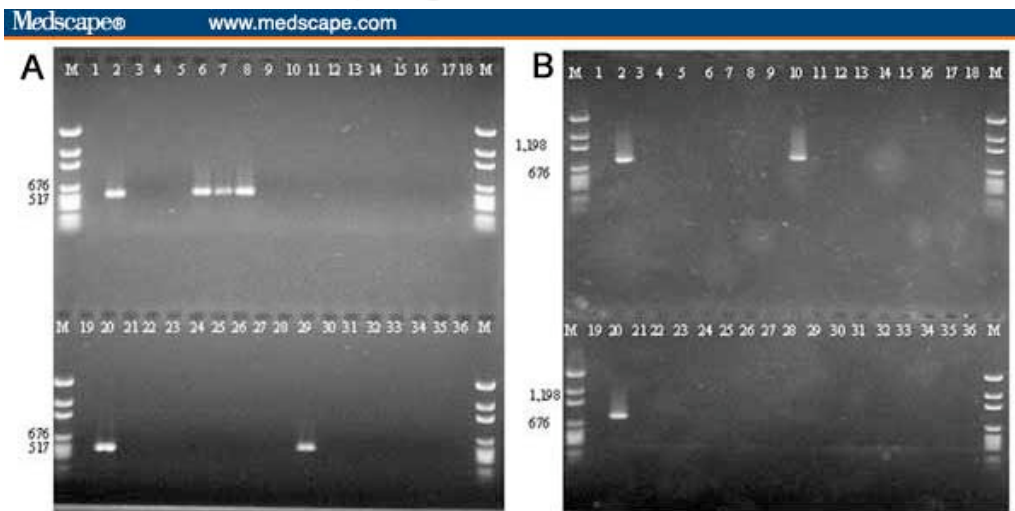
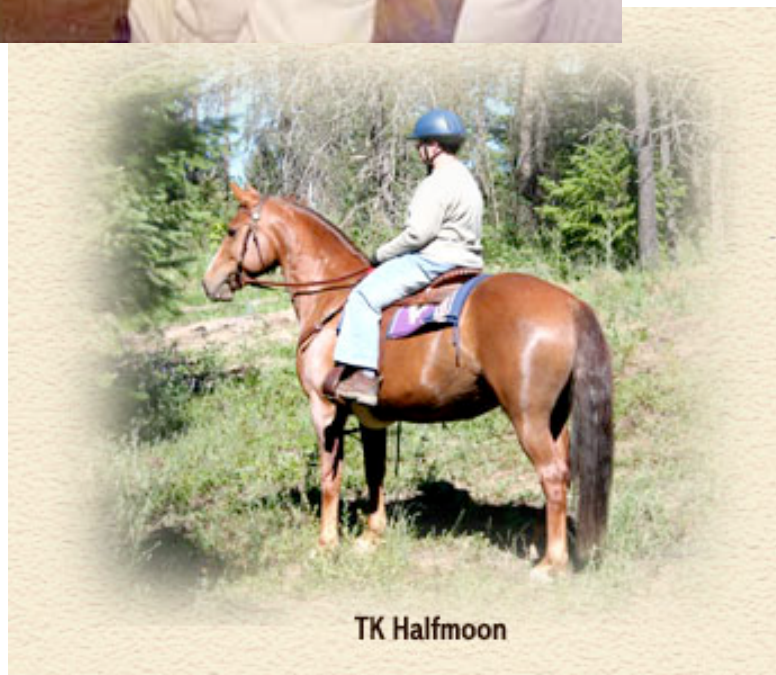
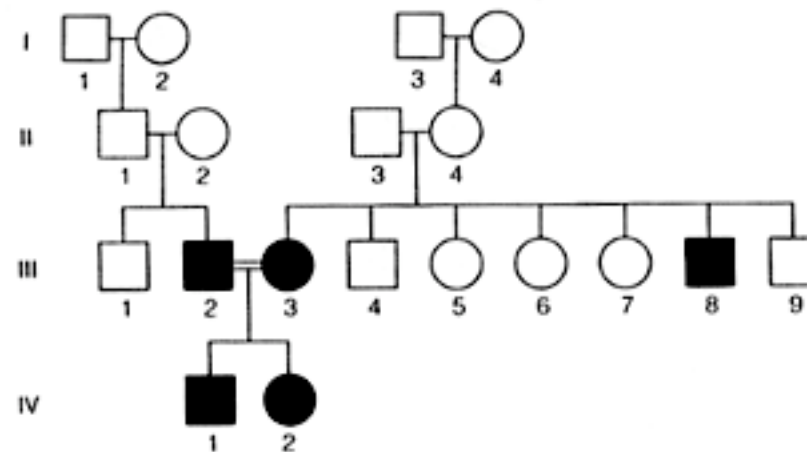


