

UNIVERSITY OF AGRICULTURE, ABEOKUTA
COLLEGE OF ENVIRONMENTAL RESOURCES MANAGEMENT
DEPARTMENT OF FORESTRY AND WILDLIFE MANAGEMENT

WRM – 509
WILDLIFE GENETICS, BREEDING AND CONSERVATION (2 UNITS)

Course Outline

Basic concepts of genetics. Laws of inheritance. Natural and induced breeding. Artificial insemination techniques for animals in captivity. Wildlife improvement through crossbreeding. Practical experiences in artificial insemination and induced breeding.

These notes are provided to help direct your study from the textbook. They are not designed to explain all aspects of the material in great detail; that is what class time and the textbook is for. If you were to study only these notes, you would not learn enough genetics to do well in the course.

Chromosomes and Cellular Reproduction

two major groups of organisms:

prokaryotes - lack nuclei

eukaryotes - has a formed nucleus, our discussion will be confined to eukaryotes

stages in the cell cycle

G1 -- GAP 1, the metabolically active stage following cell division

S -- DNA synthesis occurs here

G2 -- GAP 2, getting ready to divide

cell division -- either mitosis or meiosis

a chromosome consists of one or two arms, a centromere, and a pair of telomeres a kinetochore is the attachment point for the spindle fibers a centromere is the constriction in the chromosome where the kinetochores occur (these two terms are often used interchangeably, though this is not correct)

Following the S phase of the cell cycle,

- the arms and centromeres have duplicated but the centromeres are still held together by protein, thus there appears to be only one centromere
- a chromatid refers to each set of arms. Both sets are called sister chromatids.

Most eukaryotic cells are diploid, at least at one point in their life cycle, that is the chromosomes occur in pairs, one inherited from the maternal side and one inherited from the paternal side.

MITOSIS

is an asexual process for increasing the number of cells (progeny)

allows accurate replacement of old cells by new cells and ensures that all of the somatic cells of an organism have the same genetic composition

is the only means of reproducing for asexual species It is divided into four stages, which represent a continuum from one stage to the next. The boundaries between stages are not that clear in practice.

Prophase

chromosomes begin to thicken and shorten. By mid-prophase, the morphology of the sister chromatids can be determined.

nuclear membrane disintegrates

the nucleolus disappears

the centrioles, which are not present in higher plants, separate and migrate to opposite poles of the cell (the centriole/centrosome replicated during G₂ phase)

the spindle apparatus forms from the centriole

toward late prophase, the spindle fibers attach to the kinetochores. There is usually only one kinetochore associated with each chromatid and only one centromere.

Prometaphase

This is a recently recognized stage that is not used by all authors. It represents a time in what could be called late prophase when the nuclear membrane disappears and the spindle fibers attached to the centromeres

Metaphase

The chromosomes align themselves in the equatorial plane of the spindle

Anaphase

Separation of the sister chromatids with division of the centromere (which had replicated in S or G₂ phase)

chromatids are pulled to opposite ends of the cell. There is still a diploid number of chromosomes, one from each sister chromatid, at each end of the cell.

the centromeres are pulled toward the poles with the arms trailing behind

Telophase

The most significant event is cytokinesis, the division of the cytoplasm This requires the synthesis of a cell plate (destined to be a cell wall in plants) in plants or a pinching in of the cell membrane in animals

As far as the nucleus is concerned, telophase is a reversal of prophase

-reform	nuclear	membrane
-spindle	apparatus	disappears
-chromosomes		uncoil
-nucleoli reform		

MEIOSIS

Found only in sexually reproducing species. Meiosis is the only mechanism for the reduction of the chromosomal complement prior to fertilization and zygote formation. Meiosis allows a halving of the chromosomal complement such that each gamete receives one member of each homologous pair. If meiosis did not occur, during sexual reproduction the chromosome number would quickly get out of control, because the chromosome number would double with each fertilization.

characterized by two divisions

first a reductional division that produces haploid cells

second division is equational and is similar to mitosis

Meiosis I (reductional division) Prophase I (five substages)

Leptonema-first stage

the chromatin begins to condense

Zygonema-second stage

homologous chromosomes are attracted to each other and pair up or synapse. All chromosomes are paired at the end of zygonema.

synaptonemal complex begins to form between the homologues and facilitates pairing

Pachynema-third stage

chromosomes continue to shorten and sister chromatids become obvious

exchange of material between chromatids occurs (crossing-over)

synaptonemal complex begins to disappear

Diplonema-fourth stage

sister chromatids begin to separate except at chiasmata

Diakinesis-fifth stage

chromosomes continue to shorten

chromosomes continue to repel each other and the chiasmata move to the ends of the tetrad (terminalization)

spindles attach to the centromeres

Metaphase I

chromosomes align along the equatorial plane of the cell

Anaphase I

homologous chromosomes are pulled apart by the spindle fibers

centromeres do not divide, so that only homologous chromosomes are separated, not sister chromatids

this results in a dyad (pair of sister chromatids) at each pole

Telophase I

is similar to telophase in mitosis. In some species, the cell will skip this stage and go to Prophase II.

Interphase

some cells may enter an interphase, but no DNA synthesis will occur

Meiosis II (equational division)

Telophase I, interphase, and prophase II are highly variable in length depending on the species. In some cases, the phases may be essentially non-existent or may last for hours to months. prophase II, metaphase II, anaphase II, and telophase II are essentially like mitosis.

1) the sister chromatids separate during anaphase II

2) the genetic material is again reduced by half but there is no further reduction in chromosome number.

Meiosis... allows the production of new combinations of alleles by... random assortment of what were originally maternal and paternal chromosomes at anaphase I exchange of genetic material between maternal and paternal chromosomes during pachynema of prophase I maintains constant amounts of genetic material between generations

The process of creating new arrangements either by... crossing over during pachynema or independent segregation in Anaphase I is called genetic recombination These processes contribute to great diversity among the offspring, which is a strong selective advantage for sexual reproduction.

If there were only 1000 genes in the genome and 2 alleles at each gene, there would 2^{1000} possible genetic combinations among the individuals in a population. In actuality, there are many more genes than this, with more numbers of alleles per gene than two, so you can see that the possible number of combinations is very large.

Spermatogenesis

spermatogonium (2N=46)

primary spermatocyte (2N=46)

2 secondary spermatocytes (N=23)

4 spermatids

4 sperm

All sperm have equal amounts of genetic material and equal amounts of cytoplasm.

Oogenesis

oogonium (2N=46)

primary oocyte (2N=46)

In humans, meiosis I is stopped in diplotene before birth. It is not completed until ovulation.

secondary oocyte (N=23) + a polar body (lost)

In humans, meiosis II is completed after fertilization.

ootid + a polar body (lost)

ovum

a) This yields 1 ovum and 2 polar bodies

b) The first polar body does not undergo a second division and the ovum winds up with about 90% of the original cytoplasm.

Genetic Terms

Definitions of terms. While we are discussing Mendel, we need to understand the context of his times as well as how his work fits into the modern science of genetics.

Alleles- an alternate form of a gene. Usually there are two alleles for every gene, sometimes as many as three or four.

Homozygous- when the two alleles are the same.

Heterozygous- when the two alleles are different, in such cases the dominant allele is expressed.

Dominant- a term applied to the trait (allele) that is expressed irregardless of the second allele.

Recessive- a term applied to a trait that is only expressed when the second allele is the same (e.g. short plants are homozygous for the recessive allele).

Phenotype- the physical expression of the allelic composition for the trait under study.

Genotype- the allelic composition of an organism.

Punnet squares- probability diagram illustrating the possible offspring of a mating.

Gene - The fundamental unit of heredity which can be defined in three ways. i) A gene can be

defined in molecular terms as a segment of DNA carrying the information necessary to express a

complete protein or RNA molecule including the promoter and coding sequence. ii) A gene can be

defined by function with a group of recessive mutations that do not complement each other. iii) A

gene can be defined by position with a single-locus segregation pattern in a cross between lines with different alleles. Examples are a 1:3 phenotypic ratio in the F2 generation in a cross between diploid organisms.

Locus - The site on a chromosome where a gene is located. Usually defined by recombinational mapping relative to neighboring loci.

Wild-type - A standard genotype that is used as a reference in breeding experiments. Note that for human crosses there is no standard genotype and the concept of wild-type is therefore not meaningful.

Haploid - A cell or organism with one set of chromosomes (1n).

Diploid - A cell or organism with two sets of chromosomes (2n).

Incomplete dominance - The case where a heterozygote expresses a phenotype intermediate between the corresponding homozygote phenotypes.

Complementation test - A test of gene function where two genotypes with recessive alleles are combined by a cross to test whether the genotype of one parent can supply the function absent in the genotype of the other parent.

F1 - First generation produced by interbreeding of two lines.

F2 - Generation produced by interbreeding of F1 individuals.

Incomplete penetrance - Cases where certain alleles are not always expressed to give observable traits because of other environmental or genetic influences.

True-breeding - Refers to a line of individuals that on intercrossing always produce individuals of the same phenotype. This can almost always be taken to mean that the individuals are homozygous at all loci (The major exception is sex chromosome differences between males and females).

Heredity, Historical Perspective

For much of human history people were unaware of the scientific details of how babies were conceived and how heredity worked. Clearly they were conceived, and clearly there was some hereditary connection between parents and children, but the mechanisms were not readily apparent. The Greek philosophers had a variety of ideas: Theophrastus proposed that male flowers caused female flowers to ripen; Hippocrates speculated that "seeds" were produced by various body parts and transmitted to offspring at the time of conception, and Aristotle thought that male and female semen mixed at conception. Aeschylus, in 458 BC, proposed the male as the parent, with the female as a "nurse for the young life sown within her".

During the 1700s, Dutch microscopist Anton van Leeuwenhoek (1632-1723) discovered "animalcules" in the sperm of humans and other animals. Some scientists speculated they saw a "little man" (homunculus) inside each sperm. These scientists formed a school of thought known as the "spermists". They contended the only contributions of the female to the next generation were the womb in which the homunculus grew, and prenatal influences of the womb. An opposing school of thought, the ovists, believed that the future human was in the egg, and that sperm merely stimulated the growth of the egg. Ovists thought women carried eggs containing boy and girl children, and that the gender of the offspring was determined well before conception.

Pangenesis was an idea that males and females formed "pangenes" in every organ. These pangenes subsequently moved through their blood to the genitals and then to the children. The concept originated with the ancient Greeks and influenced biology

until little over 100 years ago. The terms "blood relative", "full-blooded", and "royal blood" are relicts of pangenesis. Francis Galton, Charles Darwin's cousin, experimentally tested and disproved pangenesis during the 1870s.

Blending theories of inheritance supplanted the spermists and ovists during the 19th century. The mixture of sperm and egg resulted in progeny that were a "blend" of two parents' characteristics. Sex cells are known collectively as gametes (*gamos*, Greek, meaning marriage). According to the blenders, when a black furred animal mates with white furred animal, you would expect all resulting progeny would be gray (a color intermediate between black and white). This is often not the case. Blending theories ignore characteristics skipping a generation. Charles Darwin had to deal with the implications of blending in his theory of evolution. He was forced to recognize blending as not important (or at least not the major principle), and suggest that science of the mid-1800s had not yet gotten the correct answer. That answer came from a contemporary, Gregor Mendel, although Darwin apparently never knew of Mendel's work.

The Monk and his peas

An Austrian monk, Gregor Mendel, developed the fundamental principles that would become the modern science of genetics. Mendel demonstrated that heritable properties are parceled out in discrete units, independently inherited. These eventually were termed genes.



Gregor Mendel, the Austrian monk who figured out the rules of heredity. The above photo is from <http://www.open.cz/project/tourist/person/photo.htm>.

Mendel reasoned an organism for genetic experiments should have:

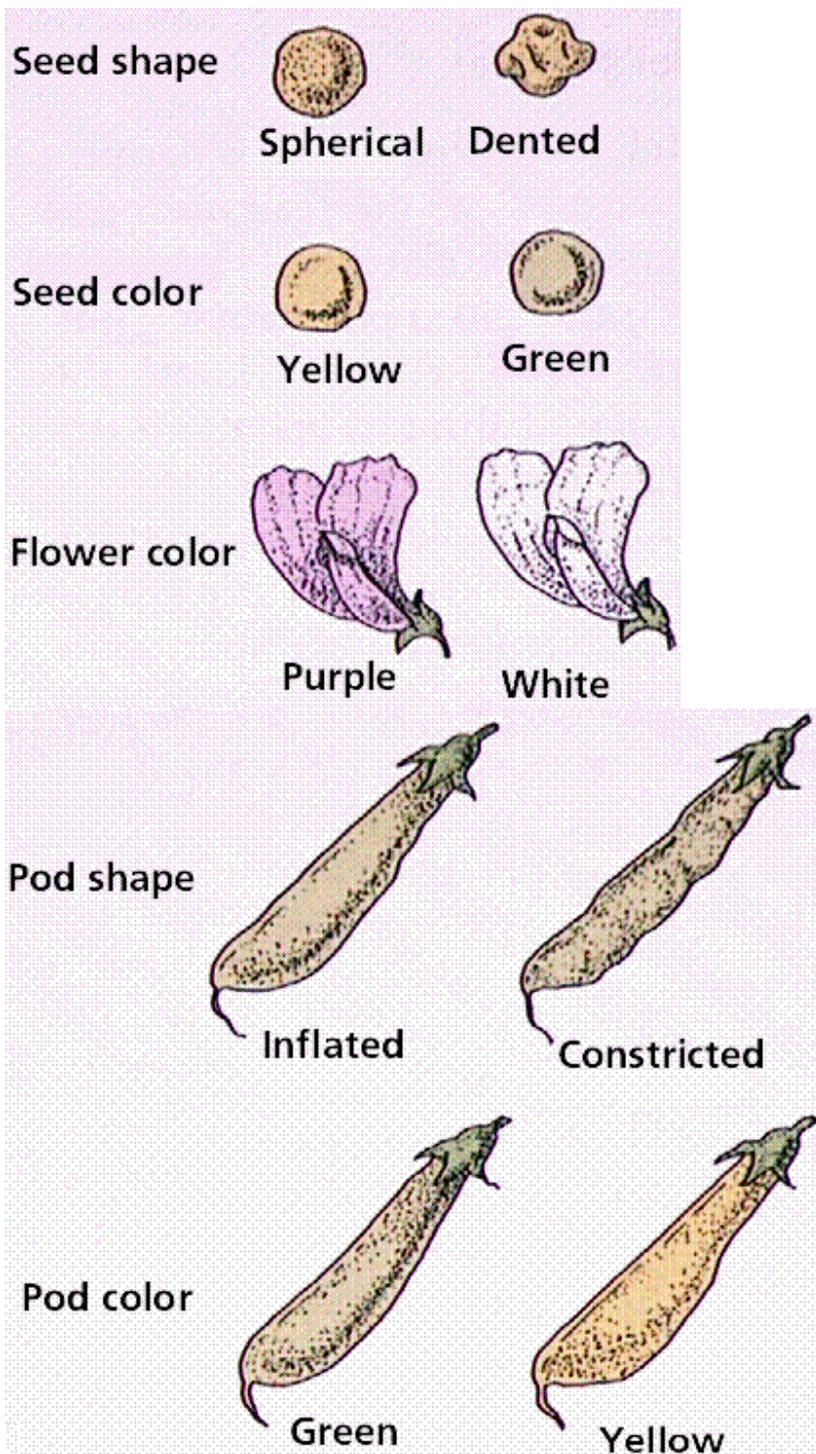
a number of different traits that can be studied

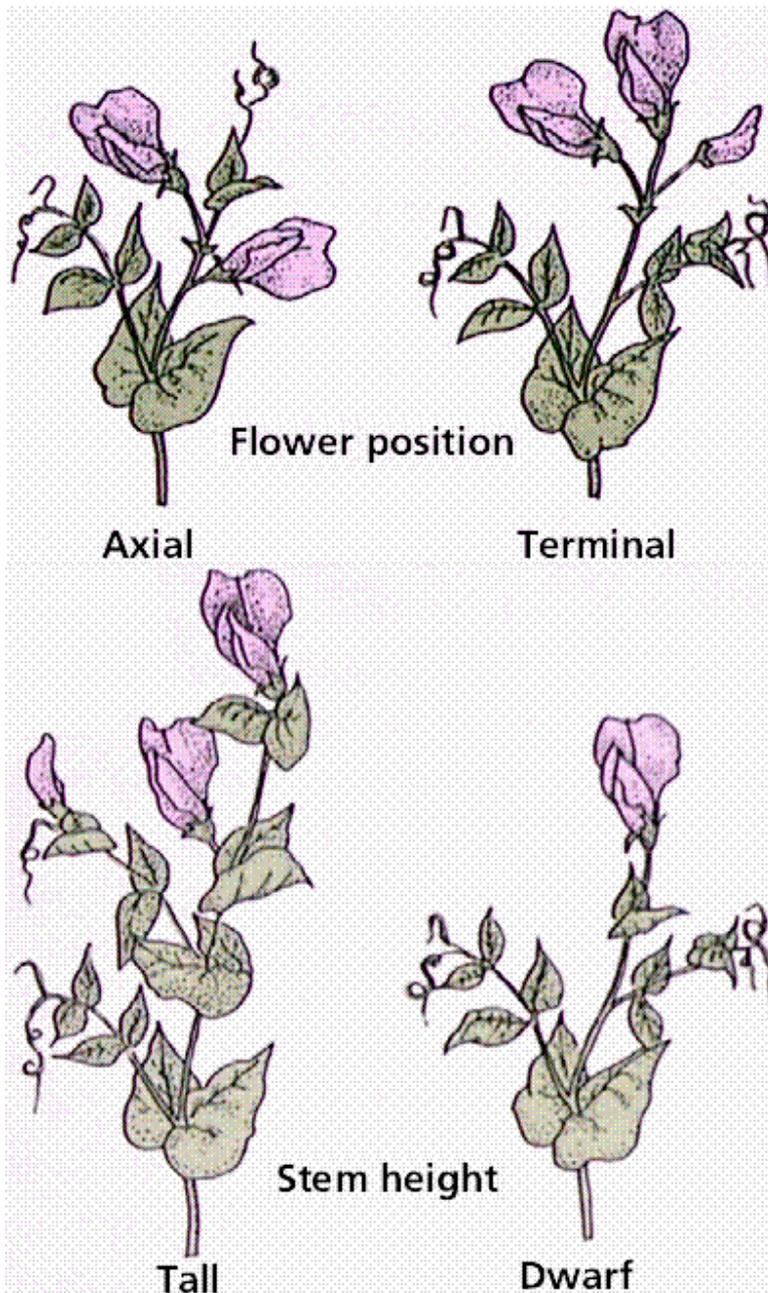
plant should be self-fertilizing and have a flower structure that limits accidental contact

offspring of self-fertilized plants should be fully fertile.

