals allows us to find regions that differ considerably between the two populations and hence to find the disease locus. Due to homozygosity by descent, individuals carrying the disease, should also be homozygous in the adjacent area of the disease locus to much larger extent than expected (cf. (Kruglyak et al., 1995) and (Lander and Botstein, 1987)).

\[ \Delta_1^i \quad \cdots \quad \Delta_1^j \quad \Delta_1^{j+1} \]

Disease-carrier

\[ \Delta_2^i \quad \cdots \quad \Delta_2^j \quad \Delta_2^{j+1} \]

Normal individual

Figure 6.2: Difference in length between marker alleles

We have thus two ways of mapping a disease gene:

1) search for approximately homozygous markers bearing in mind that homozygosity can be lost due to mutations.

2) to amplify the affect in i we focus on the difference in length of marker allele pairs (in Figure 6.2 called \(\Delta_i^m\) for the \(m^{th}\) marker in the disease population and \(\Delta_i^n\) for the same marker in the normal population), comparing this measure between individuals in the disease population and in the normal population. For markers tightly linked to the disease locus, the \(\Delta_1^i\) distance will be very small due to homozygosity by descent but the \(\Delta_2^i\) distance will be normal. Looking instead at markers situated at the other end of the chromosome, hence not linked to the disease locus, the distances \(\Delta_1\) and \(\Delta_2\) will both be normal.

To find a measure to help us search for the disease locus by comparing these two populations, we can form the distance measure \( \Delta = |\Delta_1 - \Delta_2| \). This measure is calculated for a sample of individuals from each
population by performing a gene-scan. When $\Delta$ is large in some sense for a marker, it will be close to the disease locus.

Consider an individual homozygous for the disease locus, and thus hopefully for the marker locus. Let $D_1 = I - J$, where $I$ is the length of the marker allele on the first chromosome in the pair, and $J$ is the length of the marker allele on the second. $I$ and $J$ are independent and have the distribution in the formula above $\left( \frac{M^t}{1 + \lambda} \right)$. Then $D_1$ can be considered as a distance between the two alleles. Since $\mathbb{E}[D_1] = 0$, $D_1$ cannot give us that much information so we need to find a better measure, for example

$$\Delta_1 = D_1^2 = (I - J)^2 = I^2 + J^2 - 2IJ.$$ 

Hereby, we get that

$$\mathbb{E}[\Delta_1] = 2\mathbb{E}[I^2] - 2(\mathbb{E}[I])^2 = 2(\mathbb{E}[I^2] - (\mathbb{E}[I])^2) = 2\text{Var}(I).$$

Now we need the variance of $I$, that is the variance of the length of the marker allele. Recall the distribution of $I$, $f(t) = (1-r)^tU^t + (1-(1-r)^t)P$. That is, we have a mixture of $f_1(t) = U^t$ and $f_2(t) = P$. Let $\mathbb{E}_1$ and $\text{Var}_1$ refer to the former and $\mathbb{E}_2$ and $\text{Var}_2$ to the latter. We have that

$$\mathbb{E}_2[I] = \sum_k k\pi_k = \mu_2,$$

where $\pi_k$ is the stationary distribution, and

$$\text{Var}_2(I) = \sum (k - \mu_2)^2 \pi_k = \sigma_2^2$$

is the variance.

Let $I = \sum_i X_i$, where $X_i$ is the mutation in step $i$. We use the strict stepwise mutation model (SMM) with equal probabilities of adding and subtracting one, that is, $P(X_i = 1) = P(X_i = -1) = \theta/2$ and $P(X_i = 0) = 1 - \theta$ (see Figure 2.2) and, of course, the $X_i$’s are considered independent. We get

$$\mathbb{E}_1[I] = 0$$

and

$$\text{Var}_1(I) = \mathbb{E}_1[I^2] = \text{Var}_1\left( \sum_i X_i \right) = \sum_{i=1}^t \text{Var}_1(X_i) = t\theta.$$
Notice that this formula shows that, knowing $\text{Var}(I)$ and $\theta$, $t$ can be estimated.

Now, the expectation of $\Delta_1$ can be calculated:

$$E[\Delta_1] = 2 \text{Var}(I) = 2(1-r)^4 t \theta + 2(1-(1-r)^4) \sigma_2^2 + 2(1-r)^4 (1-(1-r)^4) \mu_2^2.$$  

As mentioned earlier, the effect of homozygosity mapping can be amplified by looking for regions with large $\Delta$-values between affected and normal individuals. For this reason, it is of interest to consider the case of a person showing no symptoms of the disease. We are interested in a rare disease, and thus very few people in the population should be carrying the disease allele in any of the two loci. We thereby assume that the people in this category carry some other allele than the disease allele.