Section 14. Pedigree Analysis and Molecular Markers



Source: Emerg Infect Dis © 2003 Centers for Disease Control and Prevention (CDC)

Basic Pedigree Analysis

In humans, one mating (one pair of parents) rarely has enough children to give reliable ratios. In that case one can still do pedigree analysis. Modern pedigree analysis is much more sophisticated than anything we can do; we will use pedigrees mainly as good practice in basic genetic analysis.

Mendel could control his crosses, mate any pea plant with any other. We have to take advantage of "natural experiments":

1. Find individual with unusual trait whose inheritance is to be studied (= propositus).
2. Examine as many relatives as possible for presence of trait, and construct pedigree.
3. Analyze to determine mode of inheritance.

Very important in medical genetics and genetic counseling.

For students who want an extra source of practice problems or another trimmed-down introduction to genetics, some students in previous classes have used Schaum's Outline of Genetics. It is available from the bookstore on order, delivery time one or a few days.

On the exam, if you answered 20,000 to the second part of question 15, return your exam to us today or next week and we will give you 3 points. This is because an error in the lecture Section 11 on Sex and Meiosis would lead you to give that answer. The corrected version of Section 11 is on the web. The corrected sequence of events in male and female animal gametogenesis is:

spermatogonia –mitosis-> primary spermatocytes –MI-> secondary spermatocytes
-MII-> spermatids -> differentiate-> sperm

oogonia –mitosis-> primary oocyte –MI-> secondary oocyte –MII-> ovum (egg)
+ polar body + polar body

NOTE: This does NOT affect the answer to the first part; one oocyte gives rise to only one egg.

This will increase the mean score somewhat so I won't post the statistics until we have corrected the grades.

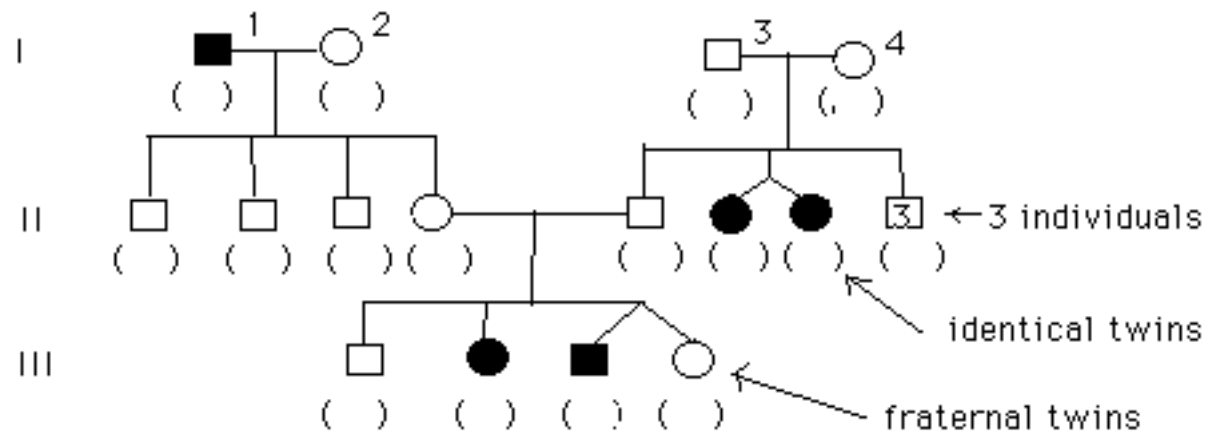
Generations labelled roman numerals I, II, ...

Individuals labelled arabic numerals 1, 2, ...

Males square, females round.