Mendel's experimental organism was a common garden pea (*Pisum sativum*), which has a flower that lends itself to self-pollination. The male parts of the flower are termed the anthers. They produce pollen, which contains the male gametes (sperm). The female parts of the flower are the stigma, style and ovary. The egg (female gamete) is produced in the ovary. The process of pollination (the transfer of pollen from anther to stigma) occurs prior to the opening of the pea flower. The pollen grain grows a pollen tube which allows the sperm to travel through the stigma and style, eventually reaching the ovary. The ripened ovary wall becomes the fruit (in this case the pea pod). Most flowers allow cross-pollination, which can be difficult to deal with in genetic studies if the male parent plant is unknown. Since pea plants are self-pollinators, the genetics of the parent can be understood easily. Peas are also self-compatible, allowing self-fertilized embryos to develop as readily as out-fertilized embryos. Mendel tested all 34 varieties of peas available to him through seed dealers. The garden peas were planted and studied for eight years. Each character studied had two distinct forms, such as tall or short plant height, or smooth or wrinkled seeds. Mendel's experiments used some 28,000 pea plants.

Mendel's contribution was unique because of his methodical approach to a definite problem, use of clear-cut variables and application of mathematics (statistics) to the problem. Mendel was able to demonstrate that traits were passed from each parent to their offspring through the inheritance of genes.





Some of Mendel's traits as expressed in garden peas. Images from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com).

Mendel's work showed:

1. Each parent contributes one factor of each trait shown in offspring.

2. The two members of each pair of factors segregate from each other during gamete formation.
3. The blending theory of inheritance was discounted.
4. Males and females contribute equally to the traits in their offspring.
5. Acquired traits are not inherited.
6. He suggested two laws: Random Segregation and Independent Assortment
7. He crossed purebred peas, which differed in clearly observable traits
8. Purebred original stocks are called the P_1 generation
9. First generation hybrid crosses are called F_1 for filial generation 1
10. Second generation, F_1 crossed with F_1 (in peas, self fertilized) are called F_2 for filial generation 2
11. F_1 crosses are also called hybrid crosses
12. When just one character and two traits are under consideration, it is called a monohybrid cross
13. When two characters are being considered, it is called a dihybrid cross
14. Dominant traits are those exclusive traits that appear in the F_1 generation.
15. Recessive traits are the traits that are hidden or masked in the F_1 generation.
16. Alleles are different forms of a gene, each diploid individual has two, each gamete has one, the union of gametes re-establishes a pair.
17. The genotype is the actual genes in the individual or the alleles the individual has in its genome.
- 18 The phenotype is the physical appearance or biochemical activity caused by the genotype.
19. The genotype could be Aa ; the phenotype would be A , or what A makes the organism look or function like.
20. A genotype of aa would have the phenotype a or what a makes the organism look like.

Crossing

The genotype AA produces gametes with A or A.

The genotype Aa produces gametes with either A or a.

The genotype aa produces gametes with a or a.

Homozygous is when the diploid individual has a pair of identical alleles (AA or aa) for the character.

Heterozygous is having a pair of different alleles (Aa).

Proportions of progeny (offspring from the cross) will be close to those expected, but not necessarily exactly the expected.

It is like tossing a coin. If you toss a coin ten times, the expected value of 5 heads and 5 tails will only be observed sometimes.

The Punnett Square

Punnett squares deal only with probability of a genotype showing up in the next generation. Usually if enough offspring are produced, Mendelian ratios will also be produced.

Step 1 - definition of alleles and determination of dominance.

Step 2 - determination of alleles present in all different types of gametes.

Step 3 - construction of the square.

Step 4 - recombination of alleles into each small square.

Step 5 - Determination of Genotype and Phenotype ratios in the next generation.

Step 6 - Labeling of generations, for example P1, F1, etc.

The Punnett square is a useful device to recombine alleles of gametes and generate all possible outcomes to determine their relative proportions.

For any cross, Punnett square(s) may be used, but their best contribution can be realized if used on one character at a time.

In the below square, a simple monohybrid cross is diagrammed. The males generate two kinds of gametes, those with an A allele and those with an a. The same is true for the females.

Female - >	A	a
Male		
A	AA	Aa
a	Aa	aa

The outcome is a genotypic ratio of 1AA : 2Aa : 1aa.

To express as proportions, simply add the values of the ratios and use the total as the denominator.

In the above example: 1 + 2 + 1 = 4. The denominator would be 4.

The proportions would be 1/4 AA, 2/4 (or 1/2) Aa and 1/4 aa.

The percentages would be the proportions times 100. For example, 1/4 X 100 = 25%.

GENETIC LAWS

1. The Rule of segregation

- a gamete receives only one allele from the pair of alleles possessed by an organism; fertilization re-establishes the double number

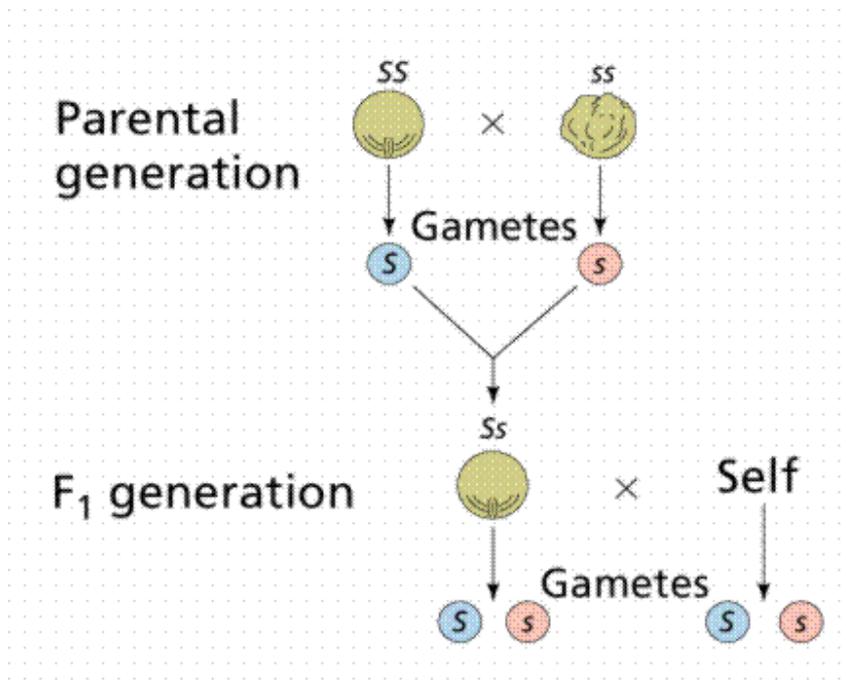
OR IN OTHER WORDS

- two members of a gene pair (alleles) segregate from each other during the formation of gametes. As a result, half the gametes carry one allele and the other half carry the other allele.

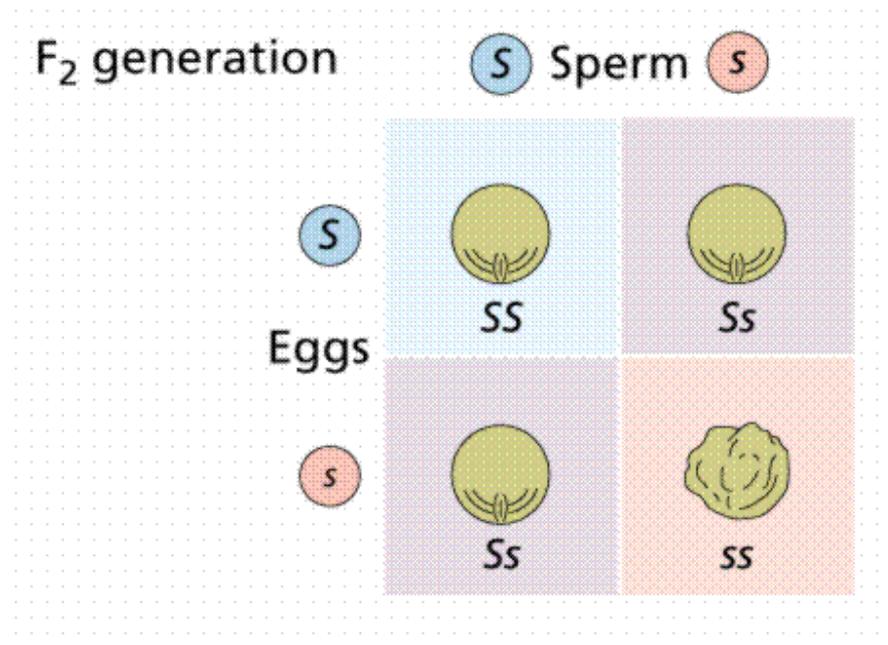
Mendel continued to test his rule of segregation by carrying out breeding experiments through the F₆ generation. In each case, he could predict the phenotypic ratios in the offspring. He also used what he called a test cross. Like any good scientist, Mendel wanted a different kind of cross to test his rule of

segregation. The test cross gave him such a test. By crossing a homozygous recessive individual with a heterozygous individual, the rule of segregation would predict a 1:1 phenotypic ratio among the offspring. When Mendel did such a cross, he did observe a 1:1 ratio. This confirmed his prediction and supported the theory (rule of segregation) upon which the prediction was based. Today, because the rule of segregation is so strongly supported, we use test crosses to determine the genotype of an individual with the dominant phenotype assuming of course, that the rule of segregation is still valid.

Mendel studied the inheritance of seed shape first. A cross involving only one trait is referred to as a monohybrid cross. Mendel crossed pure-breeding (also referred to as true-breeding) smooth-seeded plants with a variety that had always produced wrinkled seeds (60 fertilizations on 15 plants). All resulting seeds were smooth. The following year, Mendel planted these seeds and allowed them to self-fertilize. He recovered 7324 seeds: 5474 smooth and 1850 wrinkled. To help with record keeping, generations were labeled and numbered. The parental generation is denoted as the P1 generation. The offspring of the P1 generation are the F1 generation (first filial). The self-fertilizing F1 generation produced the F2 generation (second filial).



Inheritance of two alleles, S and s, in peas. Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com).



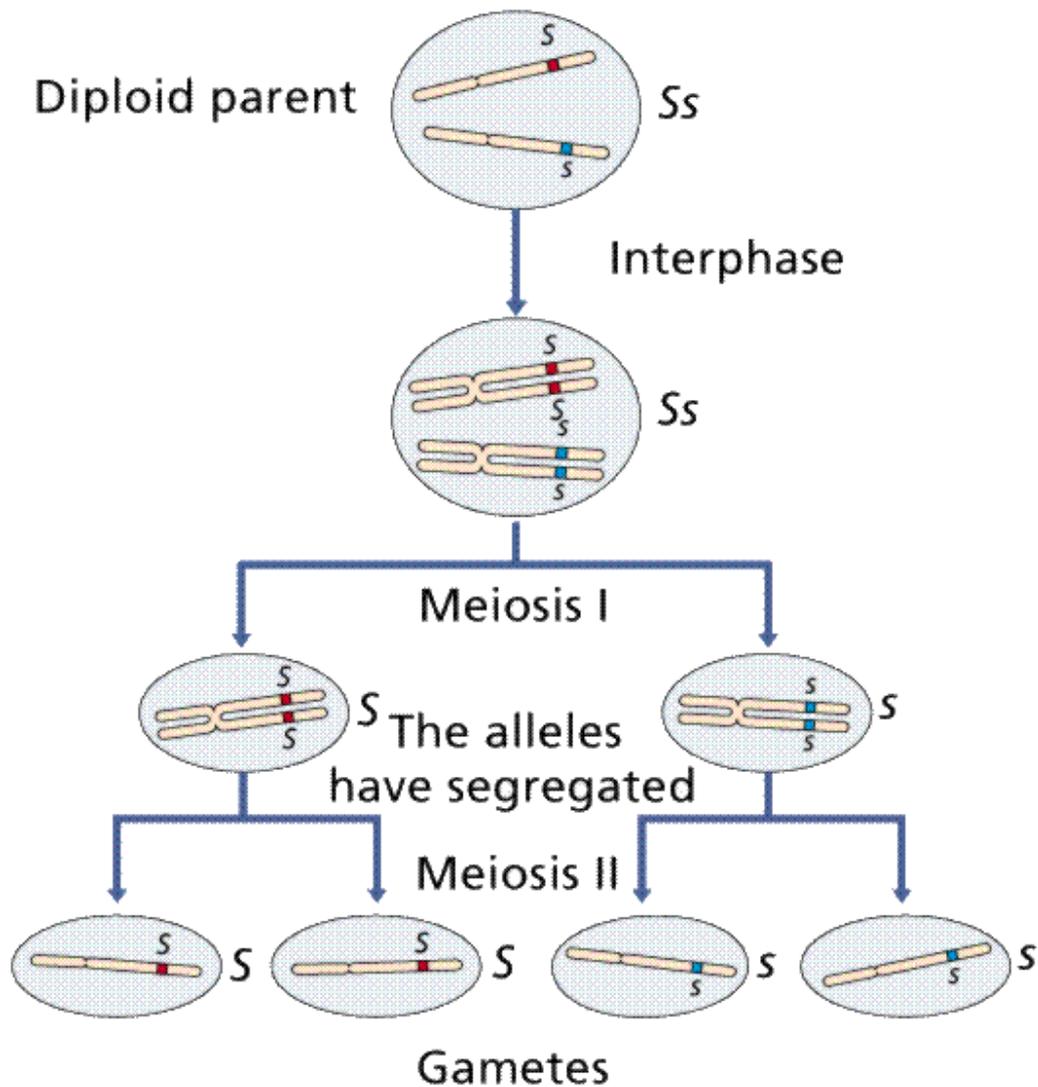
Punnett square explaining the behavior of the S and s alleles. Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com), used with permission.

P1: smooth X wrinkled

F1 : all smooth

F2 : 5474 smooth and 1850 wrinkled

Meiosis, a process unknown in Mendel's day, explains how the traits are inherited.



The inheritance of the S and s alleles explained in light of meiosis. Image from Purves et al., *Life: The Science of Biology*, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com), used with permission.

Mendel studied seven traits which appeared in two discrete forms, rather than continuous characters which are often difficult to distinguish. When "true-breeding" tall plants were crossed with "true-breeding" short plants, all of the offspring were tall plants. The parents in the cross were the P1 generation, and the offspring represented the F1 generation. The trait referred to as tall was considered dominant, while short was recessive. Dominant traits were defined by Mendel as those which appeared in the F1

generation in crosses between true-breeding strains. Recessives were those which "skipped" a generation, being expressed only when the dominant trait is absent. Mendel's plants exhibited complete dominance, in which the phenotypic expression of alleles was either dominant or recessive, not "in between".

