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Department of Fundamental Medical Disciplines

Medical Biology Practicals: Classical Genetics and Cytogenetics

by

Boris M. Sharga, Margarita Yu. Hliudzyk-Shemota,
Diana B. Pylypiv, Volodymir P. Feketa

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Authors are staffs of the Department of Fundamental Medical Disciplines:

Boris M. Sharga, CSc (PhD), docent
Margarita Yu. Hliudzyk-Shemota, CSc (PhD), assistant
Diana B. Pylypiv, 6 year student of the Medical Faculty N1, part-time technician
Volodymir P. Feketa, DSc, professor, vice-rector of the Uzhhorod National University

Reviewers:

Fábry Zoltán, DSc, professor, Department of Biochemistry and Pharmacology of the Medical Faculty №1.

Taras M. Hanych, DSc, professor, Department of Internal Medicine of the Medical Faculty №1.

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Introduction

The training of modern health professionals is impossible without the study of fundamental disciplines. Medical biology is an important and comprehensive subject for the training of students who have chosen medicine as their future profession.

This handbook is prepared for students to master important sections of this subject, such as classical genetics and cytogenetics. It was compiled according to the program of the course "Medical biology". It consist of ten sessions, seven of which provide detail basic theoretical principles, examples of solving problems for monohybrid and dihybrid inheritance of traits and diseases, gene interactions and linkages, linkage chromosomes, analyses of family tree and human populations. There are also tasks for students to solve on their own. Two classes cover cytogenetic problems, such as the effects of structural and numerical chromosome aberrations and the causes of these variations. One lesson presents approaches to karyotyping, reading chromosome idiograms and protocols for chromosome staining methods for their genetic analysis. For each lesson, in addition to a detailed overview of the topic, there is a list of available articles, books, textbooks, Internet sources from which students can gain additional knowledge.

This publication will help students master the basic knowledge of genetics, cytogenetics and learn to use them for genetic analysis for medical counseling of individual patients and families and for genetic analysis at the population level. The handbook can be useful for medical students and for all biology students also

Authors

CONTENT

Practical 1. Monohybrid Genetics. Mendelian inheritance of human traits.

Theoretical background. Mendelian Genetics is among the most interesting fields of Medical Biology. However, before starting to solve the problems, we need to refresh our knowledge of some categories and to get an understanding of Mendelian Genetics principles, which form a basis for Human Genetics also.

Gregor Mendel started his studies of heredity in 1843. He knew nothing about chromosome (chr) or the nature of genes at that time. Nevertheless, his studies of garden pea traits inheritance allowed him to open very important lows and to introduce quantitative approach into its evaluation. He predicted the existence of genes by observing mathematical ratios in the offsprings of genetically different parental plants.

When he crossed pure (homozygous) lines of garden pea, round × wrinkled seeds, yellow × green seeds, purple × white petals, inflated × pinched pods, green × yellow pods, axial × terminal flowers, long × short stems, he received in 1st generation (*F1*) all plants of one parental phenotype in each cross: round, yellow, purple, inflated pods, green pods, axial flowers, long stems, respectively. However, in *F2* obtained from self-pollination of the *F1*, he discovered the segregation by phenotypes round: wrinkled seeds, yellow: green seeds, purple: white petals, inflated: pinched pods, green: yellow pods, axial: terminal flowers, long: short stems as 3:1. The study of next generation *F3* revealed that 3:1 phenotypic ratio corresponded to 1:2:1 genotypic ratio and this was true for each of 7 characters. As example, for stem size he discovered: 3/4 long stem (1/4 pure-breeding long + 2/4 "impure" long) and 1/4 pure-breeding short. G. Mendel explained the 1:2:1 ratio by next ideas [2]:

1. Mendel saw no blending of phenotypes, so he made a conclusion about the existence of hereditary determinants. We now call them genes.

2. Each pea plant has 2 determinants (now genes) — a determinant pair (now gene pair) — in each cell for each character studied. Mendel's reasoning here is obvious: the *F1* plants, e.g., must have had one gene for the dominant phenotype and another gene for the recessive phenotype, which showed up only in later generations.

3. The determinant pairs (gene pairs) segregate equally to the gametes (eggs, sperm).

4. Thus, each gamete carries only one member of each determinant pair (gene pair).

5. The union of one gamete from each parent to form the zygote of a new progeny individual is random — that is, gametes combine without regard to which member of a determinant pair (gene pair) is carried [2].

Using A for gene representing dominant phenotype and a for recessive phenotype gene, Mendel illustrates these points diagrammatically (Fig.1). To test his model G. Mendel, for instance, crossed F1 plant grown from a yellow seed with *F1* plant grown from the green seed. Next generation ratio 1:1 was predicted. This Mendel's prediction is pictured in Fig. 2, where *Y* stands for the gene that determines dominant phenotype (yellow seeds) and *y* stands for the gene that codes for recessive phenotype (green seeds). From this cross he gathered 52 green (*yy*) and 58 yellow (*Yy*) seeds, close enough to the predicted 1:1 ratio. This also confirmed the concept of *Y* and *y* equal segregation in *F1* plants.

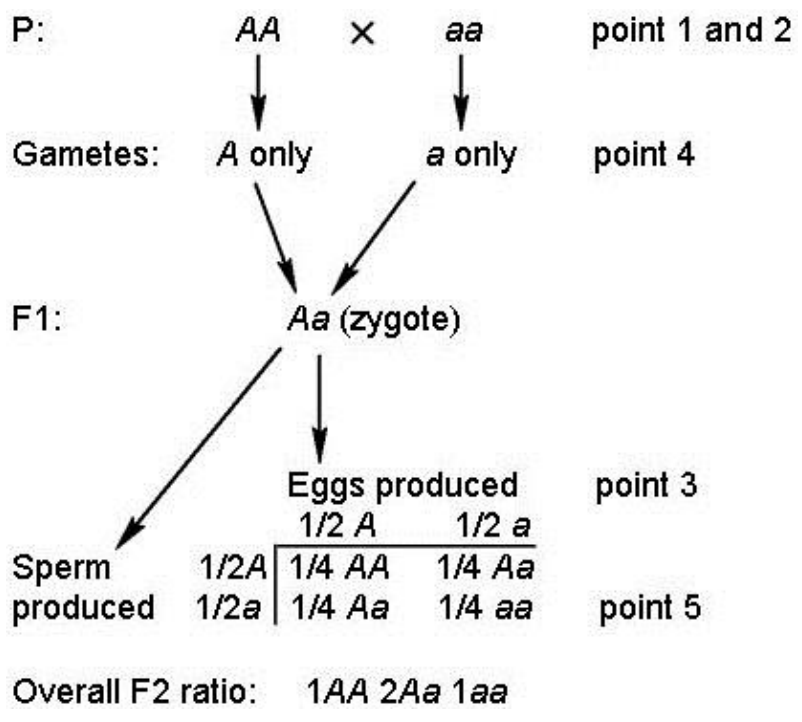


Fig. 1. Mendel's model for the hereditary determinants of a character difference in P, F1 and F2. *By D.B. Pylypiv.*

The concept was recognized as **Mendel's First Law:** *The two members of a gene pair segregate from each other into the gametes, so that one-half of the gametes carry one member of the pair and the other one-half of the gametes carry the other member of the pair.*

The individuals represented by *Yy*, *Aa* or *Bb* are called heterozygotes or hybrids, while the *yy*, *aa* or *bb* represent the pure line or homozygotes ("hetero-" for "different" and "homo" for "same").

Thus, an *AA* organism is homozygous dominant and *aa* is homozygous for the recessive gene, or homozygous recessive. The designated genetic constitution of the character(s) under study is called the genotype. Thus, *YY* and *Yy*, e. g., are different genotypes coding for the same phenotype of the seeds (yellow). Underlying the 3:1 phenotypic ratio in the *F2* there is a 1:2:1 genotypic ratio of *YY : Yy : yy*. The dominant phenotype is established in analysis by the appearance of *F1*. Mendel showed that the dominance of one phenotype over another is in fact due to the dominance of one member of a gene pair over the other.