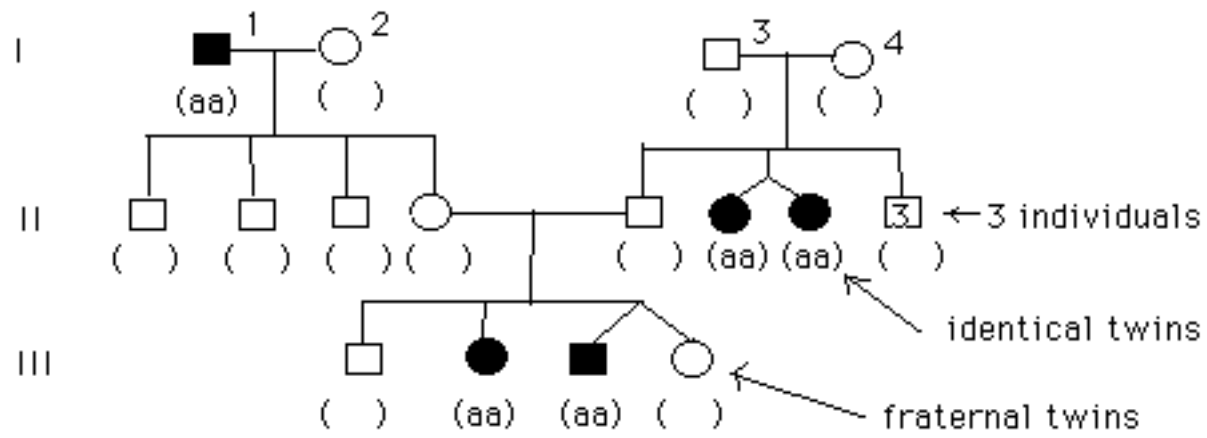
Shaded = affected.

Mated individuals connected by "marriage line".



Try simplest hypothesis first: 1 gene, 2 alleles, complete dominance, affected are homozygous recessive. Fill in genotypes in steps:

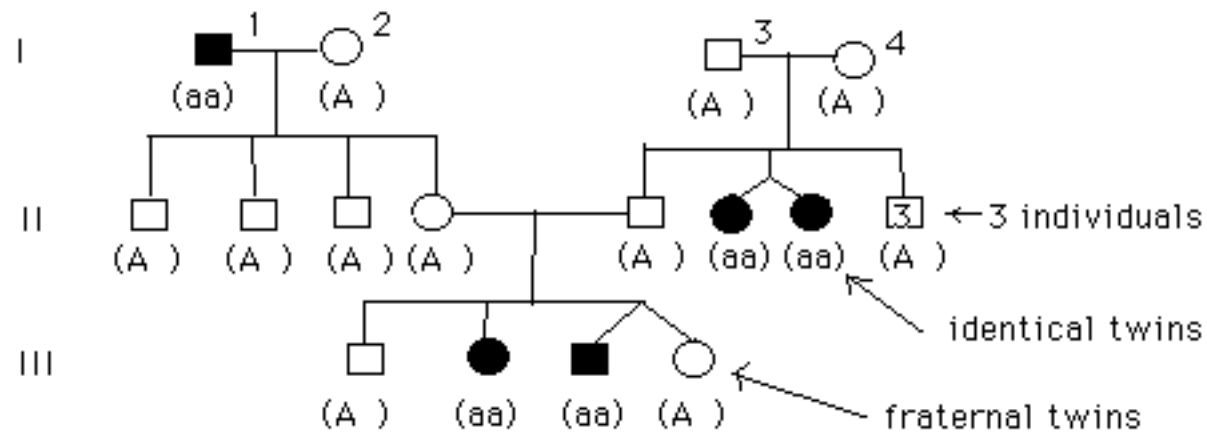
(1) All affected are homozygous recessive.



Try simplest hypothesis first: 1 gene, 2 alleles, complete dominance, affected are homozygous recessive. Fill in genotypes in steps:

(1) All affected are homozygous recessive.

(2) All unaffected have at least one dominant allele.