When members of the F1 generation were crossed, Mendel recovered mostly tall offspring, with some short ones also occurring. Upon statistically analyzing the F2 generation, Mendel determined the ratio of tall to short plants was approximately 3:1. Short plants have skipped the F1 generation, and show up in the F2 and succeeding generations. Mendel concluded that the traits under study were governed by discrete (separable) factors. The factors were inherited in pairs, with each generation having a pair of trait factors. We now refer to these trait factors as alleles. Having traits inherited in pairs allows for the observed phenomena of traits "skipping" generations.

Summary of Mendel's Results:

The F1 offspring showed only one of the two parental traits, and always the same trait.

Results were always the same regardless of which parent donated the pollen (was male).

The trait not shown in the F1 reappeared in the F2 in about 25% of the offspring.

Traits remained unchanged when passed to offspring: they did not blend in any offspring but behaved as separate units.

Reciprocal crosses showed each parent made an equal contribution to the offspring.

Mendel's Conclusions:

Evidence indicated factors could be hidden or unexpressed, these are the recessive traits.

The term phenotype refers to the outward appearance of a trait, while the term genotype is used for the genetic makeup of an organism.

Male and female contributed equally to the offsprings' genetic makeup: therefore the number of traits was probably two (the simplest solution).

Upper case letters are traditionally used to denote dominant traits, lower case letters for recessives.

2. Rule of Independent Assortment

Mendel extended his experiments to dihybrid crosses. From these data, he postulated the Rule of Independent Assortment. The parental generation consisted of a round yellow pea (RRYY) and a wrinkled green pea (rryy). The F1 generation was round and yellow (RrYy). The F1 generation was selfed to yield the F2 generation.

The F2 generation consisted of... (figures 3.11 and 3.12)

9 round, yellow

3 round, green

3 wrinkled, yellow

1 wrinkled, green

Mendel's second principle states ...

that, during gamete formation, the alleles at one locus segregate into the gametes independently of the pair of alleles found at a different locus.

Dihybrid Crosses

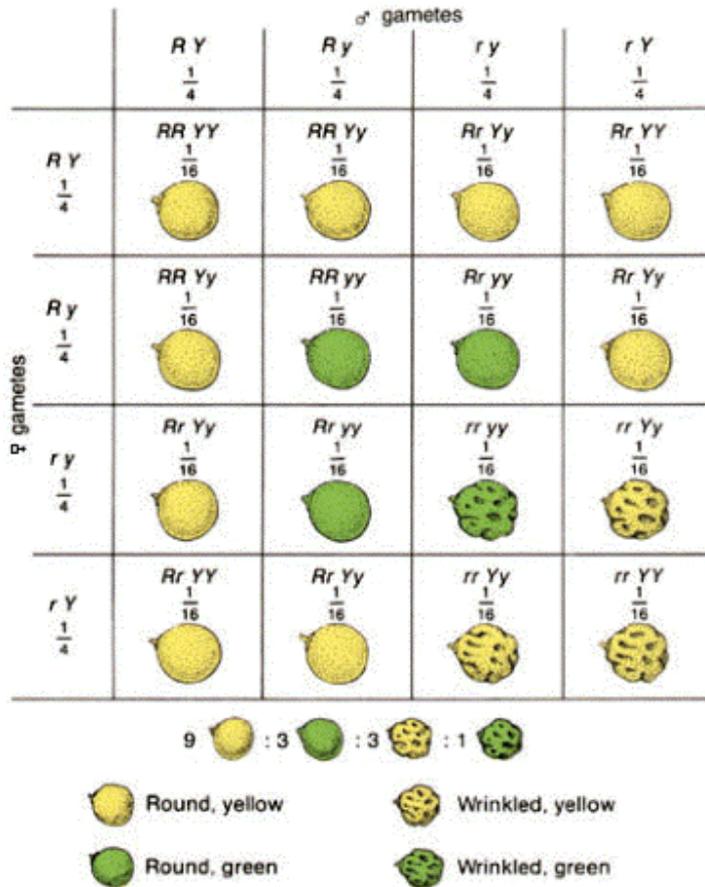
When Mendel considered two traits per cross (dihybrid, as opposed to single-trait-crosses, monohybrid), The resulting (F2) generation did not have 3:1 dominant:recessive phenotype ratios. The two traits, if considered to inherit independently, fit into the principle of segregation. Instead of 4 possible genotypes from a monohybrid cross, dihybrid crosses have as many as 16 possible genotypes.

Mendel realized the need to conduct his experiments on more complex situations. He performed experiments tracking two seed traits: shape and color. A cross concerning two traits is known as a dihybrid cross.

Crosses With Two Traits

Smooth seeds (S) are dominant over wrinkled (s) seeds.

Yellow seed color (Y) is dominant over green (g).



Inheritance of two traits simultaneously, a dihybrid cross. The above graphic is from the Genetics pages at McGill University (<http://www.mcgill.ca/nrs/dihib2.gif>).

101 wrinkled yellow

32 wrinkled green

Mendel analyzed each trait for separate inheritance as if the other trait were not present. The 3:1 ratio was seen separately and was in accordance with the Principle of Segregation. The segregation of S and s alleles must have happened independently of the segregation of Y and y alleles. The chance of any gamete having a Y is 1/2; the chance of any one gamete having a S is 1/2. The chance of a gamete having both Y and S is the product of their individual chances (or $1/2 \times 1/2 = 1/4$). The chance of two gametes forming any given genotype is $1/4 \times 1/4$ (remember, the product of their individual chances). Thus, the Punnett Square has 16 boxes. Since there are more possible combinations to produce a smooth yellow phenotype (SSYY, SsYy, SsYY, and SSYy), that phenotype is more common in the F2.

From the results of the second experiment, Mendel formulated the Principle of Independent Assortment-- that when gametes are formed, alleles assort independently. If traits assort independent of each other during gamete formation, the results of the dihybrid cross can make sense. Since Mendel's time, scientists have discovered chromosomes and DNA. We now interpret the Principle of Independent Assortment as alleles of genes on different chromosomes are inherited independently during the formation of gametes.

VARIATION AND MENDEL'S LAWS

1. An example is partial dominance (also called incomplete dominance).

Characteristic of partial dominance is an intermediate expression: Red (AA) X White (aa) yields Pink (Aa).

In this case, the genotypes can be determined by observing the phenotypes.

The genotype AA would be red and the phenotype of Aa would be pink.

Another important indicator of whether a gene exhibits incomplete dominance is the ratio of progeny found in the F2 generation, which is 1:2:1 if incomplete dominance is the mode of inheritance.

In the example above the ratio would be 1 red : 2 pink : 1 white. (1AA : 2Aa : 1aa)

Female - >	A	a
Male		
A	AA Red	Aa Pink
a	Aa Pink	aa White

Visual appealing example of incomplete dominance.

2. Another exception is codominance where both alleles are expressed.

M / F	A	A
A	AA Black	Aa Black & White
A	Aa Black & White	aa White

Why are there dominant and recessive alleles? What is the molecular basis of dominance?

Usually, dominant alleles are functional forms of the gene, whereas recessive alleles are non-functional forms of the gene. Often genes code for enzymes. If the gene is

functional, the enzyme it codes for works. If non-functional, then the enzyme either fails to work or it is not synthesized.

Therefore, recessive traits tend to be the lack of something. For example, the lack of pigment might mean the color white is observed instead of the dominant brown color.

How can partial dominance be explained? What is the molecular explanation for partial dominance?

Snapdragons are the commonly used textbook example of partial dominance. Red, pink and white snapdragon flowers are commonly seen.

The red flowered plants are homozygous for the red allele. White flowered plants are homozygous for the white allele. Pink flowered plants are heterozygous.

Red flowered plants produce twice the amount of pigment than pink. White flowered plants produce no red pigment.

In the typical dominant /recessive mode of inheritance, one functional copy of the gene, such as that found in a heterozygote, is sufficient to generate a phenotype that is indistinguishable from the homozygous dominant individuals.

In snapdragons and other cases of partial dominance, a dose difference is observable.

What molecular explanation describes codominance?

The ABO blood types of humans are the commonly used textbook example of codominance.

However, only the A and B blood types demonstrate the codominant mode of inheritance.

Ignoring the o, an individual could be either A, AB or B blood type. The A blood type in this hypothetical would be AA. The B would be BB and the heterozygous individuals would be AB.

Both the A and B alleles are functional. However, they code for different forms of a cell surface antigen, which is distinguishable by testing with antibodies.

Humans also have an MN blood antigen system, which is codominant.

(example, MN blood group in humans).

1) there are two alleles L^m, L^n

2)	genotypes	phenotypes
	$L^m L^m$	M blood
	$L^n L^n$	N blood
	$L^m L^n$	MN blood

e.g. sickle cell anemia

1) two alleles Hb^a (normal), Hb^s

2)	genotypes	phenotypes
	$Hb^a Hb^a$	normal
	$Hb^a Hb^s$	normal
	$Hb^s Hb^s$	sickle cell anemia

Answers to a few frequently asked questions

Q: What is meant by wild type allele? A: The most commonly found phenotype and the allele that causes it.

Q: What is a mutant type allele? A: Deviants from the wild type phenotype, even if they are observed in the wild, and the allele that causes it.

Q: Why are there two determinants, gene copies or alleles per individual? A: Because in humans, other animals and in the plants first studied, the diploid form of the organism was studied, and the diploid has two copies of each type of chromosome. The determinants are at specific locations on a certain chromosome. We humans get one of each chromosome from our mother and one from our father (ignoring for the moment, the sex chromosomes).

3. Epistasis: When Genes Affect Each Other

Webster's New World Dictionary definition for epistasis is: "the suppression of gene expression by one or more other genes".

This definition provides a sense of what epistasis is; however, the gene expression might not be and often is not suppressed, but the effect of the gene on the phenotype of the organism is altered or interfered with by another gene.

Another textbook definition is: "an interaction between genes, in which the presence of a particular allele of one gene determines whether another gene will be expressed".

It also means to stand on. The expression of one allele prevents or interferes with the expression of alleles at another locus.

This definition is closer to the actual events that usually occur in epistasis. However, there seems to be two different meanings for the term expression in genetics. According to one definition, a gene is expressed when it is used to generate an RNA molecule and then a protein. The word usage in these definitions is different. It implies that expression is when a gene reveals itself in an observable phenotypic trait.

Often, the gene that is masked still codes for an enzyme. However, the enzyme lacks its substrate because another gene that would have produced it is defective.

By definition, the epistatic gene masks the expression of the hypostatic gene.

In the preceding sections, genes affected just one character. An allele was associated with a trait.

In fact there are cases where one gene can affect many traits. This is called pleiotropy.

In the discussion of epistasis in a general genetics course including this one, the influence of one gene on the phenotypic effects of another is studied. All follow the dominant/recessive mode of inheritance. You can imagine that in nature much more complex examples of epistasis exist.

Obviously, there is a biochemical basis for epistasis. It is enjoyable to hypothesize about the nature of the biochemical basis of a case involving epistasis. This is possible once the ratio has been determined.

The most basic genetic crosses used to study epistasis are F1 dihybrid. The F2 generation's phenotypic ratios deviate from the 9:3:3:1 expected from genes following the dominant recessive mode of inheritance.

A F2 phenotypic ratio of 12:3:1 is an example of one involving epistasis. A grouping of one or more of the ratios occurs. In this case it is the grouping of 9 and 3.

Examples of deviations from the normal phenotypic ratio indicative of epistasis:

9 : 7 is a variation of the normal ratio. (9 : 3 : 3 : 1)

9 : 3 : 4 is a variation of the normal ratio. (9 : 3 : 3 : 1)

15 : 1 is a variation of the normal ratio. (9 : 3 : 3 : 1)

9 : 6 : 1 is a variation of the normal ratio. (9 : 3 : 3 : 1)

Here is an example of a problem that generates a 9:7 phenotypic ratio in the F2 progeny, and a biochemical hypothesis to explain how. Assume we have two different strains of mice, which have alleles for black and white coat color, but for different genes. The F1 dihybrid population is all black. If individuals homozygous for either recessive allele are white then the outcome of a dihybrid cross would generate a ratio indicative of epistasis.

(Alone) Gene 1: 3 black : 1 white would be expected from a monohybrid F1 cross.

(Alone) Gene 2: 3 black : 1 white would be expected from a monohybrid F1 cross.

But, an F1 cross for the dihybrids, i.e., CcPp X CcPp would yield:

3 Black : 1 White (cc)

3 Black : 1 White (pp)

9 Black : 3 White (cc) : 3 White (pp): 1 White (ccpp)

or 9 Black : 7 White

For further explanation of the above, it can be understood like this:

(a) Recessive Epistasis:

In mice, as in many mammals, there are actually 5 loci that control coat color. We will look at two of these loci.

agouti pattern (gray) results from the banding pattern of pigments deposited in each hair

two alleles

A is dominant to a and causes banding

AA, Aa are agouti

aa produces no bands and the individual is black

another locus controls the expression of the black pigment

two alleles

C is dominant to c and causes production of the black pigment

CC, Cc produces black pigment

cc produces no black pigment and is white (albino)

P homozygous black CCaa x ccAA homozygous white

F₁ CcAa all agouti

dihybrid cross of F₁ x F₁

F₂ 9 agouti: 3 black: 4 albino

This phenotypic ratio is similar to the 9:3:3:1 ratio expected for the F₂ generation in a dihybrid cross with alleles exhibiting complete dominance, except that the last two categories (3+1) have been combined.

one locus whose dominant allele (C) is necessary for the development of color and

another locus whose dominant allele (A) is necessary for the banding pattern to produce the agouti color precursor -----> black pigment -----> agouti pattern (allele

C) (allele A)

The recessive genotype of the color gene (cc) is epistatic to, or it interferes with, the dominant allele of the agouti pattern.

(b) Duplicate recessive epistasis

Epistasis in corn

P homozygous purple AABB x aabb homozygous white

F₁ AaBb all purple

dihybrid cross of F₁ x F₁

F₂ 9 purple:7 white

9:3+3+1 = 9:7

genotype	phenotype	
A-, B-	purple	9
A-, bb	white	3
aa, B-	white	3
aa, bb	white	3

"-" equals a wild card

The biochemical pathway is . . .

precursor (colorless)----->intermediate (colorless)----->purple pigment
(allele A) (allele B)

Thus aa is epistatic to B and bb is epistatic to A.

Sex Determination and Sex-linked Characteristics

In the late 1800's and early 1900's, various scientists discovered that the chromosomes within the nucleus divide longitudinally during cell division the divided chromosomes were distributed in equal numbers to the daughter cells the total number of chromosomes remains constant in all cells of an organism except during gamete formation the chromosome number varies greatly from species to species

in 1903, Sutton and Boveri noted that the transmission of chromosomes from one generation to the next paralleled the transmission of genes from generation to generation

They proposed the Chromosome Theory of Heredity which states that the chromosomes are the carriers of the genes. As there are many more loci/genes in an organism, it follows that a single chromosome has many loci.

Sex Determination In dioecious species (separate sexes) there are several means to determine sex. The chromosomes involved in sex determination are called sex chromosomes. All other chromosomes are called autosomal chromosomes or autosomes. Although sex chromosomes provide the most common means of sex determination, it is not the only mechanism.

in bees, males are haploid (N) while females are diploid (2N) sex may be determined by a single allele or multiple alleles as in some wasps by environmental factors as in some turtles, these have indeterminate genetic sex-determining mechanisms. The temperature at which the eggs are incubated determines the sex of the turtles. In some species, warm nests yield mostly males and cool nests yield mostly females. In other species of turtles this is reversed.

We will limit our discussion to chromosomal sex determining mechanisms as this is most common and is the mechanism seen in mammals.

The autosomes occur in homologous pairs with each chromosome possessing one copy (allele) of each gene. Segregation and reassortment lead to the pattern of inheritance that we have seen so far, which is called Mendelian inheritance. The sex chromosomes may be genetically distinct thus homologous pairs may not exist and this leads to inheritance patterns that are different from autosomal inheritance.

There are four basic types of chromosomal mechanisms

XX-XY (figures 4.4 & 4.5) in which females are homomorphic XX and males are heteromorphic XY. This is found in mammals including humans and some insects including *Drosophila*.

In humans, females have 23 homomorphic pairs and males have 22 homomorphic pairs plus a heteromorphic pair. During meiosis, females produce only one kind of gamete all having one X chromosome. Males produce two kinds of gametes, one with an X and the other with a Y chromosome. Females are homogametic and males are heterogametic.