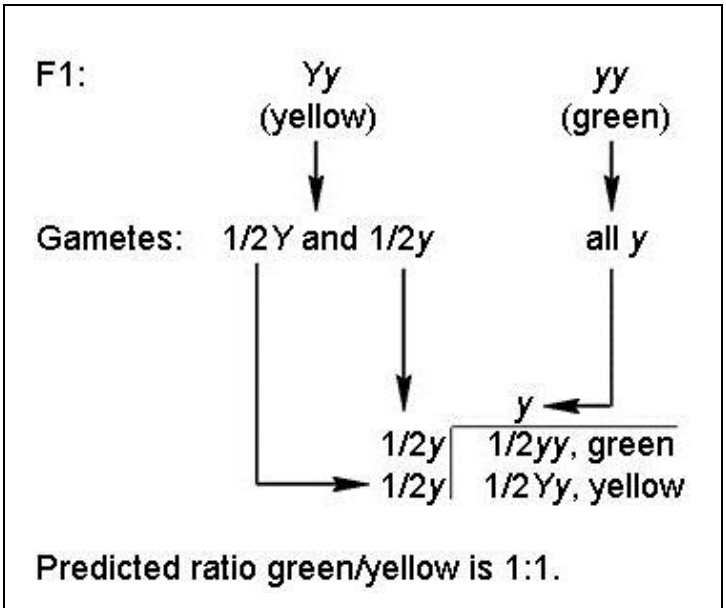


Fig. 2. F1 cross between yellow and green peas. *By D.B. Pylypiv.*

Mendel developed an analytic scheme for the identification of genes regulating any biological character or function. For example, in the petal color character (purple/white) Mendel showed that the difference was determined by one gene pair. Actually, Mendel managed with a gene of petal color. The gene exists in two alternative forms: one is dominant for purple color (*C*) and another is recessive (*c*) for white color of the petals. The pea plants have one of the gene pairs *CC*, *Cc* or *cc*. Both members of the pair affect the same character, the petal color. The alleles *C* and *c* on

molecular level may differ only at one or few nucleotides and they are versions of one basic gene. The gene pair consists of identical alleles in homozygous (*CC* or *cc*) and of different alleles in heterozygous (*Cc*).

To estimate the heterozygote, it is necessary to cross an organism (suspected heterozygote, for example, *Cc*) with a monozygotic individual (*cc*). If segregation *Cc* : *cc* (1:1) is observed in offspring, the organism is heterozygous. The controlled mating for genetic analysis in humans cannot be made. The pedigree or family tree analysis, a record of mating within the families is made instead of that. The first member of a family with an unusual trait or mutation is called propositus. The geneticists draw the family tree with use of special symbols (Fig.3) and tracing the propositus character within the family history.

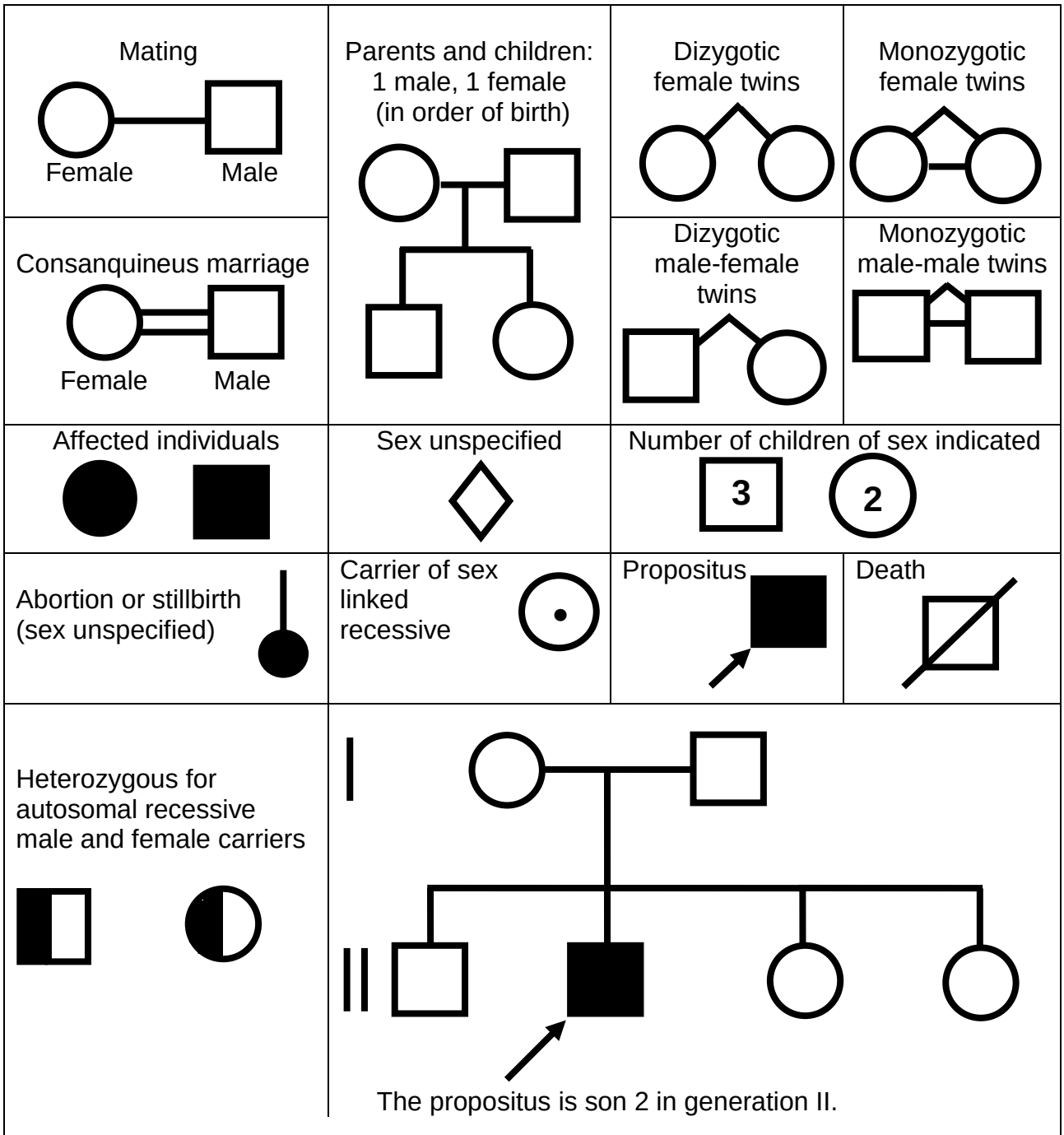


Fig. 3. Current symbols for human pedigree analysis. *By D.B. Pylypiv.*

Many of human traits are inherited as contrasting alleles (alternative states of the gene) in the way of simple Mendelian genetics. The family tree analysis can reveal the inheritance patterns of many human disorders. The condition of recessive trait, e.g., albinism, cystic fibrosis or phenylketonuria (Table 1) is determined by recessive allele and alternative unaffected phenotype is coded by dominant allele. The dominant allele is usually marked in capitals (*A, B or C*, etc.) and recessive allele is written as *a*, *b* or *c*, etc.

Table 1. Autosomal recessive disorders

Disease	*Prevalence*
Sickle cell anaemia [18]	1 : 625
Cystic fibrosis [6]	1 : 2,000
Tay-Sachs disease [19]	1 : 3,000
Phenylketonuria [16]	1 : 12,000
Mucopolysaccharidoses [14]	1 : 25,000
Lysosomal acid lipase deficiency [12]	1 : 40,000
Glycogen storage diseases [9]	1 : 50,000
Galactosemia [8]	1 : 57,000

The Mendelian ratios are rarely observed in families, because the sample size (children number) is too small [1]. The pedigree of recessive traits is usually with few shaded symbols only within groups of affected siblings. Their grandparents, parents and later generations tend not to be with this particular trait. Usually, people who have disease-coding recessive alleles are heterozygous and the dominant gene diminishes the effect of recessive allele. This does not allow to manifest the recessive phenotype.