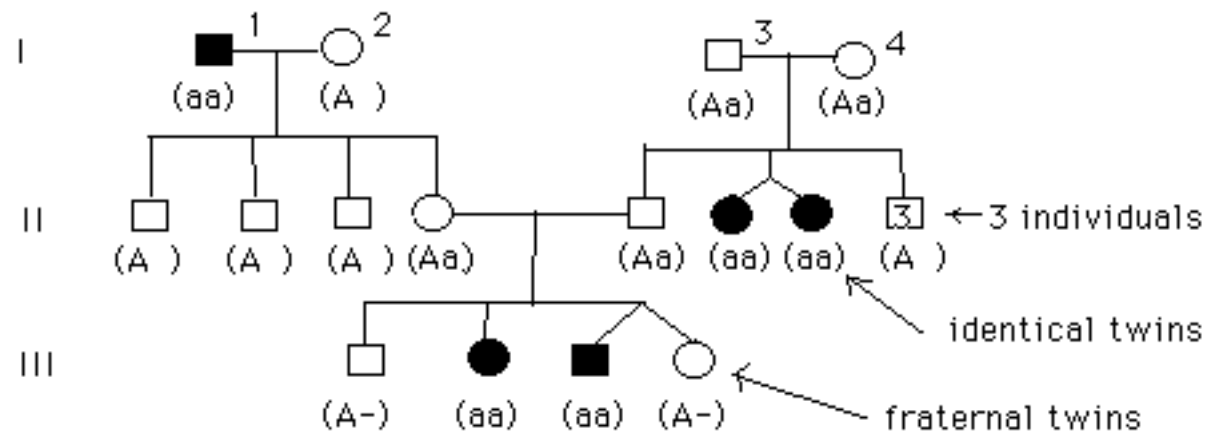


Try simplest hypothesis first: 1 gene, 2 alleles, complete dominance, affected are homozygous recessive. Fill in genotypes in steps:

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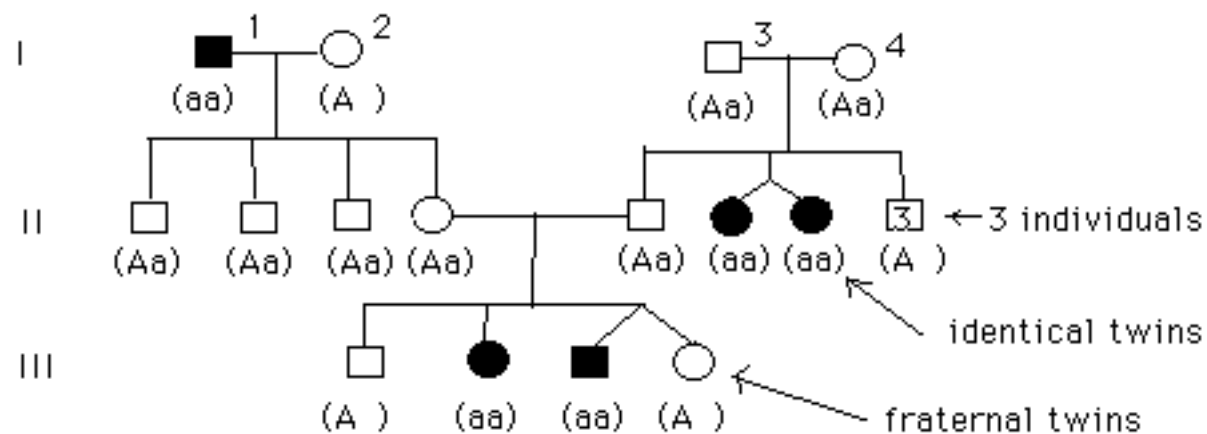
(2) All unaffected have at least one dominant allele.

(3) All homozygous recessive must get one recessive allele from each parent.