ZZ-ZW system in which females are heteromorphic ZW and males are homomorphic ZZ. This occurs in birds, some fishes, and moths. It is essentially the opposite of XY in mammals.

XX-XO system in which females have 2 X chromosomes. Males have only 1 X and no additional sex chromosomes. Females have an even number of chromosomes and males have an odd number of chromosomes. This occurs in many species of insects. This was the first sex determining mechanism discovered, and the sex determining chromosome was named the X in 1905. Gametes of males have either an X chromosome or no sex chromosome.

compound chromosome system These can be very complex with multiple numbers of X and Y chromosomes. e.g. in *Ascaris incurva*, a nematode, there are 26 autosomes, 8 X chromosomes, and 1 Y chromosome Males have 26A + 8X + Y for 35 chromosomes Females have 26A + 16X for 42 chromosomes This type of system is also common in spiders.

Sex determination in the XY system is the most studied because it is found in humans and *Drosophila*. Does the X chromosome or the Y chromosome determine the sex? It varies from species to species. In *Drosophila*, the greater the number of X chromosomes relative to the autosomes, the more likely the individual will be female.

Phenotype	chromosomal complement	# of X/# of autosomal sets
normal female	XX + 2N autosomes	1.00
normal male	XY + 2N autosomes	0.50
metafemale	XXX + 2N autosomes	1.50
metamale	X + 3N autosomes	0.33
intersex	XX + 3N autosomes	0.67

Sex balance theory or genic balance theory states that the X chromosome determines the sex of the individual and that sex is a dosage phenomena, where the ratio of the amount of the X relative to the autosomes determines the sex. In addition, environmental effects can influence the development of the intersex flies.

Further studies have shown that sex is ultimately determined by the locus sex-lethal on the X chromosome, though several other loci on the X chromosome and the autosomes are also needed for sex determination.

The sex balance theory was assumed to apply to other XY systems, including humans. However, cytologic evidence (chromosomal studies) of mice and humans showed that . .

1) XO were female (Turner)

2) XXY were male (klinefelter)

which is opposite of what the sex balance theory would predict. All males have at least one Y and all females have no Y's, regardless of the number of X's. The reason is that on the Y chromosome, there is a gene that causes an undifferentiated gonad to become a testis. This gene is called the sex determining region Y (sry). Its mode of action is basically to control a number of other genes that effect the development of the sexual characteristics.

X-linked inheritance

In animals with XY sex determining mechanism, the X chromosome has many loci, many that have nothing to do with sex as such. The Y is usually smaller and possesses fewer loci that are not the same loci as that on the X chromosome. Thus females that have the same allele at a locus on the X chromosome are homozygous. Different alleles would be

heterozygous. Males, because they have only one X, are hemizygous and can have only one allele at a locus. Because of this, one copy of a recessive allele will be expressed in the phenotype in males.

In sex-linked inheritance, crosses are not reciprocal. The X-linked pattern is called the criss-cross pattern of inheritance because fathers pass the trait to daughters who pass it on to sons.

Sex-limited traits: are traits that are autosomally inherited, and they are expressed in one sex, but not in the other. Some examples include sexually dimorphic plumage in birds, milk yield in mammals, antlers in deer, beards in humans.

Sex-influenced traits appear in both sexes but more so in one sex than another. Male pattern baldness in humans is an example. The male hormone testosterone is needed for full expression of baldness. Because of this hormone difference, the allele for baldness behaves as a dominant trait in males (expressed when heterozygous), but behaves as a recessive allele in females (must be homozygous to be expressed).

Pedigree Analysis

Pleiotropy- a gene that causes or affects the phenotypic development in two or more characteristics. For example, the many symptoms that are seen in individuals who are homozygous for cystic fibrosis - one trait is the build-up of thick mucus in the lungs of affected individuals and a second trait is the abnormal development of the vas deferens in males, which often leads to sterility.

Penetrance- an allele that produces the same effect in every individual of the proper genotype is said to have complete penetrance. If this is not the case, the allele is said to be incompletely penetrant).

of individuals with correct phenotype

% penetrant = -----

of individuals with genotype coding for phenotype

Most alleles show complete penetrance. Here are some examples of alleles that do not.

- 1) the autosomal dominant allele that causes retinoblastoma
- 2) the autosomal dominant allele that causes polydactyly
- 3) the sex-linked dominant allele that causes rickets
- 4) the autosomal recessive allele that causes eyelessness in *Drosophila*

Expressivity- once a gene is expressed, it may have different degrees of expression.
examples

eyeless in *Drosophila*. If it penetrates (85%) the phenotype can range from totally eyeless to eyes barely reduced.

polydactyly in humans. If it penetrates, it can range from one extra finger or toe to several extra fingers or toes.

We can now apply what we know about X-linked and autosomal inheritance to determine the inheritance pattern of human traits.

We must use pedigree analysis because obviously we cannot do the appropriate test crosses to determine inheritance pattern. This often leads us into the situation where we can eliminate some patterns and are left with a couple of possible patterns.

Many times, we do not have enough progeny to make strong statistically valid statements about the mode of inheritance.

We must be concerned with the certainty of paternity. In many studies in the U.S. and Europe, 15-25% of children do not have the father of record.

To do pedigree analysis, we must start with a family tree (figures 6.2 & 6.3 for definitions of symbols).

Four Basic Patterns of Inheritance

Autosomal dominant inheritance

If the trait is rare, most matings are between heterozygotes (affected) and homozygous (unaffected) recessives, which leads to predominantly 1:1 phenotypic ratios for all children, regardless of sex

the dominant phenotype should appear in every generation, unless there is reduced penetrance

Autosomal recessive inheritance

generations are often, but not always, skipped there should be equal distribution among the sexes if both parents are affected, all children will be affected often found in consanguineous marriages most affected children have normal parents

Sex-linked dominance no generations are skipped affected males must come from affected mothers all the daughters, but none of the sons, of an affected father are affected approximately half of an affected female's sons and daughters are affected

Sex-linked recessive males are most affected affected males have carrier mothers, who are known to have affected brothers, fathers, or maternal uncles affected females come from affected fathers and carrier mothers affected female's sons must be affected approximately half the sons of carrier females should be affected

X-inactivation

The stainable material in a chromosome is called chromatin. In a G_1 stage-chromosome, it consists of a single strand of DNA, plus several types of protein. There are two such types of chromatin.

euchromatin is uncoiled or uncondensed during interphase heterochromatin remains coiled or condensed during interphase and is replicated late during the S phase.

There are two kinds of heterochromatin.

facultative heterochromatin - this heterochromatin may revert to euchromatin depending upon the physiological or developmental conditions of the cell. An example of this is X-inactivation due to the formation of a Barr body.

constitutive heterochromatin - is permanently condensed and never euchromatic. The centromeric region is constitutive heterochromatin, but there may be other regions as well.

In males, the single X chromosome is euchromatic. In females, one of the two X chromosomes becomes condensed heterochromatin on the 16th day of embryonic development. In the interphase nucleus, this appears as a dark spot near the edge of the nucleus.

A person will have $n-1$ Barr bodies where n equals the number of X chromosomes. Thus.

a normal female (XX) will have one Barr body

a normal male (XY) will have no Barr bodies

a Turner female (XO) will have no Barr bodies

a Klinefelter male (XXY) will have one Barr body

The result of X-inactivation is to produce a mosaic of phenotypes within females that are heterozygous at an X-linked locus. The inactivated X remains inactivated through all subsequent mitotic events within that cell line.

In human beings, females can be heterozygous for the enzyme glucose-6-phosphate dehydrogenase. Skin cells taken from these individuals can be grown in tissue culture, where each cell grows into a cell line that expresses only one form (allele) of glucose-6-phosphate dehydrogenase. This also occurs in cats, where the locus for coat color is found on the X chromosomes. Males, because they are hemizygous, are either black or orange. Females that are heterozygous the black and tan alleles produce a calico coat color because of random X-inactivation during embryonic development. Males that are calico, which are quite rare, always turn out to be XXY and are thus heterozygous at the coat color locus and produce the same pattern as females.

Recessively Inherited Human Disorders

A recessive allele that causes a disorder is usually a defective version of the normal allele.

Defective alleles that cause disorders code for either a malfunctional protein or none at all.

Heterozygotes can be phenotypically normal, if one copy of the normal allele is sufficient to code for the needed quantities of the protein.

Recessively inherited disorders range in severity from nonlethal traits to lethal diseases.

Since these disorders are caused by recessive alleles:

The phenotypes are expressed only in homozygotes (aa) who inherit one recessive allele from each parent.

The frequency of this event approximates the square of the frequency of the allele in the population.

Heterozygotes (Aa) can be phenotypically normal and act as carriers, making transmission of the recessive allele to their offspring possible.

The vast majority of people afflicted with recessive disorders are born to normal parents, both of whom are carriers.

The probability is $1/4$ that a mating of two carriers (Aa X Aa) will produce a homozygous recessive zygote.

The probability is $2/3$ that a normal child from such a mating will be a heterozygote, or a carrier.

Genetic disorders are not usually distributed evenly among all racial and cultural groups due to the different genetic histories of the world's people. Three examples of such recessively inherited disorders are cystic fibrosis, Tay-Sachs disease and sickle-cell anemia.

Cystic fibrosis is the most common lethal genetic disease in the United States, striking 1 in every 2,500 Caucasian. It is much rarer in other races.

The frequency of this allele of the gene in the United States population is 2%.

The probability is $1/4$ that a mating of two carriers (Aa X Aa) will produce a homozygous recessive zygote.

The probability is $2/3$ that a normal child from such a mating will be a heterozygote, or a carrier.

The dominant allele codes for a membrane protein that pumps chloride ions out of cells. These pumps are lacking or are defective in recessive homozygotes, so chloride accumulates abnormally in the cells causing the osmotic uptake of water from the surrounding mucus. Disease symptoms result because the thickened mucus builds up in the pancreas, lungs, digestive tract, and other organs.

Tay-Sachs disease occurs in 1 out of 3,600 births. The incidence is about 100 times higher among Ashkenazic (central European) Jews than among Sephardic (Mediterranean) Jews and non-Jews.

Brain cells of babies with this disease are unable to metabolize mucopolysaccharides, because a crucial enzyme, hexosaminidase, does not function properly.

As mucopolysaccharides accumulate in the brain, the infant begins to suffer seizures, blindness and degeneration of motor and mental performance. The child usually dies after a few years.

Sickle-cell anemia is the most common inherited disease among African-Americans. It affects 1 in 400 African-Americans born in the United States.

The disease is caused by a single amino acid substitution in hemoglobin.

The abnormal hemoglobin molecules tend to link together and crystallize, especially when blood oxygen content is lower than normal. This causes red blood cells to deform from the normal disk-shape to a sickle-shape.

The sickled cells clog tiny blood vessels, causing pain and fever characteristic of a sickle-cell crisis.

About 1 in 10 African-Americans are heterozygous for the sickle-cell allele and are said to have the sickle-cell trait.

These carriers are usually healthy, although some suffer symptoms after an extended period of low blood oxygen levels.

Carriers can function normally because the two alleles are codominant (heterozygotes produce not only the abnormal hemoglobin but also normal hemoglobin).

The high incidence of heterozygotes is thought related to the fact that in tropical Africa where malaria is endemic, heterozygotes have enhanced resistance to malaria compared to normal homozygotes. Accordingly, heterozygotes have an advantage over both homozygotes.

Chromosome Variation

Changes in chromosome number

Most organisms are diploid, or have two sets of chromosomes. Many others are haploid, or have one set of chromosomes.

euploidy- a general term that refers to any number of sets of chromosomes

aneuploidy- a general term that refers to at least one chromosome more or less than the diploid number

polyploidy- refers to a cell having more than two sets of chromosomes

1 set = haploid

2 sets = diploid (the "normal" condition for most eukaryotic cells)

3 sets or more = polyploid

3 sets = triploid,

4 sets = tetraploid

Euploidy

As it turns out, many species of plants are polyploid descendants of diploid ancestors.

Polyploidy is tolerated rather well in many species of plants.

In animals, polyploidy is not tolerated and very few polyploid species are known to exist. Those that do exist are usually asexual, parthenogenetic, or hermaphroditic. Most of the problems resulting from polyploidy occur during synapsis of homologues during prophase I.

As plants do not have a chromosomal mechanism for sex determination, synapsis and subsequent disjunction is not as great a problem. In fact, most plants are monoecious.

Also, organisms that have an odd number of sets, for example triploid or pentaploid, are usually sterile because during prophase I, three homologues may synapsis. Disjunction at anaphase can result in two one way and one the other way. This leads to variable numbers of chromosomes in the gametes. Another possibility is two of the three homologues synapse and the third does not synapse at all. This also leads to unbalanced gametes.

Those organisms that have an even number of sets, for example tetraploid or hexaploid, will have an even number of homologues synapsis, and have a better chance of getting the same number of chromosomes to each gamete, though still not very likely.

Any individual that has multiple sets from the same genome. . . $2N \rightarrow 4N$ is called an autopolyploid. This individual will have four homologues synapsis during prophase I of meiosis. This is better than three or five, but there are still some problems.

Polyploids can be created artificially by treatment with colchicine, which blocks spindle formation so that the chromosomes do not separate. Eventually, the nucleus reforms but with double the number of chromosomes. Within somatic cells, for example liver cells, many individual cells may be tetraploid, with no apparent ill effects for the

individual. If, however, the sets represent separate genomes, things are better in terms of how meiosis proceeds and ultimately in terms of fertility. With two sets from two different genomes (allopolyploidy), the synapsis involves only two chromosomes, and works well. Allopolyploidy can come about in several ways.

1.

a) Haploid gametes of two distinct species combine to form a hybrid.

The hybrid reproduces vegetatively until somatic doubling occurs in a cell of the floral meristem, which produces a flower structure that has all chromosomes existing as homologous pairs.

At this point, meiosis is normal and sexual reproduction can occur via self-fertilization.

2. Two distinct species produce unreduced (diploid) gametes, which fuse to produce an allopolyploid, which is fertile if it can self-fertilize.

Aneuploidy

Aneuploidy refers to at least one more or one less chromosome than the diploid number. If an individual has $2N+1$, in which one of the chromosomes has three copies, the individual is trisomic. Two extras ($2N+2$) is tetrasomic and only one copy ($2N-1$) is monosomic. Aneuploidy results from nondisjunction or failure of either homologous chromosomes in anaphase I or sister chromatids in anaphase II to separate at some stage of meiosis. This produces a gamete with one fewer or one extra chromosome than the normal haploid number.

Upon fertilization, a monosomic or trisomic results. In humans, the most common viable aneuploids involve the sex chromosomes, giving rise to XXX, XO, or XXY individuals.

There are no clear deleterious effects associated with the karyotype XYY. However, XYY men tend to be taller. Interestingly, there is a twenty-fold higher incidence of XYY males among the prison population than in the population as a whole, though XYY males in the non-prison population do not show an increased tendency for criminal behavior.

Within the autosomal complement, the only aberrations to have survived to birth are:

trisomy-21 = Down's syndrome (1/1000)

trisomy-13 = Patau syndrome (1/15,000)

trisomy-18 = Edward syndrome (1/7500, mostly female)

Apparently, the deleterious effects of trisomic conditions are due to overproduction of certain proteins that lead to a number of developmental problems. The only ones to have been detected in live births involve the smaller chromosomes in the human complement.

The only cases of monosomy found in live births involves the X chromosome, where XO individuals are viable, but are compromised in certain mental and physical characteristics. Monosomy in the autosomal complement is always inviable in humans, probably due to the unmasking of lethal mutations that exist in a recessive state, as well as disruption of developmental processes because of an underproduction of regulatory proteins.

30% of all fertilizations spontaneously abort within the first three months. 50% of spontaneous abortions in the United States have some sort of obvious chromosomal rearrangement, such as monosomic or trisomic conditions. These two facts indicate that nondisjunction during gametogenesis or possibly during early mitotic division is a quite common occurrence and that most aneuploid fetuses fail to survive to birth.

Centric fusion/fission

These are also referred to as Robertsonian events. It occurs when two acrocentrics fuse to produce one metacentric. The process can also go backwards. The metacentric chromosome created by a centric fusion may retain both centromeres.

These fusion/fission events change the chromosome number, but do not change the number of arms. The number of arms in the autosomal complement is referred to as the fundamental number. A centric fusion will change the number of chromosomes but will not change the fundamental number.

As a rule, an individual that is heterozygous for a centric fusion will still be fertile. Disjunction will proceed normally. Thus, some species will have individuals with different diploid numbers, but the same fundamental number.

Change in chromosomal structure

deletions

duplications

inversions

translocations

Deletions can result from one or two breaks from a single chromosome.