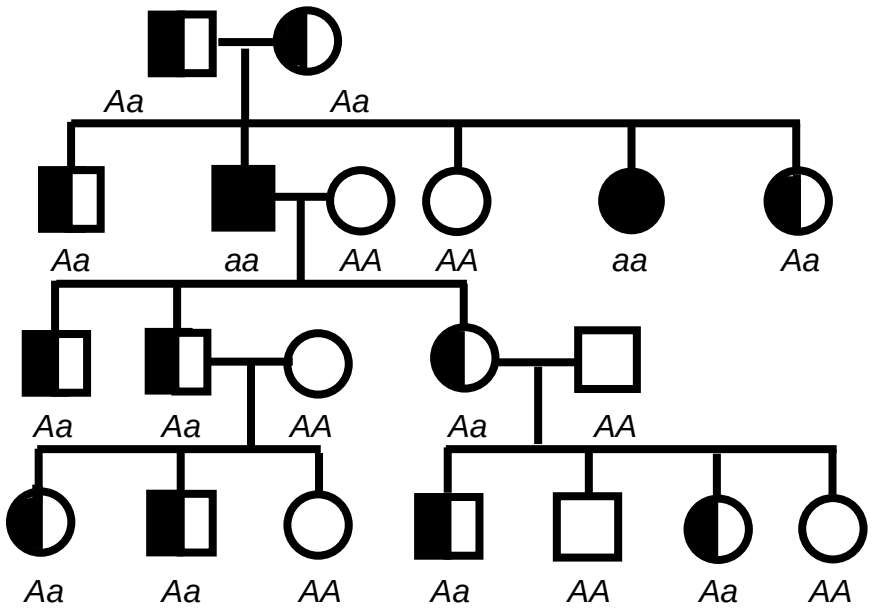


Fig. 4. Autosomal recessive disease. The squares or rings totally shaded indicate the affected members of the family, half shaded figures are healthy carriers, non shaded figures represent healthy individuals. *By D.B. Pylypiv.*

In pedigrees (Fig. 4) recessive trait is revealed by the appearance of a recessive disorder in male and female progeny of unaffected parents. By assuming that particular human community is in the Hardy-Weinberg equilibrium, we can calculate the frequencies of the three possible genotypes *AA*, *Aa* and *aa* as p^2, $2pq$ and q^2, respectively. A numerical example [2] illustrates this. If the frequency q of the recessive disease causing allele is 1/50, then frequency of the dominant allele, p, is 49/50, and the frequency of the homozygotes with the disease is $q^2 = (1/50)^2 = 1/2500$, and the frequency of heterozygotes is $2pq = 2 \times 49/50 \times 1/50 \approx 1/25$. Thus, for this example the heterozygotes are 100 times more frequent than diseased people. The birth

of the diseased individual depends on the chance of marriage of unrelated heterozygotes. The marriage between relatives (inbreeding) occurs among cousins in some countries. This significantly increases the probability of the heterozygotes mating, and thus, increases the risk of recessive homozygotes birth with the disease.

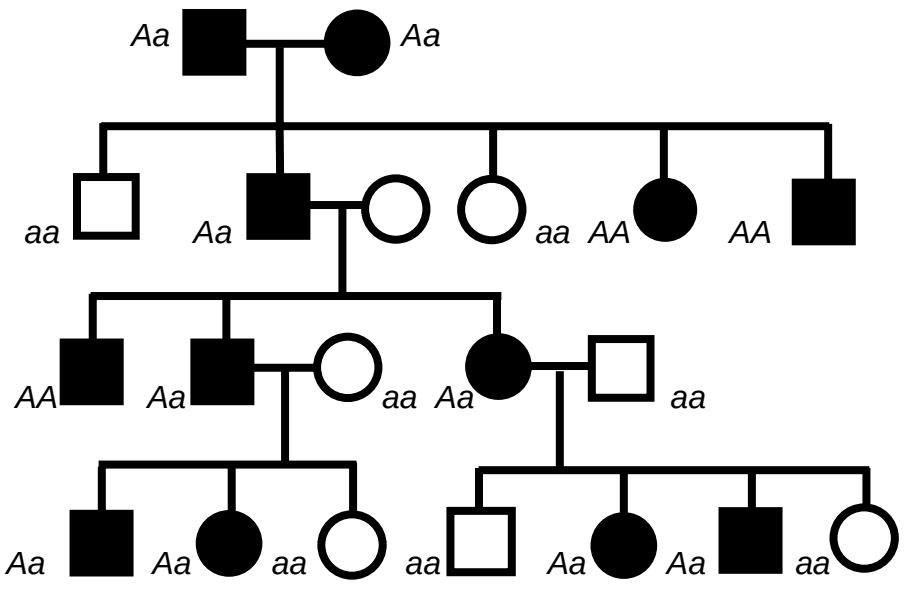


Fig. 5. Autosomal dominant disorder
The squares (males) and cycles (females) that totally shaded represent affected individuals. *By D.B. Pylypiv.*

The dominant characters are usually betrayed in every generation of the pedigree (Fig. 5) and affected fathers and mothers transmit the disease (or other trait) to their daughters and sons. Achondroplasia (a kind of dwarfism) is an example of dominant disorder with Mendelian inheritance. Homozygous by achondroplasia alleles embryos are not surviving, all living achondroplastic patients are heterozygotes.

Abnormal alleles appear *de novo* due to mutations (genetic changes). These are rare events, however, possible. Huntington disease (Table 2) is another example of a profound disorder, which is inherited in a dominant manner [4]. The symptoms are neural degeneration, leading to convulsion and premature death. It has a late onset. Usually, the disease does not appear until the reproductive age. The children of carriers have 50% probability of the dominant allele and disease inheritance. Modern molecular diagnostic techniques allow identifying the dominant allele carriers before they experience the disease onset. Genetic consulting of young couples is also important for prediction of abnormal allele possibility.

Table 2. Autosomal dominant disorders

Disease	*Prevalence*
Familial hypercholesterolemia [7]	1 in 500
Polycystic kidney disease [17]	1 in 1250
Neurofibromatosis type I [15]	1 in 2,500
Hereditary spherocytosis [10]	1 in 5,000
Marfan syndrome [3, 4, 13]	1 in 4,000
Huntington's disease [4, 5, 11]	1 in 15,000

Some other rare dominant traits in humans are polydactyly (extra fingers and toes) and brachydactyly (short digits) and piebald spotting, found in domestic animals also.

Polymorphism is a set of 2 or more common, normal, alternative, phenotypes (morphs). For example, the earlobes are dimorphic with attached and free as the 2 major morphs. The morphs and polymorphism are often determined by the alleles of one gene, inherited in the simple Mendelian manner. The polymorphism is observed on all levels of life organization, down to the level of DNA. Let us solve problems in Human Genetics

disorders and trait inherited in a simple Mendelian manner. Most of them were adopted from [1].

Solved problems

Problem 1. The man with blue eyes, both parents of which was hazel eyed, married a hazel eyed woman, father of which was hazel eyed and mother was blue-eyed. From this marriage one boy was born with blue eyes. Determine the genotype of each of the mentioned persons.

Solution: The blue eyes are less spread in the human population, than dark eyes. Child is blue eyed, and this means that phenotype of blue eyes is a recessive trait. *B* - hazel eyes – dominant over blue eyes; *b* - blue eyes – recessive character.
Grandparents, *GP*: mother's side ♀*bb* × ♂*B_*; father's side ♀*Bb* × ♂*Bb*
Parents, *P*: wife ♀*Bb* × ♂*bb* husband
Gametes, *G*: *B, b* *b*
Son, *F*: ♂*bb*

Problem 2. The man with freckles has a wife without this trait. All 3 their daughters have freckles. One of them had married the man without them. What children can they produce? Determine if freckles are inherited as dominant or recessive alleles.

Solution: *F* – freckles-dominant, *ff* - no freckles-recessive trait, Parents, *P*: ♀*ff* × ♂*F_* Children, *F*: all three ♀ *Ff*, one married with ♂ *ff* : Daughter's family: Parents, *P*: ♀*Ff* × ♂ *ff* Gametes, *G*: *F, f* Children, *F*: *Ff, ff* 1:1, 50% : 50%	*Answer*: The freckles is a dominant trait. The probability of children with freckles in daughter's family is 50%.

Problem 3. Bright-haired young man, with dark-haired parents, had married a dark-haired girl. The father of the latter was blond, and mother was dark-haired. Young family has a baby with dark hair. They like to receive the consultation about the probability of a blond child in the next birth.

Solution: *D_* - dark hair, *dd* - bright hair Grandparents, *GP*: ♀*Dd* × ♂*Dd*; ♀ *D_* × ♂ *dd* Parents, *P*: young man ♂ *dd* × ♀ *Dd* young woman	*Answer*: Probability of blond child is 1/2: *Dd* 50% dark; *dd* 50% blond.