Let $D_2 = I - J$ as before. $I$ and $J$ follow the distribution $P$, which is the distribution of alleles of the marker loci for 'normal' persons.

Again, $E[D_2] = 0$, so we use $\Delta_2 = D_2^2$ instead. $E[\Delta_2] = 2 \text{Var}(I) = 2\sigma_2^2$ is the expectation.

Under the assumption of no linkage,

$$E[\Delta_1] - E[\Delta_2] \approx 0.$$  

No linkage: $r = 1/2 \Rightarrow E[\Delta_1] - E[\Delta_2]$

\begin{align*}
&= 2(1/2)^4 t \theta + 2(1-(1/2)^4) \sigma_2^2 + 2(1/2)^4 (1-(1/2)^4) \mu_2^2 - 2\sigma_2^2 \\
&= 2(1/2)^4 t \theta - 2(1/2)^4 \sigma_2^2 + 2(1/2)^4 (1-(1/2)^4) \mu_2^2 \\
&= 2(1/2)^4 (t \theta - \sigma_2^2 + (1-(1/2)^4) \mu_2^2)
\end{align*}

What we hope to find, using a gene scan, is a marker linked to the disease allele.

In (Amos et al., 1999), a simulation study is performed which confirms the usefulness of this method. The same reference contains an application to real data.
Bibliography


APPENDIX A

Simulation, one marker

Consider the case with one marker locus linked to the disease locus, chosen at an appropriate distance.

Parameters:

• \(k = \#\) alleles at marker locus

• \(t = \#\) generations

• \(r\) is the recombination frequency

\(1 + \lambda\) is the expected number of offspring of any given person

Assumptions:

• We keep track of the number of individuals in each generation carrying one of the \(k\) marker alleles.

• For simplicity, the marker allele quantities are assumed to be uniformly distributed in the normal population.

• The \((t + 1)^{th}\) generation is generated according to the formula

\[
Z_{t+1}(i) \sim \text{Poi} \left( (1 + \lambda) \left[ (1 - r) Z_t(i) + r p_{ni} Z_{tot}(t) \right] \right),
\]

where

\[
Z_{tot}(t) = \sum_{j=1}^{k} Z_t(j) = \# \text{ of individuals in generation } t.
\]
• The age of the disease is estimated using the formula

$$\hat{t} = \frac{\ln(p_{d1} - p_{a1})}{\ln(1 - r)}$$

where $p_{d1}$ is the observed allele frequency in the disease population.

• We are sampling individuals from the last generation.
APPENDIX B

Simulation, eight markers

- Each individual is represented by haplotypes. We do not need information of the sister chromosome not carrying the disease. The rest of the genome is also irrelevant in our case.

- Since we are only interested in the carriers in the last generation, we only include carriers in the simulation. Each individual begets one or two offspring carrying the disease allele.

- We track eight markers surrounding the disease locus, four on each side of the disease locus, preferably chosen at somewhat symmetrical distances surrounding the disease locus.

- Recombination events are allowed.

The implementation is as follows: for each interval, we compare a random number with the recombination probability and if it
results in a recombination event, the marker alleles in the area from the interval of interest and the end, seen in the opposite direction of the disease locus, is exchanged. The new marker alleles are chosen from the stable population of normal alleles, here assumed to be uniformly distributed.

- The map distances are translated to recombination frequencies using the Haldane map function (see Haldane, 1919):

\[ r_{AB} = \frac{1}{2} \left(1 - e^{-2d_{AB}/100}\right) \]

Note that \( d_{AB} \) is the distance between the two loci \( A \) and \( B \), measured in cM.

- We calculate eight estimates of the number of generations, \( t \), using the formula: \( \hat{t} = \frac{\ln(\frac{1-p_1}{p_1})}{\ln(1-r)} \). As a final estimate, we use the observed mean value of these.

- Only the last generation is saved to save computer memory, so we cannot backtrack our simulation.

The computer language used to implement this method is JAVA. As long as either the number of generations or the probability of having two children is kept low, it runs painlessly.

The simulation parameters are:

\[ p = P(\text{individual begets one offspring}) \]

Thus, \( 1 - p = P(\text{individual begets two offspring}) \)

\[ g = \# \text{ of generations} \]

\( c \) is a vector describing the number of alleles of each of the eight markers.

\( r \) is the vector containing the eight distances between pairs of markers.