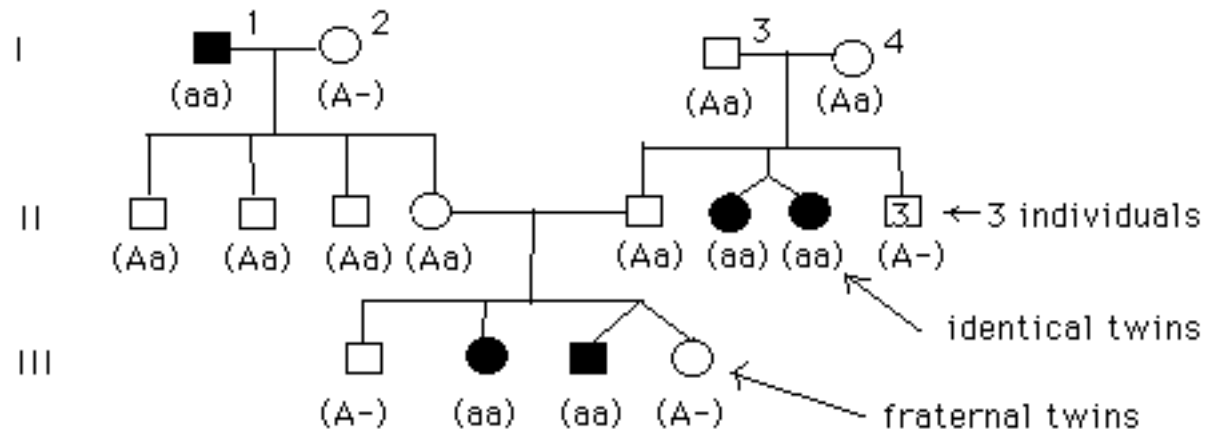
Try simplest hypothesis first: 1 gene, 2 alleles, complete dominance, affected are homozygous recessive. Fill in genotypes in steps:

- (1) All affected are homozygous recessive.
- (2) All unaffected have at least one dominant allele.
- (3) All homozygous recessive must get one recessive allele from each parent.
- (4) All offspring of homozygous recessive must have at least one recessive allele.



Try simplest hypothesis first: 1 gene, 2 alleles, complete dominance, affected are homozygous recessive. Fill in genotypes in steps:

- (1) All affected are homozygous recessive.
- (2) All unaffected have at least one dominant allele.
- (3) All homozygous recessive must get one recessive allele from each parent.
- (4) All offspring of homozygous recessive must have at least one recessive allele.
- (5) For rest of genes, use $-$ = allele unknown.



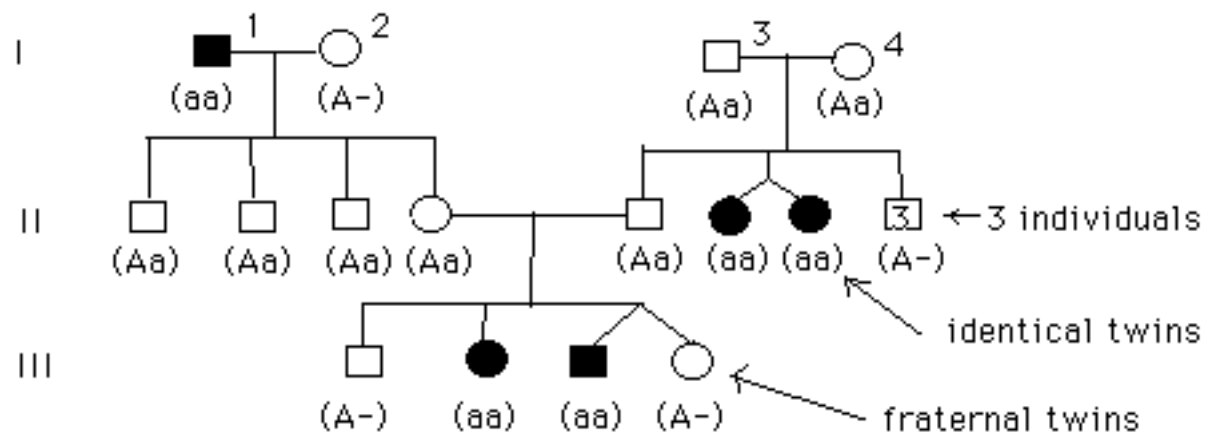
We have filled in the pedigree without finding any internal contradictions, i.e. without contradicting our hypothesis.

The genotype of I2 can be AA or Aa . Can we deduce that it is AA because it had no aa offspring?

NO: if it is Aa , the probability of getting 4 Aa out of 4 offspring is $(1/2)^4 = 1/16 > 1/20$ or 0.05, too high to reject.