Problem 4. Red hair is a recessive trait, non-red is the dominant character. At which marriages can we expect 100% probability of red-haired children being born? When can we expect 50% and 25% probabilities of red-haired children?

Solution: R- non-red, r- red hair

Parents, *P*:	♀rr × ♂ rr	♀Rr × ♂ rr	♀Rr × ♂ Rr
Gametes, *G*:	r, r r, r	R, r r, r	R, r R, r
Children, *F*:	rr	Rr : rr, 1:1	RR, Rr, Rr, rr, 1:1:1:1
Answer:	100% probability	50% probability	25% probability

Problem 5. Albinism, a total absence melanine pigent in the body, is inherited as an autosomal recessive trait. The one partner in the couple is albino. One of the twins

from this couple is albino, and another is normal. What is the probability of the albino in the next child birth?

<u>*Solution*</u>: *C* – normal; *c* – albino, Parents, *P*: ♀*Cc* × ♂*cc* Gametes, *G*: *C,c* and *c* Children, *F*: 50% *Cc* 50% *cc*	<u>*Answer*</u>: Probability of albino child (*cc*) is 50%.

Problem 6. The children's form of Tay-Sach's idiotism is inherited as an autosomal recessive trait. It has a fatal end at the children's age about 4-5 years of life. The first child died at the time, when wife was pregnant with another one. What is the probability of this disease in the next child?

<u>*Solution*</u>: *T* – normal; *t* – Tay-Sach's disease, recessive. Parents should be heterozygous as they reached the reproductive age and were without disease. Parents, *P*: ♀ *Tt* × ♂ *Tt*, Gametes, *G*: *T, t and T, t* Children, *F*: *TT 25% Tt 50% tt 25%*	<u>*Answer*</u>: Probability of Tay-Sach's disease in the next child is 25%.

Problem 7. The dark-haired man has married a dark-haired woman. The child born is red-haired. What are the parents' genotypes if dark color is the dominant trait?

<u>*Solution*</u>: *D* – dark hair, *d* - red hair; Parents, *P*: ♀ *Dd* × ♂ *Dd* Gametes, *G*: *D, d* *D, d* Children, *F*: *DD, 2Dd* (dark-haired), *dd* (red-haired)	<u>*Answer*</u>: The parents' genotypes are *Dd*.

Problem 8. The tall man has a tall father and small mother. He married a woman with high height. Her parents were low in height. The young family has a tall child. Determine the genotypes of the child's parents and grandparents. It is known that high height is a recessive character and low height is a dominant trait.

<u>*Solution*</u>: *H* –low height; *h* – high height

Parents of wife: ♀*Hh* × ♂*Hh*; parents of tall man: ♀ *Hh* × ♂*hh*;

Genotypes of the child's parents: ♀ *hh* × ♂ *hh*. Child: *hh*

Problem 9. Aniridia is a dominant trait causing blindness. It is inherited as an autosomal dominant trait. What is the probability of healthy child birth, if one of the parents (husband) suffers from the disease. Only the father of an ill person (husband) is also ill with this disease.

<u>*Solution*</u>: *A* - disease; *a* – normalcy. If only the father of the husband is ill it means that the husband is heterozygous. Couple genotypes: ♀ *aa* × ♂*Aa*. Children: 50% *Aa* and 50% *aa*.	<u>*Answer*</u>: The probability of healthy child birth is 50 %.

Problem 10. Achondroplasia is inherited as a dominant autosomal trait. Both parents are suffering from the disease, however, their child is healthy. What is the probability of two consequitive babies two be healthy ?

<u>Solution</u>: *A*- disease; *a* – normalcy Couple genotypes: ♀ *Aa* × ♂*Aa*. Gametes: *A,a* and *A,a*. Children: AA (25%) 2Aa (50%) and aa (25%).	Probability of two healthy 1/4 × 1/4 = 1/16×100= 6,25%. <u>Answer</u>:

Problem 11. Hypertonic woman married a healthy man. All their children are hypertonics. Their son married a healthy woman and daughter has a hypertonic husband. What about the possibility of having hypertonic grandchildren?

Solution: From our life experience, the hypertension is not spread in many people of the population and disease is controlled by recessive alleles of the gene. Because women marry healthy men and all their children are hypertonics, the husband must be heterozygous. Thus, *a* - disease; *A* – no disease; woman is homozygous (*aa*) and her husband is heterozygote (*Aa*) and children are *aa*. As daughter had married hypertonic man, all grandchildren from daughter must be with hypertension:

Daughter's family: *P*: ♀ *aa* × ♂*aa* (no chance to have healthy child)

In son's family we do not know if a healthy wife heterozygote is *Aa* or homozygote *AA*, wich can be with 50:50% probability 1/2 : P♀ *A*_ × ♂ aa. If this woman is *AA*, all her children should be healthy (100% healthy). However, the question is about the probability of hypertonics in her family. It could happen, if a woman has Aa genotype. With such genotype the 50% probability of ill children exists:

Parents, *P*: healthy ♀*Aa* × hypertension ♂ *aa*
Gametes, *G*: *A, a* *a*
Children, *F*: 1/2 *Aa* : 1/2 *aa*

The probability of healthy or ill child is 50%. So, the probability of ill grandchildren from son's family is a probability of two events (heterozygote wife in son's family and ill children) at one time: 1/2 × 1/2 = 1/4 = 25%.

Answer: The probability of children with hypertention is 25%.

Problem 12. Both parents are "tongue-rollers", i.e., able to roll their tongues into tubes. Their daughter is not able to do this. Provide a genetic basis for this observation.

Answer: Both parents must be heterozygous (*Rr*) for gene *R*, that codes for the ability to roll the tongue, otherwise they both can't be "tongue-rollers" and have a child without this ability. The daughter's genotype is *rr*, a recessive homozygote, the result from ♀*Rr* × ♂*Rr* mating.

Problems for homework

Problem 13. Wilson's disease is inherited as a recessive autosomal trait. What is the probability of ill children, if the wife is suffering from the disease and the husband is healthy? His parents, sister and brother are healthy also.

Problem 14. The allergy is hypersensitivity to different irritants. The allergic girl had married a healthy man, whose father was allergic. Predict inheritance of the allergy in children. Write the genotypes for all of these persons.

Problem 15. The xeroderma pigmentosum is inherited as an autosomal recessive trait. The mother of the girl is healthy and her father has this cancer problem. The girl had married a young healthy man, the carrier of this recessive gene. What about disease probability in their children and grandchildren?

Problem 16. Afibrinogenemia (absence of fibrinogen in blood plasma) often leads to death because of complications with bleeding.. It is inherited as a recessive autosomal trait. Healthy parents have ill baby. What is the probability of the next baby with this disease?

Problem 17. Galactosemia is an autosomal recessive trait. What is the probability of this disease in children if the husband is homozygous and mother is heterozygous by this allele?

Problem 18. One of the forms of cistinuria is inherited as an autosomal recessive trait. In heterozygotes an elevated level of cystine is observed only, while homozygotes suffer from cystine stones in kidneys. Estimate the probability of the cystinuria in grandchildren if both grandmothers were healthy and both grandfathers were with cystinuria.

Problem 19. Rare gene a is coding for anophthalmia, the eyes absence. The dominant gene A determines the normal eyeball development. The eye balls of heterozygotes are smaller than in average people without this allele. Both parents are heterozygotes by the allele. What is the genotype and phenotype of their children?

Problem 20. Thalassemia is inherited as a not completely dominant trait. In 90-95% of monozygotic organisms it leads to death, however, it is in moderate non-clinical form in heterozygotes. What is the probability of this disease in children, if both parents have a moderate non-clinical form of the disease?

Problem 21. The man with short lashes had married the woman with long lashes and their baby is with long lashes. The father and brother of the wife have short lashes and her mother has long lashes. Long lashes are the dominant trait, short is recessive. Determine the genotypes of all the persons and probability of a baby with short lashes.