If a single break occurs across both chromatids after replication, a dicentric fragment can result. The fragment will be lost due to the lack of a centromere. The chromosome will not move properly during anaphase and will either break or be totally lost.

If two breaks occur, the ends may rejoin and the interior fragment will be lost. The deletion can be detected due to mismatched pairs in a standard karyotype. It can be confirmed by the appearance of a bulge during synapsis at prophase I. Also, similar structures in the polytene chromosomes of fruit flies can be seen, and the absence of certain bands can be detected.

G- and C-banding
G-banding - dark stains of AT rich regions
C-banding - dark stain for heterochromatin
G- and C-banding are quite useful in detecting chromosomal changes that occur within a species, as well as chromosomal changes that characterize differences between species.

Deletions are almost always detrimental. Most species cannot tolerate the loss of any chromosomal material. The deletion of a piece of one chromosome probably allows the unmasking of lethal recessive genes present on the homologue, as seen in a monosomic individual. In addition, underproduction of regulatory proteins probably disrupts fetal development. An example in humans is cri-du-chat, a deletion in the short arm of chromosome 5. The infant makes a meowing sound like a cat, and is severely retarded.

Duplications are usually detected by repeated band sequences, especially in polytene chromosomes. They can be tandem repeats such as ABCABC, or inverted repeats such as ABCCBA. Besides being caused by chromosomal breaks, they are usually caused by unequal crossing-over between chromatids. This results in one chromatid with an excess and the other with a deletion. Serially duplicated genes are thought to have given rise to the moderately and highly repetitive DNA found in eukaryotes.

The genes alpha-globin and beta-globin of hemoglobin represent a duplication that occurred in the genome leading to the vertebrates. The alpha-globin gene duplicated itself and the repeated sequence became the beta-globin gene. Both are very similar to each other in the placement of introns and exons and both genes share many amino acid positions.

Inversions involve two breaks in a chromosome, where a piece is cut out, flipped end over end and reinserted. Crossing-over occurs between chromatids within the inversion loop. The dicentric and acentric pieces are either lost totally or give rise to abnormal gametes that produce inviable offspring. An individual heterozygous for an inversion has a 50% reduction in fertility because of inviable gametes. An inversion represents a strong barrier to interspecies reproduction, a post-mating isolating mechanism. We will examine this in more detail when we get to population genetics.

If the centromere is included within the inverted sequence, it is called a pericentric inversion. If the centromere is not included within the inverted sequence, it is called a paracentric inversion. An inversion can be detected either by a change in banding pattern or by the presence of inversion loops formed during synapsis in the heterozygous state. If the species has a mechanism to suppress crossing-over within the loop (as in *Peromyscus*), or a mechanism to sequester malformed chromosomes into polar bodies (as in *Drosophila*), the heterozygous state of the inversion loop is not detrimental. However, because the inverted segment of the chromosome has suppressed crossing-over, this region of the chromosome does not recombine and behaves as one large gene, called a supergene.

Translocation is the transfer of a part of one chromosome to another nonhomologous chromosome. If it involves one break and a transfer to another chromosome, it is called a simple translocation. If the chromosomes exchange segments, the exchange is called a reciprocal translocation.

An individual that is heterozygous for a reciprocal translocation, such as the hybrid that may result from the mating of two different species, will have a reduced fertility because of problems during meiosis.

Some chromosomal rearrangements (such as translocations) may change the position of a gene such that it finds itself in a highly active region of a chromosome. This results in increased expression of the gene. This change in expression is known as a positive effect.

An example is in Burkitt's lymphoma, in which a translocation between chromosome 8 and 14 moves a growth factor gene close to the antibody genes, which are very active in a lymphocyte. This leads to the cell becoming cancerous.

MULTIPLE ALLELES

A number of human traits are the result of more than 2 types of alleles. Such traits are said to have **multiple alleles** for that trait.

allele = (n) a form of a gene which codes for one possible outcome of a phenotype

For example, in Mendel's pea investigations, he found that there was a gene that determined the color of the pea pod. One form of it (one allele) creates yellow pods, & the other form (allele) creates green pods.

When the gene for one trait exists as only two alleles & the alleles play according to Mendel's Law of Dominance, there are 3 possible genotypes (combination of alleles) & 2 possible phenotypes (the dominant one or the recessive one).

Using the pea pod trait as an example, the possibilities are like so:

<u>GENOTYPES</u>	<u>RESULTING PHENOTYPE</u>
Homozygous Dominant (YY)	Yellow
Heterozygous (Yy)	Yellow
Homozygous Recessive (yy)	Green

where

Y = the dominant allele for yellow &

y = the recessive allele for green

If there are only two alleles involved in determining the phenotype of a certain trait, but there are *three* possible phenotypes, then the inheritance of the trait illustrates either incomplete dominance or codominance.

In these situations a heterozygous (hybrid) genotype produces a 3rd phenotype that is either a blend of the other two phenotypes (incomplete dominance) or a mixing of the other phenotypes with both appearing at the same time (codominance).

Here's an example with Incomplete Dominance:

<u>GENOTYPES</u>	<u>RESULTING</u>
BB = Homozygous Black	<u>PHENOTYPE</u>
BW = Heterozygous	Black Fur
WW = Homozygous	Grey Fur
White	White Fur

where

B = allele for black &

W = allele for white

And here's an example with Codominance:

<u>GENOTYPES</u>	<u>RESULTING</u>
BB = Homozygous Black	<u>PHENOTYPE</u>
BW = Heterozygous	Black Fur
WW = Homozygous	Black & White Fur
White	White Fur

where

B = allele for black &

W = allele for white

Now, if there are 4 or more possible phenotypes for a particular trait, then more than 2 alleles for that trait must exist in the population. We call this "Multiple Alleles".

Blood type is an example of a common multiple allele trait. There are 3 different alleles for blood type, (A, B, & O). A is dominant to O. B is also dominant to O. A and B are both codominant.

Let me stress something. There may be multiple alleles within the population, but individuals have only two of those alleles.

Why?

Because individuals have only two biological parents. We inherit half of our genes (alleles) from ma, and the other half from pa, so we end up with two alleles for every trait in our phenotype.

There are 3 alleles for the gene that determines blood type.
 (Remember: You have just 2 of the 3 in your genotype --- 1 from mom & 1 from dad).

The alleles are as follows:

<u>ALLELE</u>	<u>CODES FOR</u>
I^A	Type "A" Blood
I^B	Type "B" Blood
i	Type "O" Blood

<u>Phenotype</u>	<u>Possible Genotypes</u>
O	ii
A	$I^A I^A, I^A i$
B	$I^B I^B, I^B i$
AB	$I^A I^B$

Notice that, according to the symbols used in the table above, that the allele for "O" (i) is recessive to the alleles for "A" & "B". With three alleles we have a higher number of possible combinations in creating a genotype.

<u>GENOTYPES</u>	<u>RESULTING PHENOTYPES</u>
$I^A I^A$	Type A
$I^A i$	Type A
$I^B I^B$	Type B
$I^B i$	Type B
$I^A I^B$	Type AB
ii	Type O

Notes:

As you can count, there are 6 different genotypes & 4 different phenotypes for blood type.

Note that there are two genotypes for both "A" & "B" blood --- either homozygous ($I^A I^A$ or $I^B I^B$) or heterozygous with one recessive allele for "O" ($I^A i$ or $I^B i$).

Note too that the only genotype for "O" blood is homozygous recessive (ii).

Antigen: Protein on the surface of the blood cell. (Allele A makes A antigen. Allele B makes B antigen. Allele O makes no antigens.)

Antibody: Protein in plasma that reacts with specific antigens that enter the blood (usually something that isn't supposed to be there!). (Ex.: Anti-A is an antibody that recognizes A-antigen, binds to it (lock & key), then causes clumping together or clotting of similar A-antigens.)

Distribution and Characteristics of Human Blood Factors

Blood Type	Antigen on Red Blood Cell	Antibody in Serum Plasma	Will Clot with Blood From These Donors	Can Receive From	Can Give to:
O	None	Anti-A, Anti-B	A, B, AB	O	All
A	A	Anti-B	B, AB	A & O	A & AB
B	B	Anti-A	A, AB	B & O	B & AB
AB	A & B	None	None	All	AB

Type O Blood: Universal Donor as it contains no A or B antigens, so the receivers' blood will not clot when given the O blood.

Type AB Blood: Universal Receiver, as it contains no Anti-A or Anti-B antibodies in its plasma. It can receive all blood types.

ARTIFICIAL INSEMINATION

Artificial insemination (AI) was the first great biotechnology applied to improve reproduction and genetics of farm animals. It has had an enormous impact worldwide in many species, particularly in dairy cattle. The acceptance of AI technology worldwide provided the impetus for developing other technologies, such as cryopreservation and sexing of sperm, estrous cycle regulation, and embryo harvesting, freezing, culture and transfer and cloning.

The history of artificial insemination: Selected notes and notables

Artificial insemination (AI), as practiced by bees and many other flying insects, has played an important role in plant reproduction for a very long time. Use of AI in animals is a human invention and more recent. Undocumented tales exist of Arabs obtaining sperm from mated mares belonging to rival groups and using the sperm to inseminate their own mares. However, our story starts with recorded history, where facts are available to document noteworthy achievements. Consequently, the story is related chronologically. The developments that made AI the most important animal biotechnology applied to date include improved methods of male management and semen collection, evaluation, preservation, and insemination. Detection of estrus and species briefly included are swine, horses, sheep, goats, dogs, rabbits, poultry, and endangered species.

Swine

The AI of swine was initiated by Ivanow in Russia in the early 1900s. More extensive investigations were conducted in the 1930s. Early work was started in the United States in Missouri, in Japan in 1948, and in western Europe in the 1950s. Boars are easily trained to mount dummies. All artificial vaginas developed for boar semen collection provided a means of applying pressure to the glans penis, or a gloved hand can be used directly. F. McKenzie trained many early outstanding reproductive physiologists (Andrews, Casida, Phillips, etc.). He attended animal science meetings long after officially retiring, asking questions about the latest developments. At the 75th meeting of the American Society of Animal Science, when a roll of long-time members was called, he was the only member standing at the end, a member for 61 yr. Russian diluters for boar semen were based on glucose solutions with sodium potassium tartrate or sodium sulfate and peptone, keeping the concentration of electrolytes low. The recommended storage temperature was 7 to 12°C. When the yolk phosphate, yolk-citrate, and milk extenders were developed for bull semen, they were used or modified for boar semen, including use with cooled semen. Major efforts were made to freeze

semen, following the successful use of frozen bull sperm. Many modifications of extenders and freezing procedures were developed, including the pellet method of freezing, which was developed originally in Japan. Pregnancy rates and litter sizes are reduced with cryopreserved boar sperm, so frozen semen is limited to use in special breeding programs. Fresh or extended liquid semen is used for about 99% of AI in swine. Rapid transport of extended semen makes it feasible for swine farmers to take advantage of commercially produced boar semen. Large corporations increase the services possible from their own boars by using AI. A typical dose is 3×10^9 sperm inseminated in 80 mL. Over 50% of the sows in the United States are inseminated today, and about 80% farrow with 10 pigs per litter. Swine AI is growing at a phenomenal rate. Techniques for evaluating quality of boar sperm are similar to those used for bull sperm. Sexing of boar sperm is possible but is too slow to produce sexed sperm for commercial use.

Horses

Research on AI of horses started in Russia in 1899, stimulated particularly by the military's need for horses. Ishikawa initiated similar studies in Japan in 1912. McKenzie et al. (1939) and Berliner (1942) initiated studies on the collection, processing, and insemination of stallion and jack semen in the United States. The earliest collections of semen were obtained by placing a rubber semen collection bag in the vagina of a mare in estrus. In the 1930s and 1940s, several types of artificial vaginas were developed and they since have been modified. Semen evaluation is performed similarly to the evaluation of bull semen. Military studs provided a convenient source of stallions, but after World War II many of these were eliminated because the horse population had declined. In addition, the restrictive regulations on the use of AI by several equine breed organizations inhibited research and the application of AI. China was the major country using equine AI during this period. Advances made in cryopreserving bovine sperm stimulated interest in cryopreservation of equine semen. Research results are summarized in several congresses and symposia. Although methods have been devised to freeze stallion sperm, most equine AI is done with cooled, extended semen used within 48 h of collection during the spring breeding season.

Sheep and Goats

The early development of AI in sheep on a major scale began in Russia, where the collective farms provided an ideal arrangement for establishing AI programs. China also has extensive sheep AI programs. Artificial insemination spread to central Europe and also was widely applied commercially in France and Brazil. The techniques for semen collection and artificial insemination in sheep and goats have been described in detail.

Semen quality and breeding efficiency are affected by season. Both rams and bucks can be trained to serve the artificial vagina. However, for obtaining semen from a large number of rams in the field, electroejaculation is a useful procedure, pioneered by Gunn (1936) and applied to many species. Much of the early research in the Western world on extenders for sperm, freezing of semen, and AI techniques was done by Emmens and Blackshaw, followed by Salamon and Maxwell in Australia, and Dautier, Colas, and Cortell in France. Buck sperm cryopreservation is more successful than the cryopreservation of ram sperm. The techniques and media for freezing semen such as with egg yolk-triglycerol were modified from procedures developed for bull sperm. Frozen-thawed semen results in satisfactory fertility in goats provided that the sperm are deposited deep into or through the cervix. In the ewe this is difficult. Therefore, insemination into the uterus with the aid of a laparoscope has been necessary to achieve high fertility. Recently, Maxwell et al. (1999) used intracervical insemination successfully by adding seminal plasma to cryopreserved ram sperm before it was used for insemination. Because of the difficulty of insemination, general management and low value per animal, AI, particularly of sheep, is not widespread.

Poultry

Artificial insemination has been widely applied to poultry. Semen collection, processing, and AI have been reviewed by Sexton (1979) and Lake (1986) and more recently by Donoghue and Wishart (2000). Pioneers in the poultry field were Burrows and Quinn (1937), who developed the method of abdominal massage and pressure to collect semen. With the ease of collecting poultry semen, and proximity of hens on large breeding farms, AI is used extensively with freshly collected semen. It is used 100% for turkey breeding because mating is difficult. Freshly collected chicken semen was among the first type of semen to be frozen. However, cryopreserved poultry sperm are less fertile and freezing poultry sperm still is experimental.

Other Domestic Mammals

Although the dog was the first animal in which AI was documented, AI has only been used in special cases, such as for breeding guide dogs or for overcoming special problems. Many breed organizations do not register puppies produced by AI. Cryopreservation of dog sperm is successful, using modifications of the yolk-tris and yolk-lactose extenders developed for cryopreserving bull sperm. Rabbits have been extensively used as a model for large animals and humans. All the reproductive techniques employed with farm animals can be performed with the low-cost rabbit model, and certain placental membrane characteristics make them especially relevant for studies of human teratology.

Endangered Mammals

The first animal reproductive biotechnology used to preserve endangered species was AI. Again, many of the principles and procedures used were adapted from cattle (Watson, 1978).

Implications

In the initial stages of attempting to develop AI there were several obstacles. The general public was against research that had anything to do with sex. Associated with this was the fear that AI would lead to abnormalities. Finally, it was difficult to secure funds to support research because influential cattle breeders opposed AI, believing that this would destroy their bull market. The careful field-tested research that accompanied AI soon proved to the agricultural community that the technology applied appropriately could identify superior production bulls free from lethal genes, would control venereal diseases, and did result in healthy calves. Thus, fear was overcome with positive facts. The extension service played an important role in distributing these facts. The knowledge gained from the AI experience was extremely helpful in stepwise development of each successive reproductive technology, such as frozen semen, superovulation, embryo transfer, and, eventually, cloning. Simultaneously, the public became better informed and more willing to accept that technology developed with worthy goals, and built-in ethical application, could produce positive change, benefiting the whole community. Worthy goals, development of the necessary knowledge and skills, and ethical considerations all are essential components of any technology that will result in a positive impact on society and the environment. Thus, the impact of AI was much more profound than simply another way to impregnate females.

Advantages of Artificial Insemination

Artificial insemination (AI) is an important technique to ensure rapid genetic improvement in any species. In addition it has several other advantages as listed below.

- (i) It gives more efficient use of genetically superior males.
- (ii) Prevention of diseases.
- (iii) Eliminates the need for transportation of animals.
- (iv) Elimination of behavioural problems.

(v) Preservation of semen can extend the reproductive life of the good males, as it can be used long after the death of the male.

Difficulties Associated with Artificial Insemination

There are however, certain difficulties associated with AI as laid out below:

- (i) Poor post ejaculation sperm motility due to the gelatinous nature of the semen.
- (ii) Lack of standard techniques for freezing semen.