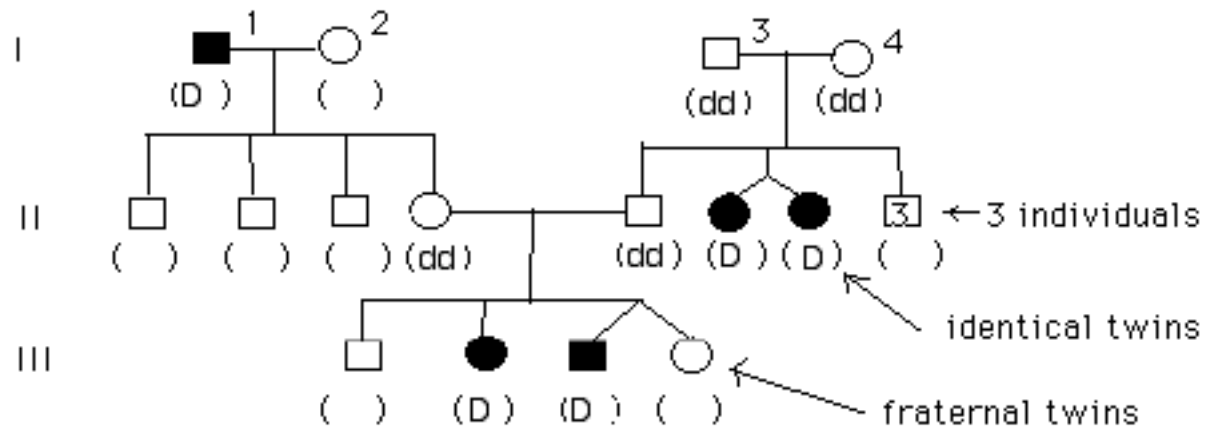
In general, one cannot use ratios to determine genotypes in pedigrees, because the sample size is too small.

However, one can calculate the probabilities that I 2 is AA or Aa , taking into account the information from the offspring. Important in genetic counseling, where I 2 may want to know the probability that her next offspring will be affected. Uses a method of conditional probability called Bayes' theorem. The conditional probability that I 2 is homozygous is 0.6124; this is the probability that would be used in genetic counseling.



Return to pedigree with albinism. Try with other hypothesis, with albinism due to dominant allele D , normals dd :

Problem: the identical twins in generation II, and III 2 and 3, must have dominant allele, must have got from one or other parent, so one of their parents would have to be albino. Internal contradiction.



Molecular Markers

A major problem in studying and treating human hereditary diseases is our inability to identify heterozygous carriers of recessive genetic defects. Need to do so to counsel them about having children. (Also problem in doing genetics with any diploid organism.)

Many parents would like to use amniocentesis to find out if the embryo they are carrying will develop hereditary defects, at a developmental stage when abortion is still an option.

Even some dominant defects cannot always be detected in time, because symptoms may not appear before individuals reach reproductive maturity. Show incomplete penetrance = failure to be expressed in all individuals of the appropriate genotype.

e.g. Huntington's disease (Huntington's chorea):

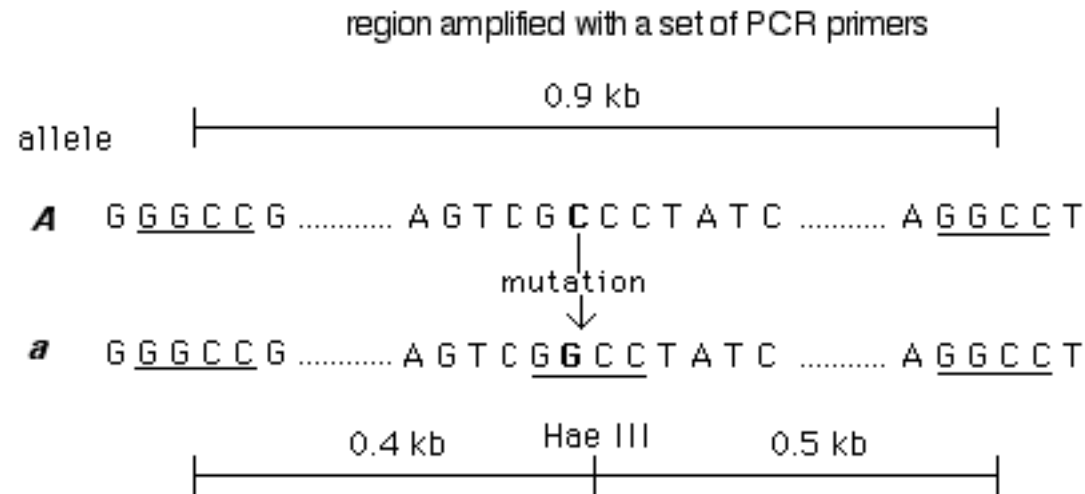
age penetrance = proportion of individuals who are known to carry the genotype for the disease and actually show symptoms by this age

| | |
|----|------|
| 30 | 0.1 |
| 40 | 0.3 |
| 50 | 0.6 |
| 60 | 0.85 |
| 70 | 0.95 |

Molecular markers can solve these problems. Many ways of detecting polymorphic differences.