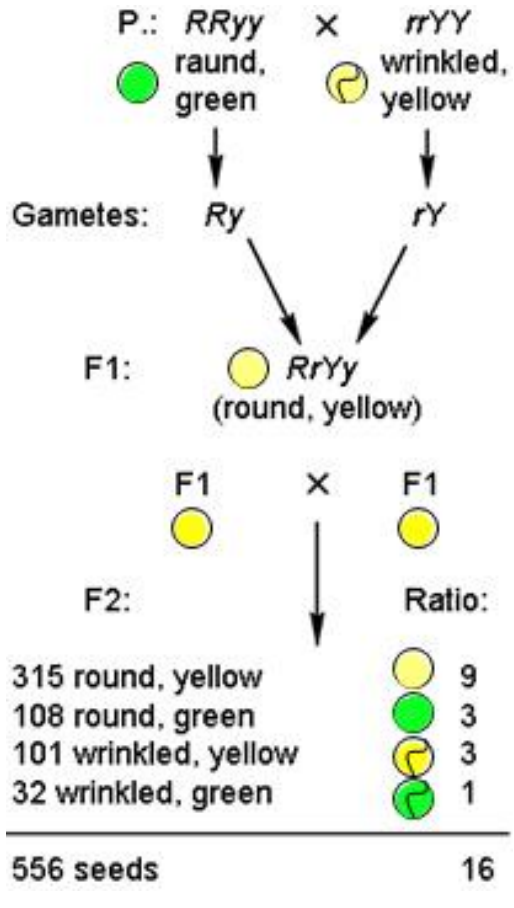
Literature

1. Барна І.В. Загальна біологія: збірник задач.- Тернопіль: підручники і посібники, 2013.- 736 с.
2. Griffits Anthony J.F., Wessler S.R., Carroll S.B., Doebley J.F. Introduction to genetic analysis.- 11th edition.- W.H. Freeman & Company, N.Y., 2015.- 868 p.
3. Keane M.G., Pyeritz R.E.// Circulation. – 2008.-Vol. 117, N21.- p. 2802–13.
4. Vos T. et al. // *Lancet (London, England)*.- 2016.- Vol. 388(10053).- P.1545–1602.
5. Walker F.O. // Lancet.- 2007.- Vol. **369** (9557).- p. 218–28. doi:10.1016/S0140-6736(07)60111-1. PMID 17240289.
6. https://en.wikipedia.org/wiki/Cystic_fibrosis
7. https://en.wikipedia.org/wiki/Familial_hypercholesterolemia
8. https://en.wikipedia.org/wiki/Galactosemia
9. https://en.wikipedia.org/wiki/Glycogen_storage_disease
10. https://en.wikipedia.org/wiki/Hereditary_spherocytosis
11. https://en.wikipedia.org/wiki/Huntington%27s_disease
12. https://en.wikipedia.org/wiki/Lysosomal_acid_lipase_deficiency
13. https://en.wikipedia.org/wiki/Marfan_syndrome
14. https://en.wikipedia.org/wiki/Mucopolysaccharidosis
15. https://en.wikipedia.org/wiki/Neurofibromatosis_type_I
16. https://en.wikipedia.org/wiki/Phenylketonuria
17. https://en.wikipedia.org/wiki/Polycystic_kidney_disease
18. https://en.wikipedia.org/wiki/Sickle_cell_disease
19. https://en.wikipedia.org/wiki/Tay%E2%80%93Sachs_disease

Practical 2. Dihybrid Genetics. Mendelian inheritance of human traits.

Theoretical background. Dihybrid Genetics studies what happens in crosses, in which pure parental lines differ in two genes that control two different characters.

To indicate the genotypes of pea seed color Gregor Mendel used *Y* and *y* for yellow and green seeds and *R* or *r* for round or wrinkled seeds, respectively. When he crossed two lines *RRyy* and *rrYY*, round yellow seeds were obtained, as expected for *F1*. The *F2* result is summarized in Fig.1. In this and other similar experiments using other pairs of traits in many dihybrid crosses in each case he obtained 9:3:3:1 ratios. Mendel discovered that the ratio of 3:1 for each of the traits in each of the crosses is preserved. By using squares of Punnett we can follow Mendel's analysis of *F2*.



♂G: / ♀G:	*RY* 1/4	*Ry* 1/4	*rY* 1/4	*ry* 1/4
RY 1/4	*RRYY* round, yellow, 1/16	*RRYy* round, yellow, 1/16	*RrYY* round, yellow, 1/16	*RrYy* round, yellow, 1/16
Ry 1/4	*RRYy* round, yellow, 1/16	*RRyy* round, green, 1/16	*RrYy* round, yellow, 1/16	*Rryy* round, green, 1/16
rY 1/4	*RrYY* round, yellow, 1/16	*RrYy* round, yellow, 1/16	*rrYY*, wrinkled, yellow, 1/16	*rrYy* wrinkled, yellow, 1/16
Ry 1/4	*RrYy* round, yellow, 1/16	*Rryy* round, green, 1/16	*rrYy* wrinkled, yellow 1/16	*rryy* wrinkled, green 1/16

round, yellow – 9/16;
round, green – 3/16;
wrinkled yellow – 3/16;
wrinkled green – 1/16.

Total genotypes – 16; round : wrinkled, 12 : 4 = 3 : 1; yellow : green, 12 : 4 = 3 : 1.

Fig. 1. *F2* generation analysis from one of the dihybrid cross experiments of G. Mendel. *By D.B. Pylypiv, based on the data by G.Mendel* [4].

As we can see, there is nothing difficult in dihybrid crosses. They can be regarded as two combined monohybrid matings.

Similarly, by using the Punnett square you can work out the probabilities of particular genotypes and phenotypes that children can have at particular marriages.

During the solution of problems in Genetics it is often necessary to calculate the probability of particular genotypes. The probability, *p* is defined as:

$$p = \frac{Number\ of\ times\ an\ event\ is\ expected\ to\ happen}{Number\ of\ opportunities\ for\ an\ event\ to\ happen\ (or\ number\ of\ trials)}$$

For instance, the probability of rolling a 5 on a die in a single trial is written *p* (of a 5) = 1/6, because the die has six sides. Thus on the average one 5 should turn up for each six rolls.

The probability that two independent events will occur simultaneously is the product of their respective probabilities. For example, rolling of 5 on a die twice is 2 independent events, and

p (of two times 5) = 1/6 × 1/6 = 1/36 (The product rule).

When two dice are rolled together, the probability either one of two independent events is equal to sum of their individual probabilities:

p (of two 4s or two 5s) = 1/36 + 1/36 = 1/18 (The sum rule).

Mendelian Genetics most often also regards two kinds of events: 1) mutually exclusive, when the occurrence of one event excludes the possibility of the others and 2) independent, for which the occurrence or the nonoccurrence of any one of them does not affect the probable occurrence of any of the others.

If we deal with mutually exclusive events, the combined probability of two or more events is the sum of their individual probabilities. The probability that two or more independent events will occure is the product of their individual probabilities [2, 3].

For instance, the probability of dominant phenotype in offsprings from a *Aa* × *Aa* merriage is 3/4, the sum of 1/4 (the probability for *AA)* and 1/2 (the probability for *Aa).* The probability of independent events, two consecutive babies with recessive phenotype is 1/16, the product of 1/4 (the probability for one infant with genotype *aa*) and 1/4 (the probability for next *aa* child).

The genotype of any particular offsprings result from random and independent combining the alleles of the gametes from the two parents, the proportions of the different genotypes will be the product of the individual gamete probabilities [2, 3].

The *F2* profile from the pea dihybrid cross can be predicted if the mechanism for placing *R* or *r* is independent from placing of *Y* or *y* into the gametes. The frequency of gamete types can be calculated by determining their probabilities according to the rules just given. Thus, if you pick a gamete at random, the probability of picking a certain type of gamete is the same as the frequency of that type of gamete.

According to Mendel's first law, the heterozygote produces gametes in the proportions: *R gametes* = *r* gametes = 1/2; *Y gametes* = *y gametes* = 1/2.

An *RrYy* plant forms 4 types of gametes. The probability of each gametic combination (also present in Punnett square in Fig. 1) can be calculated according to the product rule: *p(RY)* = 1/2 × 1/2 = 1/4; *p(Ry)* = 1/2 × 1/2 = 1/4; *p(ry)* = 1/2 × ×1/2 = 1/4; *p(rY)* = 1/2 × 1/2 = 1/4.

Branch diagram is also useful to solve some problems. For instance, the phenotypic ratio 9:3:3:1 can be pictured as a branch diagram with application of the

product rule to estimate frequencies (Fig. 2). The diagram can be extended to a trihybrid cross if you draw another set of branches at the end. However, the branch chart becomes complicated when we analyze polyhybrid cross with many of alternative characters. The number of phenotypes and genotypes are calculated as 2^n and 3^n, where *n* is a number of segregating gene pairs [2, 3].