Methods of Semen Collection

The main techniques used nowadays are i) electro-ejaculation and ii) by artificial vagina. A brief account of both methods are given below.

Electro-ejaculation - For semen collection by electro-ejaculation, sedation or general anaesthesia of the animal may be required. In camels the use of detomidine hydrochloride (Dormosedan) 30 - 35 mg/kg bodyweight bwt, i.v., or 70 – 80 mg/kg bwt, i.m., is superior to other sedatives such as xylazine and acepromazine. In camels electro-ejaculation can be achieved by giving two sets of stimulation, each of 10 - 15 pulses of 3 to 4 secs duration at 12 v and 180 mA, with a rest of 2 - 3 min between the two series of impulses. Several collection tubes should be at hand, because of the possibility of urine contamination. Also, due to the short duration of ejaculation, the semen obtained is often of poor quality. The volume of semen recovered by electro-ejaculation is usually less than that collected by artificial vagina (AV), but the other semen parameters are similar.

Artificial Vagina - For collection by AV in camels a modified bull vagina (30 cm long, 5 cm internal diameter) has given the best results. However, care must be taken to avoid the ejaculate making contact with the rubber liner of the AV, since this has been shown to adversely affect sperm motility. Hence, a shortened AV may be used, allowing the semen to pass directly into the collection flask. Alternatively, an additional disposable plastic inner liner may be inserted to avoid contact with the rubber material but I find that the camels do not accept this very well. The AV is filled with water at 55 - 60°C to give an internal temperature of 38 - 40°C. A clear, glass water-jacketed (35 - 37°C) semen vessel is attached to the apex of the cone shaped internal latex rubber liner to enable visualisation of ejaculation. Observation of natural matings suggested that the highly mobile urethral process of the camel penis may need to gain entry to the cervix to stimulate ejaculation during the extended copulatory process. For this reason we inserted a foam imitation cervix of about 8 cm in length inside the AV. The semen is

then collected using the following method: (i) A sexually receptive female is first teased by the male in order to make olfactory contact (ii) The bull is then lead up from behind to the sitting female with the operator on the left side of the female. (iii) As soon as the bull has sat down on the female and makes a few thrusts, the operator grasps the bull's sheath and directs his penis into the AV and holds it there by manual pressure at the base of the scrotum. (iv) The bull will make several thrusts, interspersed by periods of rest, until ejaculation is completed. The ejaculate is usually expected in fractions and this whole process can take between 5 and 10 min, although it may occasionally last for 20 min or even longer!

Semen Evaluation

Volume - The volume of the semen is highly variable depending on the male, method of semen collection and the length of sexual stimulation.

Colour - The colour of camelid semen is greyish-white depending on the ratio of the gelatinous fraction, which is grey, to the sperm rich fraction, which is white. If the semen appears slightly yellow in colour it has probably been contaminated with urine.

Viscosity - One of the main physical characteristics of camelid semen is its high viscosity which makes handling and estimation of sperm concentration difficult. This viscosity is usually attributed to the presence of mucopolysaccharides from secretions of the bulbourethral gland or the prostate, but the degree of viscosity depends on the individual male and on the quantity of the gelatinous fraction in the ejaculate. If the semen is allowed to stand at room temperature for 10 - 20 min. it may partially liquify and the spermatozoa can attain motility, but other studies have shown that liquification can take up to 8 h. This problem may be overcome by using hydrolytic enzymes for liquefaction of semen. Trypsin at 1:250 concentration is effective at liquifying alpaca semen but in other attempts to eliminate the viscosity of semen in 2 - 3 min., collagen (0.5 mg/ml) seemed to be a better agent than trypsin. The reaction was irreversible however, i.e., the liquified semen did not regain its viscosity.

Sperm Concentration - Concentration of semen is best estimated using a haemocytometer. The semen sample is diluted 1:100 or 1:200 in formal citrate before the number of sperm are counted.

pH - pH can be directly measured using small volume electrodes or pH paper. Seminal plasma is slightly alkaline and ranges from 7.8 - 8.6 in the dromedary.

Semen Motility - Motility is best estimated by placing a drop of semen diluted in extender onto a pre-warmed glass slide and examining it under a microscope. It varies

greatly depending on the viscosity of the semen. Other methods of determining sperm motility such as a computerized semen motility analyzer are difficult in camelids due to the gelatinous nature of the semen. Semen used for artificial insemination or preservation should have at least the characteristics.

Short-term Semen Preservation

Semen Extenders - Semen extenders for camelidae are mostly adapted from studies in other species and contain:

- (i) a source of energy (glucose or fructose)
- (ii) a protein for protection against cold shock (lipoprotein from egg yolk or casein from milk).
- (iii) a buffering system and antibiotics.

In dromedaries the best results to date have been achieved with three commercial extenders, Green buffer (IMV, L'Aigle, France) with 20% (v:v) egg yolk added; Laiciphos (IMV) and Androhep or an extender containing 11% (w:v) lactose and 20% (v:v) egg yolk, as compared with other extenders such as skimmed milk extender which contains 2.4% (w:v) skimmed milk powder, 4.9% (w:v) glucose, 0.2 Mega penicillin/100 ml extender and 735 units/ml streptomycin. As the collection procedure can be rather prolonged it can be advantageous to add about 1 - 2 ml of extender to the collection vessel before the collection takes place to help preserve the semen.

Dilution

In the dromedary and camels, semen is diluted at a ratio of 1:1 or 1:3 (semen:extender) depending on the concentration of the ejaculate. The extender should be at a temperature of 30 - 35°C when added slowly to the semen. Further work is required to determine the optimum number of spermatozoa per insemination, but initial studies indicated that good results are obtained in dromedaries if 300×10^6 motile sperm are inseminated.

Cooled Semen

Diluted semen can be stored in the refrigerator at +4°C for 24 - 36 h or in an Equitainer®. An Equitainer is a thermally insulated container standing about 45 cm in height that has a hollow core in which two coolant cans, that have been in a freezer for at least 24 h prior to use, are placed. The semen is sealed in a plastic universal tube which is wrapped in two thermal balast bags (provided in the equitainer) at room

temperature and placed within a plastic cup inside the equitainer before closing the lid. The semen is inseminated 24 h later providing it has a motility of at least 35 - 40%. However whereas pregnancy rates of 50 - 60% have been reported in camels inseminated with fresh semen diluted as above, and inseminated within 30 minutes of collection, the conception rate dramatically decreases to 25 - 30% in camels inseminated with diluted semen cooled for 24 h; all the pregnancies were achieved from cooled semen diluted in Green buffer + 20% egg yolk.

Frozen Storage of Semen

Split samples of semen have been used to compare different freezing methods by assessing post-thawing sperm motility and morphology and by testing viability in a physiological solution of 1% sodium chloride at 38°C. It was determined that the best freezing method was a modification of the technique developed for boar semen by Westendorf et al, in 1975.

Cooling extender - 11% lactose solution and 20% egg yolk.

Freezing extender - 11% lactose solution, 20% egg yolk, 6% glycine and 1.5% OEP-Equex (emulsifying agent).

Thawing of Semen

Thawing is best carried out in a water bath:

small straws - 40°C for 10 secs

large straws - 50°C for 40 secs

ampoules - for 1 - 2 mins

The quality of post-thawed semen is improved by using small straws (0.25 ml) as the freezing vehicle, but the use of larger straws used to be recommended to ensure that an adequate amount of semen was deposited in the uterus to provide the

Scheme 1 for freezing camel semen

Raw semen 25 - 30°C

First re-suspension; Add cooling extender (1:1 v:v) Cool to +15°C in 2.5 h

Second re-suspension; add freezing extender to give a sperm concentration of 150 x 10⁶/ml after 2.5 h Cool to +5°C in 1.5 h

Add freezing extender up to a sperm concentration of 100 x6/ml +5°C

Place in large (4 ml) straws (Fa. Makrotub, Landshut, Germany)

Freeze 20 min over liquid N₂ from +5°C to -120°C

Plunge into liquid N₂ from -120°C to -196°C

Scheme 2 for freezing camel semen

Raw semen 25 - 30°C

Re-suspend semen (1:1, v:v) in Green buffer + 20% egg yolk Cool to +4°C in 2 h

Add equal volume of Clear Buffer +20% egg yolk as Green buffer

added above (i.e. if added 5 ml Green buffer add 5 ml Clear buffer) Equilibrate at 4°C for 30 min

Load into 0.25ml straws

Freeze 20 min over liquid N₂ from +4°C to -140°C

Plunge into liquid N₂ from -140°C to -196°C

factors necessary for inducing ovulation. However this necessity has now been overcome if the insemination is preceded by an injection of 3000 i.u. human Chorionic Gonadotrophin (hCG) or 20 mg Buserelin 24 h before the insemination takes place.

Fertility Results for Frozen-Thawed Semen

Although remarkable fertility has been reported for camels inseminated with frozen semen, such results have not yet been reported for dromedary camels. Although post-thaw motility has looked promising, no pregnancies have been reported as yet after inseminating dromedary camels with frozen semen. Therefore, further experiments are required to improve the fertility of frozen-thawed dromedary semen.

Methods and Timing of Insemination

Using ultrasonography the ovarian follicles should be greater than 13 mm before ovulation is induced and insemination takes place. Insemination can be carried out with the female in a standing or sitting position. In the latter case the animal may be restrained in her position which is much safer. The perineal region should be cleaned and the semen deposited into the cranial part of the cervix or the body of the uterus. This can be achieved by using an insemination gun passed through the vagina and cervix

with one hand in the rectum holding the cervix, similar to the standard technique used in cattle. In the dromedary camel ovulation occurs between 36 - 48 h after GnRH or hCG injection and the optimum time for insemination with either fresh or frozen-thawed semen is 24 h after treatment. Good results (50%) have been obtained with fresh semen when insemination occurred 24 h after mating with a vasectomized male.

Artificial Insemination of Poultry

Artificial insemination was first practiced in America during the 1920's and became widely used in Australia with the introduction of laying cages in the late 1950s.

Some of the advantages that have been claimed for artificial insemination in the past have been:

Increased mating ratio: In a flock it is usually one cockerel mated to six to ten hens. With artificial insemination it is claimed this ratio could be increased four fold. In both cases it depends on the strain and breed of the birds. In my commercial farming days with white leghorn cockerels I used from five to seven cockerels with a pen of a hundred hens. When heavy meat birds came along it was about one cockerel to about eight or ten hens in a large flock.

Use of older males from outstanding performers: Older male birds that have been flock improvers can be used for several generations. Whereas under natural mating their useful life is limited.

Able to use an injured bird: Valuable male birds that have been injured in the leg can still be used for artificial insemination.

Elimination of preferential mating: When there is poor fertility cause by preferential mating it can be eliminated.

Laying cages can be used: Laying cages are no longer a problem when fertile eggs are needed. Selected hens can be inseminated and remain in the cage. The exact pedigree of the chickens hatched from these fertile eggs is known. Several commercial farms used colony cages with several hens and one rooster, fertility always seemed to be a problem; artificial insemination did solve this problem for some, until this type of housing went out of favour generally. Although there is still one large farm only an hours drive from my office that still uses this system for its breeding stock. See the

photo of a colony cage from my farm, which I used for layers but not for breeding purposes.

Successful cross breeding: Usually cross breeding is very successful under natural conditions, but some times there is a kind of colour discrimination: some hens will not mate with a male of a different colour unless they have been reared together. During my commercial poultry farming when the white leghorn Australorp cross were the layers of the day, I always reared the white leghorn cockerels with the Australorp pullets right from day old onwards. This practice produced very good fertility in this cross. But for small breeders or poultry fanciers rearing them together is not always possible, artificial insemination could be a solution to a fertility problem.

Recommended housing of the rooster.

The male birds can be housed in individual cages, but they need to have enough room to be able to crow. A suggested cage size is 45 cm wide, 60 cm deep and 60 cm high. The feed and water containers should be hung on the outside of the cage. Male birds respond to the people handling them and a quiet, unhurried approach is necessary with careful handling. During the collection of semen, it is essential that visitors remain outside the shed. This will prevent the birds from becoming frightened. It is a good idea that the males are housed in close- proximity to the hens so that the time between collection and in- semination is kept to a minimum. Prior to use, the selected male birds should be examined for external parasites, particularly poultry lice, and treated accordingly. It is also a good idea to clip the feathers from around the vent area to give easy access to the male organ. This applies particularly to loose-feathered breeds of poultry.

Semen Collection.

For this operation two people are needed, one for holding and collecting the semen (holder), the other (operator) to stimulate the control flow of semen. The holder rests the male bird's keel on the palm of his right hand in a horizontal position so that the head is between the holder's side and elbow, the bird's legs being free to move. It is important to hold the male bird loosely to gain the desired result. The holder's left hand is used to collect the semen.

The operator holds the rooster's legs loosely but firmly in his right hand and strokes the back of the bird from neck to tail with his left hand. The stroke is firm but not tight and the fingers and thumb follow the lateral contours of the body. After a few strokes, the male organ swells and protrudes outwards and downwards. The white semen will be

seen in the central furrow of the organ. The semen is milked down by firm finger pressure either side of the vent into the collecting tube.

The male bird should be milked three or four times before insemination is required to check semen quantity and colour. If the male bird refuses to produce semen after 10 days of handling or if the semen, which should be white, is discoloured due to contamination by faecal material or blood, then it is probably useless to persevere with him.

Insemination of the Hen.

The hen is held by the left hand being placed over the breast with the bird's back forced against the holder's body, the head pointing to the ground. The right hand is placed over the vent so that the thumb is above and forefinger below the vent. A sudden pressure exerted around the breast area and, at the same time, using the thumb and forefinger to spread apart the cloaca, resulting in the turning of the cloaca inside out. The operator, with 0.1 ml of semen or 0.2 ml of diluted semen drawn up in the inseminating tube, places this tube as far as possible in the exposed oviduct opening seen at the left side of the intestinal opening. The semen is introduced at the same time as the holder releases the pressure and the cloaca returns to its normal position.

Regularity of Insemination.

Inseminations should be carried out on two consecutive days the first week and then once each week thereafter while fertile eggs are required.

As poultry semen has a very limited life, insemination of hens should be complete within one hour of semen collection.

It is a good idea to carry out the operation at the same time each day, the best time being between 2.00pm and 4.00pm. The reason for this is that during the morning, most hens have an egg in the oviduct, thus obstructing the free passage of semen to the ovary. Another point in favour of inseminating the hens in the afternoon is that it is generally cooler and the hens are less likely to be affected by heat, particularly in late spring.

Observation has shown that eggs are fertile after the second day of insemination and can remain fertile for two weeks or more. If another male is to be used on the same hen in a breeding program, it is suggested that a period of three weeks elapse before the second male is used.

If large numbers of male birds are to be used for artificial insemination, it is suggested that, prior to their use, a sample of the semen be examined under a microscope to check sperm motility as there is a good correlation between sperm movement and fertility.

Equipment Required.

The equipment need not be lavish or expensive. It consists of a glass or plastic test tube for collecting semen from the male, a 3 cc hypodermic syringe with 0.1 ml graduations, a rubber connection (bicycle valve rubber), and a 0.5 cm external diameter glass inseminating tube 9 cm in length. Sometimes, a small plastic funnel is used where semen collection may be difficult. Equipment is illustrated below.



Artificial Insemination Equipment From top, clockwise: Syringe with inseminating tube and rubber connection. Ringer's Solution. Glass Tube

In order to increase the number of hens that can be inseminated from the same rooster, the semen may be diluted with a solution known as modified Ringer's solution. The composition of this solution is as follows:

Sodium chloride 68 grams

Potassium chloride 17.33 grams

Calcium chloride 6.42 grams

Magnesium sulphate 2.50 grams

Sodium bicarbonate 24.50 grams

Distilled water 10 000 cc

Rather than go to the trouble and expense of preparing this solution, it can be purchased from some pharmaceutical companies. The degree to which semen can be diluted is in the ratio of one part semen to 2 parts diluent.

Crossbreeding Systems For Animal Production

Commercial producers can improve productivity and efficiency by understanding and applying genetic principles. Improvement through genetics can be achieved using two different methods:

1. Selection

2. Crossbreeding

Selection: In order for commercial producers raising straightbred, single breed cattle to make genetic improvement, they must utilize selection, and provide an optimal environment for those cattle selected to express their genetic potential. Selection is an excellent tool with traits of moderate to high heritability such as growth rate and carcass traits. However, some of the most important traits related to beef cattle production, such as reproductive rate and calf survival, are of low heritability. This means the success of selection programs for these traits will be very limited.