RFLP = restriction fragment length polymorphism: some individuals in a population have a particular restriction site; others lack it.

Can be due to difference in single base pair (single nucleotide polymorphism = SNP), or to insertions or deletions.



Now to see whether individual is *AA*, *Aa*, or *aa*, isolate a small sample of DNA, amplify this region, restrict the amplified DNA with HaeIII, run on gel to separate fragments and estimate their sizes.

| genotype | fragments |
|-----------|---------------|
| <i>AA</i> | 0.9 |
| <i>Aa</i> | 0.9, 0.5, 0.4 |
| <i>aa</i> | 0.5, 0.4. |

Mutations that are due to a transposable element can be detected by PCR of the region and checking its size on a gel.

Mutations that are due to a transposable element can be detected by PCR of the region and checking its size on a gel. E.g. *wrinkled* allele in peas, *white* in *Drosophila*.

In these cases the molecular marker is the size difference between the alleles that we are trying to detect. More common case is when molecular marker is closely linked to the difference between our subject alleles.

Especially useful are DNA regions of tandem repeats of 2-9 nucleotides known variously as:

- microsatellites
- simple sequence repeat polymorphism (SSRP)
- simple tandem repeat polymorphism (STRP)
- short tandem repeats (STR)

Hypothetical example:

GACGACGACGAC (GAC)₄
GACGACGACGACGAC (GAC)₅

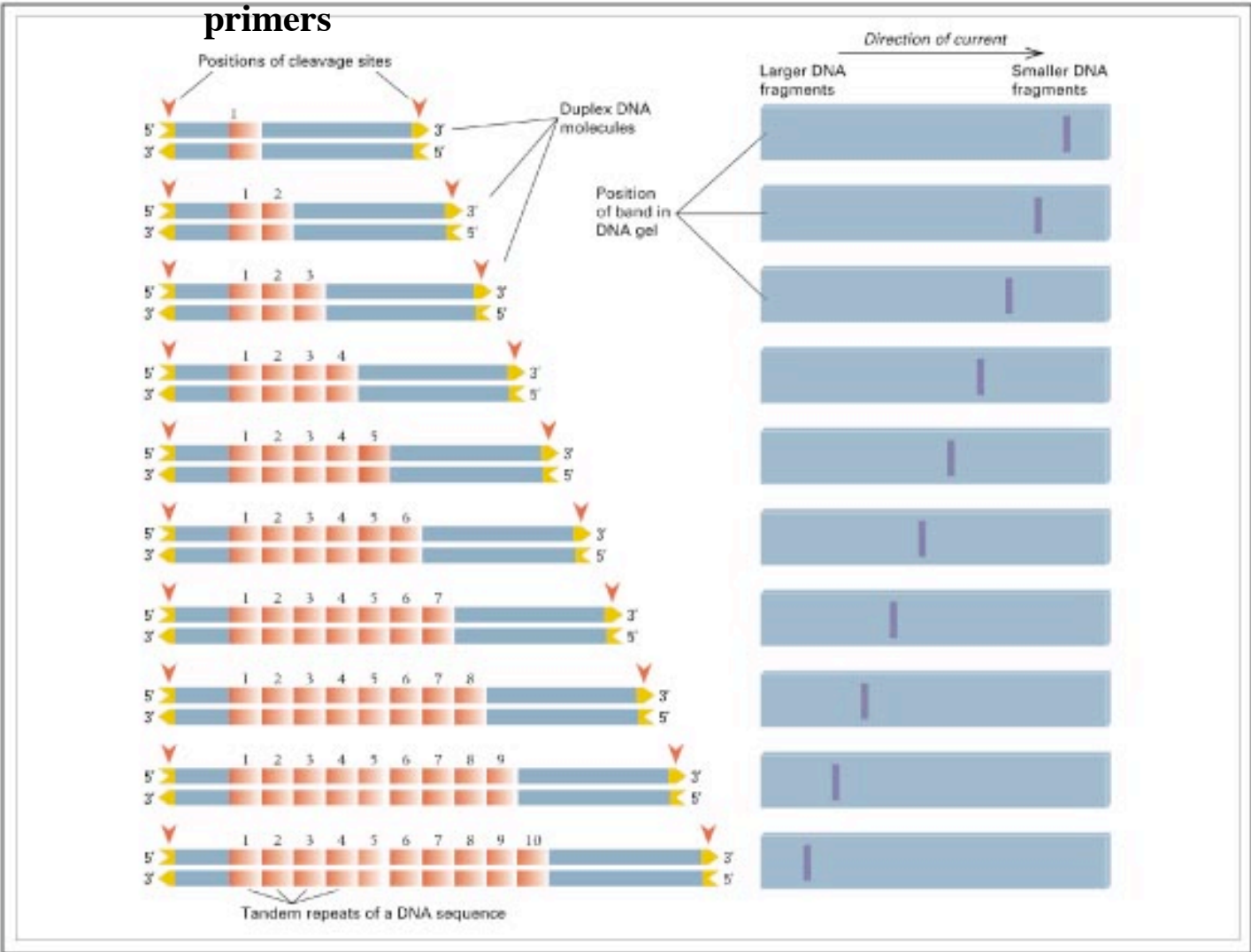
Copy number varies greatly and size differences (alleles) can be detected by PCR of the region and separating PCR products on a gel. Look for SSRP closely linked to gene and such that mutant allele of gene is closely linked to one SSRP allele while the normal allele is linked to another allele.