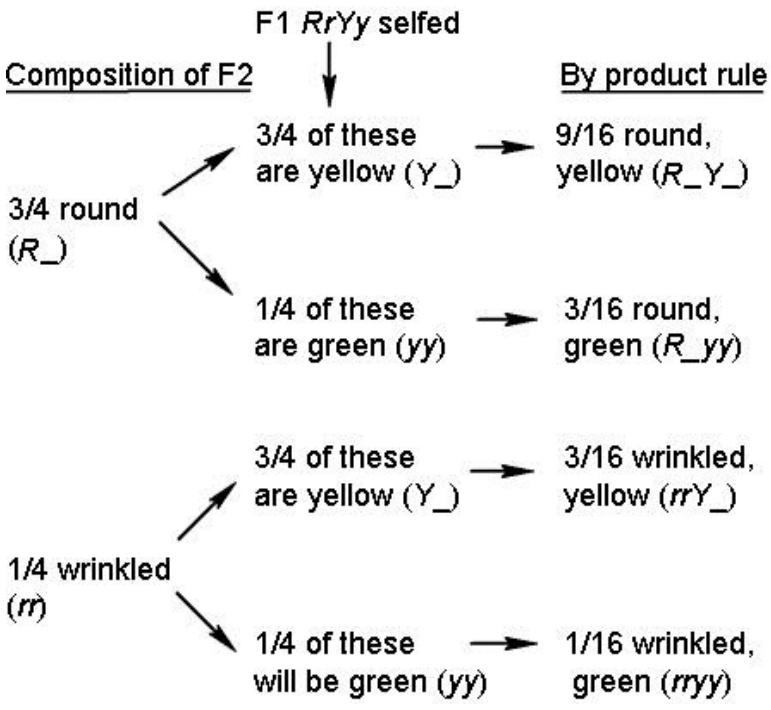


Fig. 2. Branch diagram for self cross *RrYy × RrYy*. *R_* represents *RR* or *Rr*; *Y_* is for *YY* or *Yy*. *By D.B. Pylypiv.*

In these cases we are using the product and sum rules. For example, what proportion of the progeny from the cross *AaBbCcDdEeFfGg* × *AaBbCcDdEeFfGg* will be *aaBbccDdeeffgg* if alleles assort independently? The solution is easily obtained if we apply the product rule. Thus, 1/4 of the progeny will be *aa*, 1/2 *Bb*, 1/4 *cc*, 1/2 *Dd*, 1/4 *ee*, 1/4 *ff*, 1/4 *gg*. We obtain the answer by multiplying these frequencies: $p(aaBbccDdeeffgg) = 1/4 \times 1/2 \times 1/4 \times 1/2 \times 1/4 \times 1/4 \times 1/4 = 1/4096$. The inheritance of allelic segregation is called independent assortment and its general statement is known as ***Mendel's second law***: *during gamete formation the segregation of alleles of one gene is independent of the segregation of alleles of another gene.*

To test this low G. Mendel crossed F1 dihibrid *RrYy* with recessive double heterozygote *rryy*. The progeny phenotypes reflected the gametic types of *RrYy* parent, because recessive homozygous parent contribution (*ry*) does not alter the phenotype indicated by the other hamete. G. Mendel predicted and obtained a 1:1:1:1 ratio of *RrYy*, *Rryy*, *rrYy* and *rryy*. He got the same ratio results when he tested the concept of independent assortment on 4 different combinations of characters [1]. These experiments proved the concept of equal segregation and independent assortment. The actual mechanisms of the phenomenon were unknown at that time. However, now we can explain it by chromosomal location of genes that are responsible for their equal segregation and independent assortment into gametes.

Mendel's laws were rediscovered in the late 1890s by C.E. Correns [1] (but not by E. Tschermak or H.M. De Vries). Later, the ratios 3:1, 1:1, 9:3:3:1 and 1:1:1:1 were reported for different eukaryotic organisms. This confirmed Mendel's laws as genetics laws for eukaryotes in general and, particularly, for humans.

However, in many plants and animals selfing is not possible. This problem can be overcomed by crossing identical genotypes. For instance, the *F1* animal resulting from

pure lines cross can be mated with its *F1* siblings (brothers and sisters) to produce *F2*. The *F1* animals are identical for the genes of interest, so this *F1* cross is equivalent to self pollination in plants.

Problem 1. Use the Punnett square to determine all possible genotypes from the following marriages (and their relative frequencies):

a) ♀*AaBb* × ♂*AaBb* **b)** ♀ *AaBb* × ♂*aabb* **c)** ♀ *AaBb* × ♂ *Aabb*

Solution: Punnett squares for marriages **a**, **b**, **c**

a) Parents, *P*: ♀*AaBb* × ♂*AaBb*

♂***G:*** / ♀***G:***	***AB***	***Ab***	***aB***	***ab***	*AABB* – 1, *AABb* – 2, *AAbb* – 1, *AaBB* – 2, *AaBb* – 4, *Aabb* – 2, *aaBB* – 1, *aaBb* – 2, *aabb* – 1, out of 16 genotypes.
AB	*AABB*	*AABb*	*AaBB*	*AaBb*	
Ab	*AABb*	*AAbb*	*AaBb*	*Aabb*	
aB	*AaBB*	*AaBb*	*aaBB*	*aaBb*	
Ab	*AaBb*	*Aabb*	*aaBb*	*aabb*	

Answer: genotype relative frequencies *AABB* – 1/16, *AABb* – 2/16, *AAbb* – 1/16, *AaBB* – 2/16, *AaBb* – 4/16, *Aabb* – 2/16, *aaBB* – 1/16, *aaBb* – 2/16, *aabb* – 1/16.

b) Parents, *P*: ♀ AaBb × ♂aabb

♂***G:*** / ♀***G:***	***ab***	*AaBb* – 1, *Aabb* – 1, *aaBb* –1, *aabb* 1 out of 4.
AB	*AaBb*	
Ab	*Aabb*	
aB	*aaBb*	
ab	*aabb*	

Answer: all in frequency 1/4.

c) Parents, *P*: ♀ AaBb × ♂ Aabb

♂***G:*** / ♀***G:***	***Ab***	***ab***	*AABb* – 1, *AAbb* – 1, *AaBb* – 2, *Aabb* – 2, *aaBb* – 1, *aabb* – 1 out of 8.
AB	*AABb*	*AaBb*	
Ab	*AAbb*	*Aabb*	
aB	*AaBb*	*aaBb*	
ab	*Aabb*	*aabb*	

Answer: *AABb* -1/8, *AAbb* -1/8, *AaBb* -2/8, *Aabb* – 2/8, *aaBb* -1/8, *aabb* – 1/8.

Problem 2. A blue-eyed and right-handed man had married a dark-eyed right-handed woman. One of their three children is blue-eyed and right-handed, while the other two are dark-eyed and left-handed. The man marries again. This time he has a right-handed and dark-eyed wife. They have 11 children, all right-handed and dark-eyed. Estimate the genotypes of the husband and his wives.

Solution: From our life experience, there are not many left-handed people among us. The right-handedness is the dominant allele – *R*, and left-handedness, *r* is recessive allele in human populations. The dark eyes, *D* allele, are dominant over blue eyes (*d* allele).Thus, the genotype of man is *R_dd*. Both wives were right-handed and dark-eyed (dominant traits): *R_D_*. Man has one right-handed, blue-eyed child (*R_dd*) and two left-handed, dark-eyed children (*rrDd*) from first marriage. The only way to have a left-handed child is if both parents have a left-handed allele, *r*. So, parents in first marriage are heterozygous with respect to handedness (both are *Rr*). The only way to produce a blue-eyed child is if both parents possess a blue-eyed allele, *d*. Thus, the first wife must be heterozygous (*Dd*) with respect to eye color. The father's eye color genotype is *dd*.

Therefore the genotypes must have been as follows: man – *Rrdd*, first wife – *RrDd*. The second marriage produced 11 right-handed and dark-eyed children. If new wife

were heterozygous for either character, chances for recessive phenotypes existed. However, none of the recessive traits exhibited in the offspring. Taking this into account, the second wife must be homozygous dominant for both characters (*RRDD*).