Natural selection: is a relentless process that eliminates the less suitable organisms in an environment. Natural selection, or just called selection, is a process whereby one genotype leaves more offspring than another genotype. Selection is determined by reproductive success, which has two components - fertility and survival. The genotype that leaves the most offspring is given the highest value for reproductive success. This value is called the fitness. The letter w is usually used to signify fitness and can vary from 0 to 1. Fitness is always relative to the other genotypes in the population and can vary from time to time. A variety of factors can decrease the fitness value w to below 1. The sum of the forces provides a selection coefficient, which is usually denoted by the letter S .

$$w = 1 - S$$

Types of selection
Directional selection - works by continuously removing individuals from one end of the phenotypic distribution. e.g. during the Eocene, the oldest member of the horse family appeared in the fossil record - *Hyracotherium*, about one foot high at the shoulder. Today's horses are much taller, and represent a continuous directional selection for taller horses.

Stabilizing selection - works by constantly removing individuals from both ends of a

phenotypic distribution, so that the mean is not shifted. This is the more common situation, and occurs as a population becomes optimally adapted to an unchanging environment. For example, directional selection favored an increase in the length of the giraffe's neck. However, the length today appears to be unchanging, thus stabilizing selection is acting to maintain the length of the neck.

Disruptive selection - works by removing individuals from the center of the phenotypic distribution while favoring individuals on either end. Disruptive selection is seen in the appearance of different discrete forms or morphs in the same species. An example is the polymorphic butterfly *Papilio dardanus*. This butterfly mimics several distasteful species of butterflies by its color pattern. The dominance relationships among the genotypes are such that an individual will mimic any number of models but intermediates do not occur. Intermediates would not resemble any of the models and would be rapidly eaten.

Crossbreeding: provides advantages from two main components, heterosis and complementarity. Heterosis (hybrid vigour) occurs when different breeds are mated together. One way to look at heterosis is that all purebred cattle are considered inbred as a result of breed formation and selection. Inbreeding leads to a reduction in performance, i.e. inbreeding depression. When different breeds are mated the crossbred progeny are less inbred than their parents. As a result the calves perform at a level above the average of their parents. This is heterosis, or hybrid vigour. Traits with lower heritability tend to exhibit high heterosis. Therefore, heterosis is more important for key traits relating to reproductive efficiency and calf survival, which have low heritabilities and do not respond well to selection.

Each breed has its strengths and weaknesses. Complementarity results when desirable characteristics from different breeds are combined into a crossbred. Crossbreeding achieves a higher frequency of desirable characteristics among crossbreds than that found in either single parent breed. An example of complementarity would be mating a Charolais bull (growth and retail yield) to a Gelbvieh Angus cross cow. The result — the cow has the milk and the fertility, and the calf has more growth/retail yield. The characteristics gained from the mating complement each other. This effect of breed difference is powerful, but the choice of individuals from within a breed is also very important. Poor choices of breeds and animals from within a breed will have a lasting impact on the success of any crossbreeding plan.

Why Crossbreed?

Crossbreeding animals offers two primary advantages relative to the use of only one breed: 1) crossbred animals exhibit *heterosis* (hybrid vigor), and 2) crossbred animals combine the strengths of the various breeds used to form the cross. The goal of a well-designed, systematic crossbreeding program is to simultaneously optimize these advantages of heterosis and breed complementarity.

Heterosis or hybrid vigor refers to the superiority in performance of the crossbred animal compared to the average of the straightbred parents. Heterosis may be calculated using the formula:

$$\% \text{ Heterosis} = \frac{[(\text{crossbred average} - \text{straightbred average}) \div \text{straightbred average}] \times 100}{1}$$

For example, if the average weaning weight of the straightbred calves was 470 pounds for Breed A and 530 pounds for Breed B, the average of the straightbred parents would be 500 pounds. If Breed A and Breed B were crossed and the resulting calves had an average weaning weight of 520 pounds, heterosis would be calculated as:

$$[(520 - 500) \div 500] \times 100 = 4 \%$$

This 4% increase, or 20 pounds in this example, is defined as heterosis or hybrid vigor.

The amount of heterosis expressed for a given trait is inversely related to the heritability of the trait. Heritability is the proportion of the measurable difference observed between animals for a given trait that is due to genetics (and can be passed to the next generation). Reproductive traits are generally low in heritability (less than 10%), and therefore respond very slowly to selection pressure since a very small percentage of the differences observed among animals is due to genetic differences (a large proportion is due to environmental factors). The amount of heterosis is largest for traits that have low heritabilities. This has significance for commercial breeding systems, as crossbreeding can be used to enhance reproductive efficiency. To date, the ability to select for reproduction is limited. Traits that are moderate in their heritabilities (20 to 30%) such as growth rate are also moderate in the degree of heterosis expressed (around 5%). Highly heritable traits (30 to 50%) such as carcass traits exhibit the lowest levels of heterosis.

Improvements in production from heterosis may be captured by having both a crossbred calf and a crossbred cow. The following two tables summarize the effects of individual heterosis (crossbred calf) and maternal heterosis (crossbred cow). These tables include results from numerous crossbreeding studies conducted in the Southeast and Midwest States, USA involving several breeds. The advantage of the crossbred calf is

two-fold: an increase in calf livability coupled with an increase in growth rate. Perhaps the most important advantage for crossbreeding is realized in the crossbred cow. Maternal heterosis results in improvements in cow fertility, calf livability, calf weaning weight, and cow longevity. Collectively, these improvements result in a significant advantage in pounds of calf weaned per cow exposed, and superior lifetime production for crossbred females.

Individual Heterosis: Advantage of the Crossbred Calf ¹		
Trait	Observed Improvement	% Heterosis
Calving rate, %	3.2	4.4
Survival to weaning, %	1.4	1.9
Birth weight, lb.	1.7	2.4
Weaning weight, lb.	16.3	3.9
ADG, lb./d	.08	2.6
Yearling weight, lb.	29.1	3.8

¹Adapted from Cundiff and Gregory, 1999.

Maternal Heterosis: Advantage of the Crossbred Cow ¹		
Trait	Observed Improvement	% Heterosis
Calving rate, %	3.5	3.7

Survival to weaning, %	.8	1.5
Birth weight, lb.	1.6	1.8
Weaning weight, lb.	18.0	3.9
Longevity, yr.	1.36	16.2
Cow Lifetime Production:		
No. Calves	.97	17.0
Cumulative Wean. Wt., lb.	600	25.3
¹ <i>Adapted from Cundiff and Gregory, 1999.</i>		

The other important advantage to crossbreeding is the ability to take advantage of the strengths of two or more breeds to produce offspring that have optimum levels of performance in several traits. As an example, British breeds generally excel in marbling potential whereas Continental breeds typically are superior for red meat yield (cutability). Combining the breed types results in offspring that have desirable levels of both quality grade (marbling) and retail yield (yield grade). Similarly, milk production and growth rate may be most effectively optimized by crossing two or more breeds.

It is important to realize that the crossbred offspring will not excel both of the parent breeds for all traits. In the example given previously, straightbred calves of Breed B would have had heavier weaning weights (530 pounds) than the Breed A x Breed B crossbreds (520 pounds). However, Breed B females may be larger in mature size and have higher milk production potential resulting in increased nutritional requirements and higher production costs. Limited feed resources coupled with very high milk production may result in lower reproductive performance. Therefore, the cumulative effect of crossbreeding when several traits are considered is more important than the effect on any one particular trait. Effective crossbreeding programs must be designed to optimize performance, not necessarily maximize it.

Crossbreeding Systems

The success of a crossbreeding program will depend on its simplicity and ease of management. There are several factors and challenges that need to be considered when evaluating choice of crossbreeding system, including:

- 1) Number of cows in the herd
- 2) Number of available breeding pastures
- 3) Labor and management
- 4) Amount and quality of feed available
- 5) Production and marketing system
- 6) Availability of high-quality bulls of the various breeds

The design of any crossbreeding program should take advantage of both heterosis and breed complementarity. An ideal crossbreeding program should 1) optimize, but not necessarily maximize, heterosis in both the calf crop and particularly the cow herd, 2) utilize breeds and genetics that fit the feed resources, management, and marketing system of the operation, and 3) be easy to apply and manage.

Rotational Systems

The rotational system (Fig. 1) requires establishing two or more breeding herds. In a two-breed rotational system, two groups of crossbred cows are established. Cows sired by Breed A are mated to males of Breed B, and females sired by Breed B are mated to males of Breed A. In a three-breed rotation, a third breed is added to the sequence. In rotational systems heterosis is retained at high levels, 66% in two–breed rotation, 86% in three–breed rotation. However, fluctuation in breed composition between generations can result in considerable variation in level of performance among cows and calves, unless breeds used in the rotation are similar in performance characteristics. Use of breeds with similar performance characteristics restricts the use that can be made of breed differences to optimize breed complementarity.

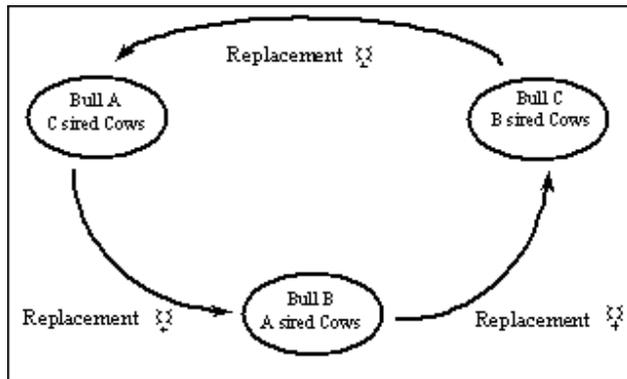


Fig. 1: Rotational System (3-breed)

Crossbred (Hybrid) Bulls

Hybrid bulls offer an alternative method of rotational crossbreeding. Using F1 bulls or composite bulls in rotational systems can significantly reduce intergenerational variance, especially if breeds chosen to produce F1 bulls optimize performance levels (ie. breed complementarity) in their crosses (i.e. continental X British). Using F1 bulls consisting of the same two breeds as the crossbred cow-herd but unrelated can result in retention of 50% of maximum possible heterosis. Supply of performance tested F1 bulls from selected and proven purebred parents may be limited.

Rotate Sire Breed

The rotation of sire breeds every 2 to 4 years provides similar benefits to a rotational breeding system for producers with small herds and limited breeding pastures. The result is simplified management, and individual and maternal heterosis. Disadvantages are the increased intergenerational variation and reduction in heterosis as breed makeup of females' swings more toward one breed and back again. This can be minimized if the breeds utilized are similar.

Rotating F1 males every 2 to 4 years can help reduce intergenerational variation but maintain complementarity if appropriate breed crosses are selected. By rotating different breeds of F1 bulls (AB, CD, EF, etc.) every 4 years, you can avoid wide intergenerational swings in biological type, if breeds A, C and E are similar in type as are breeds B, D and F.

Terminal System

In a terminal system all calves are marketed and replacement females are purchased from outside the herd (Fig 2). This allows for more intensive selection for specific traits

in the male and female lines used in the cross. Cows are usually selected for moderate frame, good milking and mothering ability. High growth potential and good carcass characteristics are important in the male line.

Heterosis benefits will be maximized when a crossbred cow (F1 female) is mated to a sire of a third breed. In a terminal system females are selected to match environment and resources while males are selected to meet end product targets (i.e. growth and carcass). High degree of complementarity and consistency of progeny is possible. Replacements need to be purchased and are price and availability dependent.

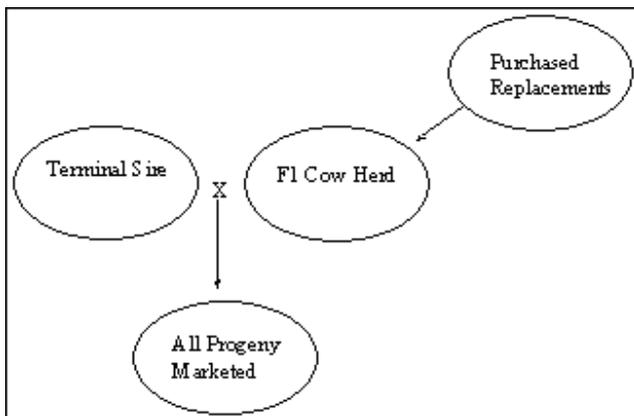


Fig. 2: Terminal System

Rotational Terminal

Figure 3 demonstrates a rotational terminal system. This system combines the best parts from the traditional rotational systems and the static terminal sire systems. The rotational part of the system provides replacement females while the terminal sire part

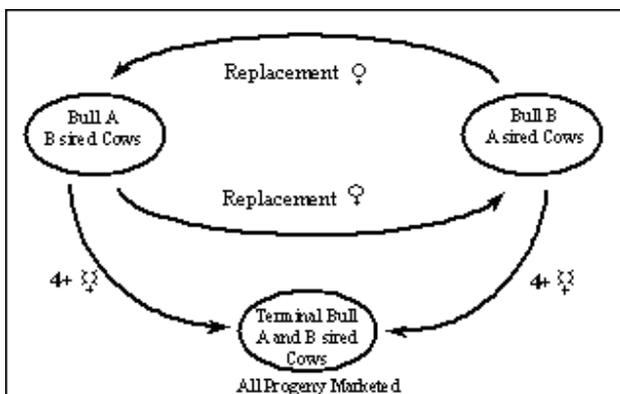


Fig. 3: Rotational-Terminal

of the system allows most of the marketed calves to be sired by growth carcass type sires. Cows remain in the rotational part of the system until they reach 4 years of age and then they move to the terminal part of the system. However a large herd size is required (at least 100 cows).

Composites

Definition:

"A population made up of two or more component breeds, designed to retain heterosis (hybrid vigour) in future generations without crossbreeding with other breeds."

Composite cattle are hybrid cattle that breed to their own kind, retaining a level of hybrid vigour we normally associate with traditional crossbreeding. Management requirement of a composite herd is similar to a straightbred herd, substantial heterosis can be maintained in composite populations so long as adequate number of sires are used in each generation to avoid inbreeding.

Heterosis will vary depending on the number of breeds that were used to form the composite. It can range from 50% of maximum possible heterosis for a 2-breed composite to 87.5% for an 8-breed composite. Selection of breeds going into the composite is also critical. Breed differences should be fully exploited so as to match the composite with the environment in which it will be used and to match it with market specifications.

Composites have the potential for "standardizing" commercial cattle, thus reducing the variation we currently see in market animals. Problem cattle today, from the feedlot and carcass perspective, are biologically extreme breeds. These extremes in market cattle are due to purebreds, high percentage animals from extreme breeds or crosses of similar extreme breeds. Often the result of poor crossbreeding decisions. With a composite breed, crossbreeding decisions are made when the breed is formed. Commercial producers just need to choose what composite breed to use. Composites are expected to be complete and balanced in performance and only those composites that fulfill this expectation are expected to survive.

Which Breeds To Use?

The environments and the resources available to raise beef cattle are as varied as the breeds themselves. Notice the tremendous variability in the available breeds. Another factor to consider is the large degree of variability that exists within a breed. Breeding decisions involve individual animals, not breed averages, so selection of the right

individuals within a breed is critical. Breed differences like these can be blamed for product inconsistency, but they can also be exploited to produce adapted animals and a consistent product.

Great variation in the seed stock and commercial cow–calf sectors of the industry is important to ensure you are positioned to match biological types of cows to environments and resources. The challenge is to design effective crossbreeding systems that allow for diversity in the cow-calf sector and that deliver consistency of end product.

A number of factors must be considered when choosing breeds to use in a crossbreeding system. Among these are:

1. individual breeding goals
2. environment
3. quantity and quality of feeds available
4. cost and availability of good seed stock
5. how breeds will complement each other in the crossing program; and
6. market-specific breed combinations may command market premiums.

Use of Artificial Insemination

The use of artificial insemination may make the application of these described crossbreeding systems more feasible provided the expertise, labor, and facilities are available to make effective use of AI. The use of AI can significantly reduce the number of breeding pastures necessary for rotational cross or rota-terminal systems. Additionally, the use of AI may significantly reduce the number of bulls (and breeds) required for natural service. As an example, in a rota-terminal system the top 50% of the cows could be mated AI for the production of replacement females. Cows that did not conceive AI as well as the other 50% of the cows could be mated naturally to the terminal sire. This would reduce the number of breeding pastures required from three to one or two (depending on cow numbers). Additionally, in any system heifers could be bred AI to calving ease sires. Another major advantage to the use of AI is genetic improvement, as semen from superior bulls in any breed could be utilized.