A repeat of 10-60 nucleotides is called a variable number of tandem repeats (VNTR).

Changes in copy number occur so often that there are many different variants in population.

Genetic Polymorphism in Which Alleles Differ in Number of Copies of a DNA Sequence

Figure 02.24



Forensic DNA Analysis

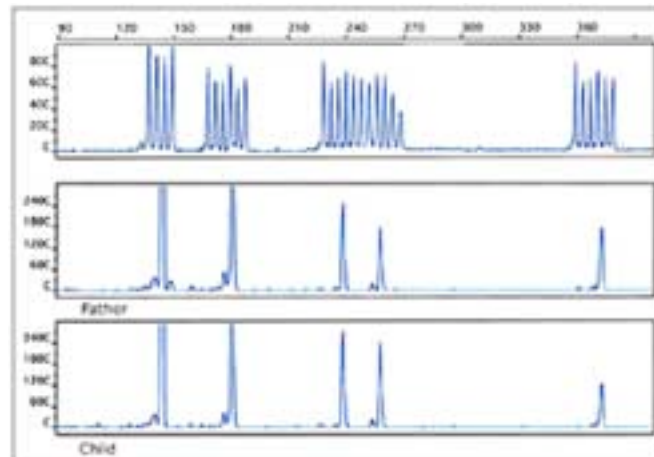
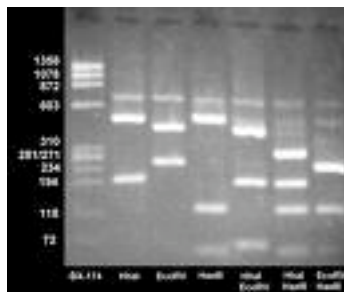
If look at several different regions where there are SSRPs or VNTRs, no two individuals are identical. Use for forensic DNA analysis: DNA fingerprinting = DNA profiling = DNA typing.

The Federal Bureau of Investigation (FBI) uses a standard set of 13 specific STR regions for the Combined DNA Index System (CODIS). CODIS is a software program that operates local, state, and national databases of DNA profiles from convicted offenders, unsolved crime scene evidence, and missing persons. The odds that two individuals will have the same 13-loci DNA profile is about one in one billion.

New:

Use 13 primer pairs to amplify different microsatellite regions. Machine separates fragments and gives profile of peaks corresponding to different alleles.

old



CODIS - combined DNA index system



- Share DNA profiles among crime labs
- 1994 - official US government approval and standards set
- 11/2005 had 124,200 forensic profiles and 2.8 million offender profiles
- 27,700 matches made thus far
- **13 core loci used**