Answer: Man is heterozygous on right-handedness and recessive homozygous for eye color – *Rrdd*, his first wife is heterozygous by both genes – *RrDd*, second wife is dominant homozygous - *RRDD*.

Problem 3. The family of healthy man and women have deaf albino child. Write the genotypes of the parents and child.

Solution: C – normal pigmentation; *cc* – albino; *D* – normal hearing; *dd* – deafness.

The parents are heterozygous by both genes, otherwise they can't produce the deaf albino children. Thus the genotypes of the family are as follows: parents, *P:* ♀*CcDd* ×♂ *CcDd*. They both are producing the same (for these particular genes) gametes, *G: CD, Cd, cD, cd*. They have a homozygous child, *F: ccdd*.

Answer: The parents have heterozygous genotypes on both genes, i.e., *CcDd*, and the child has *ccdd*, the recessive homozygous genotype.

Problem 4. The man with the 'eagle' nose and straight hair had married a woman with a wide, flat nose and super curved hair. They have 12 children and all of them have an 'eagle' nose and super curved hair. Determine the genotypes of parents and genotypes of children.

Solution: The eagle nose is dominant, *E* - eagle nose, and wide, flat nose is recessive, *e*. The super curved hair is the dominant trait – *C*, and straight hair is recessive, *c*. For this marriage we can write:

Parents, *P:* ♀*CCee* × ♂*ccEE*
Gametes, *G: Ce* *Ec*
Children, *F:* *CcEe*

Answer: The parents are homozygous by one dominant and one recessive gene: wife is *CCee* and husband is *ccEE* and all possible children are heterozygous by regarded traits - CcEe.

Problem 5. Large eyes and thick lips are dominant traits. A man with small eyes and thin lips has a wife with large eyes and thick lips. They have a son with large eyes and thick lips. Son was married to a woman with large eyes and thin lips. They produced 2 children: a boy with large eyes and thin lips and a girl with small eyes and thick lips. Determine the genotypes of all the parents.

Solution: E – large eyes; *e* –small eyes; *L* – thick lips; *l* – thin lips.

Grandparents, *GP*: wife ♀*E_L_* × ♂*eell* man
Gametes, *G*: *EL* or *El*, *eL* or *el* *el*
Son, *F*: *EeLl*
For son marriage: *P*: ♀ *Eell* × ♂ *EeLl*
Gametes, *G*: *El*, *el* *EL*, *eL*, *El*, *el*
Children, *F*: ♀*eeLl* ♂*E_ll*

Answer: The persons from this family have next genotypes: man – *eell*, wife – *E_L_*, son - *EeLl* , son's wife - *Eell*, son's children: girl – *eeLl*, boy - *E_ll*.

Problem 6. The migraine is known as a dominant trait, and deafness is a recessive trait. In the family the mother is healthy, and the father suffers from a migraine. However, mother is the carrier of deafness recessive allele, the father has normal

hearing. He is heterozygous on both genes. What is the birth probability of children with both of these diseases in this family?

Solution: From the problem information: *M* - a migraine gene, *m* - a normal (healthy) gene state; *H* - a gene of normal hearing, *h* - a deafness gene. Combinations in gametes: *MM* or *Mm* – migraine, *mm* - no migraine; *HH* or *Hh* - normal hearing, *hh* – deafness.

Parents, *P*: ♀ mmHh × ♂ MmHh

♂G: / ♀G:	***MH***	***Mh***	***mH***	***mh***
mH	***MmHH,*** migraine, normal hearing	***MmHh,*** migraine normal hearing	***mmHH,*** no migraine normal hearing	***mmHh,*** no migraine normal hearing
mh	***MmHh,*** migraine, normal hearing	***Mmhh,*** migraine and deafness (1/8=12,5%)	***mmHh,*** no migraine normal hearing	***mmhh,*** no migraine deafness

Answer. The probability of child birth with both diseases in this family is 12,5%.

Problem 7. The glaucoma of adults is inherited in two ways. One form is determined by dominant autosomal gene, and another is coded by recessive independent gene. What is the probability of child birth with both forms of glaucoma if both parents are heterozygous by alleles of both pathological genes?

Solution: *G* - glaucoma gene; *g* - gene of normal sight; *C* - gene of normal sight; c - glaucoma gene. Combinations of gametes: *GG* or *Gg* – glaucoma; gg - normal sight; *CC* or *Cc* - normal sight; *cc* – glaucoma. The probability of the child born with both kinds of glaucoma we estimate from the following mating.

Parents, *P*: ♀ *GgCc* × ♂ *GgCc*

♀G: / ♂G:	***GC***	***Gc***	***gC***	***gc***
GC	*GGCC*	*GGCc*	*GgCC*	*GgCc*
Gc	*GGCc*	‡*GGcc*	*GgCc*	‡*Ggcc*
gC	*GgCC*	*GgCc*	**ggCC*	**ggCc*
gc	*GgCc*	‡*Ggcc*	**ggCc*	*ggcc*

From the Punnett square we receive proportions: Normal sight (**ggCC*, **ggCc*) = 3/16 = or 18,75%. Both kinds of glaucoma in child (‡*GGcc*, ‡*Ggcc*) = 3/16 = 18.75 %.

Answer: The probability of the child births with both kinds of glaucoma is 18,75%.

Problem 8. Sickle cell anemia and thalassemia are inherited as incomplete dominance traits. Homozygous individuals die early in the life, while heterozygous on both genes are viable and have a special form of hemoglobin. Malarial *Plasmodium* is not able to feed on this hemoglobin, so heterozygotes do not get sick with malaria. Double heterozygotes develop mild, non-manifested (microdrepanocytary) anaemia. What is the probability of healthy child birth in a family where one parent is heterozygous in respect to sickle cell anemia but normal on thalassemia and another is normal in respect to sickle cell anemia, but heterozygous on talasemia?

Solution: Let us for simplicity abbreviate the sickle cell anemia gene allele as *A*; *a* – no anemia, normal blood allele; *T* – thalassemia gene; *t* – no talasemia, normal

blood allele. Combinations of the gametes: *AA* – profound Sickle cell anemia; *Aa* – light, non-clinical form of anemia; *aa* – healthy condition, no anemia; *TT* - profound talasemia; *Tt* - light, non-clinical form of thalassemia; *tt* - healthy condition, no talasemia.

Parents, *P*: ♀*Aatt* ×♂*aaTt*

♀ **G:** / ♂**G:**	***At***	***at***
aT	*AaTt*, 25%, light form of both diseases	*aaTt* ,25%, light form of talesamia, no anemia
at	*Aatt*, 25%, light form of anemia, no talesamia	*aatt*, 25%, healthy in respect to both diseases, but not resistant to malaria

Answer: The probability of healthy child birth is 25%.

Problem 9. In humans the right-handedness dominates over left-handedness, and achondroplasia (dwarfness as result of limb, feet and hands skeleton poor development) dominates over normal development of a skeleton. In a family where the husband and the wife are right-handed and suffer from achondroplasia (dwarfs), three girls were born: right-handed and left-handed dwarfs and right-handed with a normal structure of a skeleton. Define genotypes of children and parents.

Solution: *A* - right-handedness; *a* - left-handedness; *B*- achondroplasia gene; *b* - gene of normal development of a skeleton.

Combinations: *AA* or *Aa* - right-handedness *aa* – left-handed *BB* or *Bb* – achondroplasia, *bb* - normal development of a skeleton

Parents, *P*: ♀*AaBb* × ♂ *AaBb*

Gametes, *G*: *AB, Ab, aB, ab* *AB, Ab, aB, ab*

Children, *F*: *9A_B_*; 3 *A_bb*; 3 *aa_B*; 1 *aabb* (on empty place can be either: dominant or recessive allele of the gene).

Answer: Genotypes of parents and children: mother - *AaBb* and the father – *AaBb*; first daughter - *AABB* (or *AaBB*, *AABb*, *AaBb*); second daughter - *aaBB* (or *aaBb*); third daughter - *AAbb* (or *Aabb*).

Problem 10. Human has two forms of dummy-deafness which are defined by separate non-linked genes. What is the probability of children birth with both kinds of dummy- deafness in the family, where mother and the father suffer from the same form of dummy-deafness, and on other forms of dummy-deafness they are heterozygous?