THE POTENTIAL OF CRYOPRESERVATION AND REPRODUCTIVE TECHNOLOGIES FOR ANIMAL GENETIC RESOURCES CONSERVATION STRATEGIES

Introduction

Global diversity in domestic animals is considered to be under threat. Worldwide, a large number of domestic animal breeds is endangered, in a critical status or extinct already. Of the 6379 domestic animal breed populations, 9% is in critical condition and 39% is endangered. There is worldwide consensus about the global decline in domestic animal diversity and the need to conserve genetic diversity. The vast majority of aquatic genetic resources are found in wild populations of fish, invertebrates and aquatic plants. Domestication of aquatic species has not proceeded to the same level as it has in crop and livestock sectors. According to FAO, there are more than 1000 common aquatic species that are harvested by humans in major fisheries and thousands of additional species are harvested in small-scale fisheries. The number of species in aquaculture is growing and several important species rely on the collection of brood stock or seed from natural populations. In farm animals, trends in within-breed diversity are as important as between-breed diversity in order to be able to cope with changing requirements and future demands in breeding and selection. A small effective population size in rare or endangered breeds requires monitoring of within-breed diversity and conservation programs to maintain within breed diversity. Several authors also emphasized the reduction in effective population sizes of widely used domestic animal breeds.

There are several options to conserve genetic diversity. In general, *in situ* conservation or *conservation by utilization* is preferred as a mechanism to conserve breeds. A breed has to evolve and adapt to changing environments and efforts to create a need for products or functions of the breed should be promoted. Conservation without further development of the breed or without expected future use is not a desirable strategy. However, in addition to *in situ* conservation, methods or techniques to maintain live animals outside their production or natural environment (*ex situ live*) or through cryopreservation of germplasm (*ex situ*) are set up to preserve (germplasm of) rare breeds as well as the more widely used commercial breeds. Moreover, cryopreservation of germplasm is a very good *ex situ* strategy to conserve existing allelic diversity for future use. There is a growing interest in *ex situ* conservation strategies, serving a variety of objectives. In many countries *ex situ* conservation represents an integral component of conservation strategies. Some strategies focus primarily on preservation of germplasm of rare breeds, but in general there is consensus that *ex situ* collections

should be established for all breeds with the aim to capture as much allelic or genetic diversity in conservation programs as possible. Where *in situ* conservation or use of animal genetic resources is not necessarily dependent on high-tech approaches or facilities, the efficiency and efficacy of *ex situ* conservation strategies will certainly benefit from advances in cryopreservation and reproductive technology. Since *ex situ* conservation activities are in general rather costly, debate is going on about priorities, different methodologies and future use and benefits of cryopreservation and reproductive technology.

State of the art in cryopreservation technology

Cryobiologic principles

Cryopreservation allows virtually indefinite storage of biological material without deterioration over a time scale of at least several thousands of years, but probably much longer. Important progress in cryobiology was achieved in the second half of the previous century. Much progress resulted from empirical studies. In later years, progress was also strongly stimulated by the development of fundamental theoretical cryobiology. In so-called 'slow cooling' methods, the biological material is cooled at a range of cooling rates that are fast enough to prevent 'slow cooling damage' but are slow enough to allow sufficient dehydration of the cells to prevent intracellular ice formation (IIF). The dehydrated cells in the 'unfrozen fraction' that remains between the masses of ice will ultimately reach a stable glassy state, or 'vitrify'. In so-called vitrification methods, the water content is lowered before cooling by adding high concentrations of cryoprotective agents (CPA). Thus, no ice is formed at all, and the entire sample will vitrify. This allows fast cooling rates without risk of IIF. The CPA concentration of vitrification solutions can be minimised by using very high cooling and thawing rates. By using extremely high cooling rates, vitrification is possible even in complete absence of CPAs.

Semen

Semen of most livestock species can be frozen adequately. Also, for a large number of bird and mammal livestock species, dedicated freezing media and equipment for collecting, packing, freezing and inseminating semen have been developed and are available commercially. In the cattle AI industry, in which bulls are selected for 'freezability' of their semen, the post-thaw semen quality is quite good, featuring 50-70% motile spermatozoa. Pregnancy or calving rate is the same as that of fresh semen, provided that higher sperm dosages are used for frozen sperm. For other mammalian species the percentage post-thaw motile sperm or membrane-intact sperm is generally

somewhat lower, but a fair post-thaw viability can be expected for most species. For many species the fertility of frozen semen is found to be lower than that of fresh semen. This may depend on the site of semen deposition, the morphology of the female genital tract, and the ability to detect heat or ovulation. For instance in sheep, very poor results are obtained with cervical semen deposition when using frozen ram semen, compared to fresh. There may be considerable differences between breeds and between males, in the 'freezability' of the semen. As a consequence, frozen semen of some genetically interesting breeds or males may not be suitable as a gene bank resource, or can be used only with a poor efficiency. As to avian livestock species, semen-freezing techniques for fowl, turkey, goose, and duck, render a fair post-thaw sperm survival of up to 60% live spermatozoa. Reasonable insemination results with frozen-thawed semen have been reported for the major avian livestock species. However, there is a striking variation between studies in the reported percentages of fertilized eggs, ranging from 9-91%. Moreover, the number of spermatozoa that gives maximal fertilisation levels in chickens is much higher for frozen-thawed semen compared with fresh semen. More than 200 fish species with external fertilisation have been tested for sperm cryopreservation. The present state of the art for many species of fish seems to be adequate for the purpose of gene banking. The insemination ratios used may vary according to species and procedure between 10⁴ – 10⁷ spermatozoa per egg. Even in fish species like the African catfish, in which semen can only be obtained by testis destruction or death of the male, enough semen can be obtained from one single male to produce close to 10⁶ larvae. Thus, for gene-bank purposes, storage of only one single vial or straw would be sufficient to generate plenty of progeny of that male. Freezing media widely vary between the classes (mammals, birds, fish) but also between species within a class. Most media feature a saline or saccharide bulk osmotic support, a suitable CPA at concentrations varying from 0.2 – 1.5 M, and various protective macromolecular additives, mostly milk and egg yolk components, or lipid components from vegetal origin. Milk or egg yolk is often used in media for mammalian semen. In mammalian semen the egg yolk and milk components protect the spermatozoa during cooling and during freezing and thawing. These additives are generally not used in freezing media for avian and fish species, although in a few studies with fish semen, egg yolk was found to confer protection against cryodamage. Glycerol is widely used as a suitable CPA in mammalian, bird, and fish species. However, in poultry it is found that glycerol is contraceptive, i.e. the semen must be washed free of glycerol after thawing. The type of CPA used varies widely between species, and sometimes within one species a CPA is successfully used in one study and is found to be unsuited in another study with the same species. Glycerol is used in most mammalian species. In avian species, also DMSO, Ethylene glycol (EG), DMA and DMF are frequently used. In fish species, glycerol, DMA,

DMF, DMSO, and methanol are often used. Semen is generally cryopreserved with 'slow cooling' methods. Optimal cooling rates for freezing semen are mostly found between 10 and 100 °C/min. To some extent, the reported differences may be related to the use of different types of CPA and different CPA concentrations. An extreme example is that fowl semen can be effectively frozen at a cooling rate of approximately 600 °C/min when using dimethylacetamide as CPA, but not using glycerol. CPAs may differ widely in the cell membrane permeability, and also may affect the membrane permeability to water. These parameters greatly affect the velocity of dehydration, and therewith the optimal range of cooling rates.

Oocytes

In the last 10 years, considerable progress has been made with cryopreservation of oocytes. Viable oocytes have been recovered after freezing and thawing in a great number of species. Successes have been reported as to post-thaw oocyte maturation, fertilisation, and embryo development in a number of species. Live born young from embryos produced from cryopreserved oocytes have been reported in cattle, mouse, rat, horse and human. The present efficiency and reliability of using frozen thawed oocytes for generating offspring is still much lower compared to cryopreserved embryos. Freezing oocytes of avian and fish species is not successful, largely because of the large size, the high lipid content, and the polar organisation (vegetal and animal pole) of bird and fish ova.

Embryos or embryonic cells

In cattle, cryopreservation of embryos is highly successful. Both slow freezing and vitrification protocols are effective. The success of cryopreservation is dependent on the stage of the embryo; that is, especially good results are obtained with blastocysts. Cryopreservation of embryos resulting in live offspring has been reported for of the important (mammalian) livestock species. Cryopreservation of pig embryos has long been quite problematic, due to extreme chilling sensitivity and high lipid content of the pig embryos. However, recent studies have focussed on overcoming these problems and produced successful vitrification methods for cryopreservation of pig embryos. Embryo cryopreservation is not viable in birds and fish species, largely because of the same limitations as in the case of avian and fish oocytes, i.e. the large size, the high lipid content, and the polar organisation of the ova and the early embryos of fish and birds. However, in birds and fish species, cryopreservation of isolated embryonic cells is an option. Post-thaw survival of blastomeres was demonstrated in rainbow trout, carp and medaka. Embryonic cells and recipient embryos can be used to produce chimeric embryos. Provided that the gonads become populated with primordial germ cells from

the donor embryo, such chimeric embryos can be used to produce future progeny of the donor genotype. In the chicken, the primordial germ cells can be specifically harvested. Recently, improvement of the efficiency of producing chimerae with donor genotype germinal cells was achieved by depleting PGC from the recipient embryos using busulfan.

Somatic cells

Cryopreservation of somatic cells proved to be possible for a number of cell types. In early studies, the methods came down to adding 5 to 10% of a suitable cryoprotectant, like glycerol or dimethylsulfoxide (DMSO), to the suspension of cells in culture medium, and place tubes with a few ml of that suspension at $-80\text{ }^{\circ}\text{C}$ in a mechanical freezer. In fact this simple procedure is still effectively used today. Obviously, with this simple procedure the rate of cooling cannot be controlled; in fact in many publications the cooling rate is unknown. There are only a few studies in which controlled rate freezers were used, e.g., with skin fibroblast.

Further progress

More attention to fundamental aspects of cryobiology should enable further progress in cryopreservation methods. A fundamental approach has been taken in a number of studies concerning mammalian semen and embryos, but fewer so concerning avian and fish semen. Recently a theoretical model was presented to predict the optimal cooling program for 'slow cooling' freezing methods. The model indicated that a nonlinear cooling profile could give better results than linear freezing programs. This and other models also demonstrate that the optimal cooling rate can be expected to be inversely related to the CPA concentration, and in fact this is found in empirical studies. Therefore, it is important to address both factors in empirical optimisation studies. It can also mean that a lower concentration of CPA would become feasible provided that a higher cooling rate is used. Further improvement could result from preventing delayed ice formation or 'supercooling', e.g., by using so-called 'directional solidification' methods. Improving the freezing methods can raise the general level such that even the semen of 'bad freezers' would have an adequate post-thaw sperm survival. Attempts to vitrify spermatozoa have not been successful to date. It has recently been shown that vitrification of human spermatozoa is possible in the absence of CPA by using an extremely high cooling rate of $720,000\text{ }^{\circ}\text{C}/\text{min}$. In this way, damage due to the presence of the CPA, chilling injury and ice formation may be avoided. Further improvement of vitrification techniques is especially important for freezing cells that are sensitive to chilling, e.g., to 'outrun' spindle microtubule depolymerization in metaphase II oocytes. Very high cooling rates can be applied in the open-pulled straw (OPS) technique, or by

using the cryoloop. However, also interrupted slow cooling methods can be highly effective, as a fully normal and functional spindle can reform after thawing.

State of the art in reproductive technology

Artificial Insemination

In several species, artificial insemination (AI) techniques and strategies have been improved and knowledge on the fate of sperm in the female genital tract (e.g., phagocytosis) improved during the last decades. However, there are large differences between species in insemination techniques and pregnancy rates using fresh or frozen semen. In cattle and pigs existing AI infrastructure allows easy collection and future use of semen, but only in cattle the use of frozen semen replaced the use of fresh semen. In pig production disadvantages of using frozen semen (reduced fertility, high freezing, storage and transport costs) are still larger than the advantages. In sheep, surgical (laparoscopic) AI gives much better pregnancy rates than cervical AI. However, laparoscopic AI is more laborious and also more invasive than cervical AI. In a study, it was shown that the difference in pregnancy rates between surgical and non-surgical AI was even larger with frozen semen compared to fresh semen: 20% versus 70% pregnancy with 180×10^6 vs. 10×10^6 sperm. It is believed that frozen-thawed sperm are less motile and lack stamina to transverse the highly viscous cervical mucus, but phagocytosis of the sperm by leukocytes is also considered as a cause of the reduced fertility. Development of a nonsurgical technique to reach the oviductal end of the uterine horns as closely as possible would enhance the efficiency and ease of use of cryopreserved semen in sheep. Such deep intrauterine insemination techniques have been developed in pigs and may, in general, contribute to the more efficient use of semen (less sperm per insemination). AI can be used successfully in poultry, but is not used extensively in any domestic avian species except turkeys where it is used almost exclusively for commercial flock production.

Embryo Transfer

Surgical embryo transfer is in principle possible in all mammalian livestock species. In contrast, non-surgical embryo transfer is only possible in cattle (routinely performed), horses and also pigs, though still not as efficient as in cattle and horses. For embryo transfer purposes, embryos can either be flushed from donors or can be produced *in vitro*. Surgical embryo collection is in principle possible in all mammalian livestock species. In contrast, non-surgical embryo collection is only possible in cattle and horses. After surgically shortening of the long uterine horns of the pig, non-surgical recovery of embryos has been proven to be possible in pigs too. Although ethical issues have

prevented the further use of this method, it may be used in specific situations, e.g., to collect in a relatively short time large numbers of embryos in very rare pig breeds. The efficiency of non-surgical embryo collection in cattle, and to a lesser extent in horses, can be improved by hormonal induction of superovulation. *In vitro* production of embryos by *in vitro* maturation and fertilisation of oocytes is possible for major livestock breeds, although the efficiency varies between species. Oocytes for this purpose can either be collected by aspiration of immature oocytes from ovaries from slaughtered (or deceased) animals or by the use of ovum pick-up techniques in live animals. The latter techniques are presently mainly in use in cattle and horses, but could also be used in other livestock species.

Reproductive cloning

Reproductive cloning involves collection of oocytes, culture and *in vitro* maturation of oocytes, enucleation of oocytes, transfer of (somatic) nuclei to (or fusion of the somatic cells with) enucleated oocytes, culture of the resulting embryos, and finally, embryo transfer into recipients of the same or a highly related species. The use of nuclear transfer means that the original mitochondrial genotype of the nucleus donor is lost. In mammals, live offspring have been obtained from embryos generated from somatic cells in a number of species, i.e. sheep, cattle, mice, pigs, goats, horse, rabbits and cats. Until now, cloning has failed in rat, rhesus monkey and dog. Amazingly, some success (embryo development but no live offspring) was even obtained when bovine oocytes were used as recipients for somatic nuclei from other mammalian species. However, it must be emphasised that current techniques are inadequate to be used safely and efficiently for procreation. In all published research, only a small proportion of embryos produced by using somatic cells developed into live offspring, i.e., typically less than 4%. The low overall success rate is the cumulative result of inefficiencies at each stage of the cloning process. Many pregnancies are terminated by abortion, and full term pregnancies not seldom result in abnormal offspring. Therefore it seems that current cloning techniques introduce errors that affect prenatal development. Even apparently healthy live born offspring could have anomalies that only become apparent later in life, or in the next generation of animals. On a long time horizon it is very likely that cloning methodology will become both reliable and efficient. In fish, successful cloning has been reported. To our knowledge no successful cloning has been reported in poultry.

Miscellaneous emerging reproductive technologies

Transplantation of ovarian tissue and germ cells (e.g., primordial germ cells (PGCs) or spermatogonial stem cells(SSCs)) are emerging technologies with potential for future use in conservation programs. Autotransplantation of ovarian tissue has been

developed to restore fertility in woman after aggressive chemotherapy resulting in ovarian failure. Successful transplantation of ovarian tissue has been reported in rodents, sheep, marmoset monkeys and humans. Successful whole sheep ovary cryopreservation and autotransplantation has recently been reported. Germ cell transplantation research has been developed as a unique approach for the study of gametogenesis and germ line manipulation. To date, successful germ cell transplantations have been reported in several livestock species, e.g., transplantation of SSCs in cattle and goats, but also in poultry and fish. As far as application of this technology in fish is concerned, fascinating results of allogenic transplantation in rainbow trout have been reported. By transplanting PGCs of donors into the peritoneal cavity of hatching recipient embryos, live fry with donor-derived phenotype were produced from gametes of PGC-recipients. Although many hurdles have to be taken, in the long term these emerging technologies may enable production of gametes or offspring of rare or extinct breeds by abundantly available individuals of related common breeds. First steps to overcome limitations for homologous transfer of ovarian tissue and germ cells are now underway. The development of an effective recipient preparation protocol in mice is an example of this.