Solution: *A* - a gene of normal audition, *a* - a gene is dummy- deafness *D* - a gene of normal audition, *d* - a gene is dummy- deafness. Combinations of alleles in gametes - genotypes: *AA* or *Aa* - normal audition; *aa* - is dummy- deafness *DD* or *Dd* - normal audition; *dd* - is dummy- deafness.

Parents, P: ♀ *Aadd* × ♂ *Aadd*

Gametes, G: *Ad, ad* *Ad, ad*

Children, F: 1 *AAdd* (25%), 2 *Aadd* (50%), 1 *aadd* (25%)

Answer: the probability of children birth with both kinds of dummy-deafness is 1/4 or 25%.

Problem 11. Anomalies of the eye lens and eye corneas can be the causes of congenital blindness. These are recessive characters which are inherited independently.

Mother and father are healthy, but are carriers of recessive alleles of blindness. What is the probability of healthy and sick children in the family?

Solution: *A* - normal lens; *a* - lens problem; *B* - normal corneas; *b* – corneas problem.

Parents, P: ♀*AaBb* × ♂*AaBb*

♂***G:*** / ♀***G:***	***AB***	***Ab***	***aB***	***ab***
AB	*AABB*	*AABb*	*AaBB*	*AaBb*
Ab	*AABb*	☼*AAbb*	*AaBb*	☼*Aabb*
aB	*AaBB*	*AaBb*	●*aaBB*	●*aaBb*
ab	*AaBb*	☼*Aabb*	●*aaBb*	‡*aabb*

There are 9 healthy genotypes; 3 genotypes are causing cornea problems only (☼); 3 code for the lens problems only (●); 1 is causing both: lens and cornea problems (‡). The ratio of phenotypes is 9:3:3:1.

Answer: The probabilities of healthy and diseased children being born are: 9/16 (56,25%) and 7/16 (43,75%), respectively.

Problem 12. In humans some forms of short-sightedness dominate over normal vision and brown eyes dominate over the blue. Genes of both pairs of traits are situated in different chrs. What children can be expected from parents, which are heterozygous by both genes?

Solution: S - short-sightedness; s - normal vision; B - brown eyes, b – blue eyes.

Parents, P: ♀*SsBb* × ♂ *SsBb*

♂**G:** / ♀**G:**	***SB***	***Sb***	***sB***	***sb***
SB	*SSBB*	*SSBb*	*SsBB*	*SsBb*
Sb	*SSBb*	*SSbb*	*SsBb*	*Ssbb*
sB	*SsBB*	*SsBb*	*ssBB*	*ssBb*
sb	*SsBb*	*Ssbb*	*ssBb*	*ssbb*

From the Punnett square 3 genotypes (*ssBB, ssBb, ssBb*) provide normal vision and brown eyes. The genotype *ssbb* provides information for blue eyes and normal vision. All other genotypes code for the short-sightedness: 3 - in blue and 9 – in brown eyes; 9+3=12.

Answer: Short-sightedness is expected in 12 out of 16 genotypes. The probability of short-sighted children is 12/16 or 75%.

Problems for homework

Problem 13. How many phenotypic and genotypic classes can be produced in dihybrid mating where one parent is homozygous and another is heterozygous for both pairs of genes?

Problem 14. Rough fur and black coat color are the dominant traits for guinea pigs, the laboratory animal often used to study human diseases. The albino, rough guinea pig was mated with a black, smooth guinea pig. The offspring types produced over a period of several years after multiple matings are black, rough and black, smooth only. What are the genotypes of parents?

Problem 15. Brown (*C*) is dominant to albino (*c*) (no pigment) in coat and eyes and rough coat (*R*) dominates over smooth coat (*r*) in mice. Two animals are selected for breeding and their genotypes are *CCRR* and *ccrr*. Determine the expected genotypic and phenotypic ratios for *F1* and *F2* generations and in cross between an *F1* mouse and a mouse with the genotype *CcRR*.

Problem 16. Two individuals who are heterozygous for dark and light hair color and the ability to taste phenylthiocarbamide (PTC) are just married. List all possible genotypes and phenotypes of their children, taking into account that ability to taste PTC and dark color of the hair are dominant traits.

Problem 17. Both parents have no hearing problems. One of them has smooth hair and another has curling hair. Their first child was born deaf with smooth hair. Their second child has curling hair and good hearing. Both pairs of genes are located in different chrs. What is the probability of the next birth of deaf children with curly hair in the family, knowing that the curly gene dominates over the gene of smooth hair, and deafness is a recessive trait?

Problem 18. In humans there are two types of blindness and each is determined by their autosomal recessive gene. Genes of these two types of blindness are situated in different pairs of chrs. What is the probability of blind child birth, if
a) the mother and father suffer from the same type of hereditary blindness and they are normal in respect to the pair of genes coding for other type of blindness?
b) the mother and father are suffering from different types of hereditary blindness; are they homozygous in both pairs of these pathological genes?
c) the parents are sighted, however, both grandmothers suffer from the same type of hereditary blindness, and by the second pair of genes they are normal and homozygous. There was no blindness found in grandfather's pedigree.
d) the parents are sighted, however, both grandmothers suffer from different types of hereditary blindness, and by the second pair of genes they are normal and homozygous. There was no blindness found in grandfather's pedigree.

Problem 19. The marriage of man and woman of unknown genotypes resulted in the birth of black haired, hazel-eyed boy; black haired, blue-eyed boy; bright haired, blue-eyed boy and bright haired, hazel-eyed boy. Determine the phenotypes and genotypes of parents. Dark hair and hazel eyes are dominant traits.

Problem 20. Mother has free ear lobe (dominant trait) and smooth chin, while father has attached ear lobe and hole onto chin (dominant trait). Their son has a free ear lobe and hole onto chin, while the daughter has the mother's traits. Write possible genotypes of these parents and children.

Literature

1. Correns C.//*Berichte der Deutsch. Botan. Gesellschaft*.–1900.-18.-S.158–168.
2. Griffits Anthony J.F., Wessler S.R., Carroll S.B., Doebley J.F. Introduction to genetic analysis.- 11th edition.- W.H. Freeman & Company, N.Y., 2015.- 868 p.
3. Kowles R.V. Solving problems in genetics.- Springer Science+Business Media, New York, 2001.- 479 p.
4. Mendel G. Versuche über Plflanzenhybriden. Verhandlungen des naturforschenden Vereines in Brünn, Bd. IV für das Jahr 1865, Abhandlungen, 1866.- S. 3–47.

Practical 3. Genes interaction. Dominance, incomplete dominance, codominance and lethal alleles.

Theoretical background. There is nothing difficult about understanding the ***dominance*** of a particular gene. Here one (dominant) gene product totally suppresses the effect of other (recessive) genes. For example, the dominance of the genes for yellow seed color and round pea shape over the genes of green seed color and wrinkled pea shape were shown in Mendel's experiments.

The ***incomplete dominance*** could be regarded as "dilution" of the effect of particular gene by the effect of other gene product in heterozygote, resulting in new phenotype (as it could be seen in pink flower color in snapdragons or level of lip protrusion, shape of hair, voice pitch, Tay-Sachs disease severity or hands size).

The ***co-dominance*** allows manifestation of both genes' effects, like it is observed, for example, in human blood groups.

Although an organism can carry only 2 alleles of one gene, many alleles of a single gene can be present in the population (*multiple allelism*) and the set of alleles itself is called allelic series, *e.g.*, self-incompatibility alleles in plants. When the same alleles meet in pollen grains and in egg cells no pollination takes place. This encourages the exchange of genes between different plants in the population. Multiple alleles determine the chevron pattern on the leaves of white clower. The coat color in rabbits is controlled by multiple alleles with dominance row $C > c^{ch} > c^{h} > c$ (Table 1) [6].

Table 1. *C* gene in rabbits

Coat-color Phenotype	***Genotype***
Full color	CC or Cc^{ch} or CC^{h} or Cc
Chinchilla	$c^{ch}c^{ch}$ *or* $c^{ch}c^{h}$ *or* $c^{ch}c$
Himalayan	$c^{h}c^{h}$ *or* $c^{h}c$
Albino	cc

Another example is the human ABO blood-group alleles. There are four blood types (or phenotypes) in the ABO system (Table 2). There are 3 major alleles (***i, I^A,*** and ***I^B***) but, of course, any person has only 2 of the 3 alleles (or 2 copies of 1 of them). The alleles ***I^A*** and ***I^B*** each determine a unique antigen; allele ***i*** confers inability to produce an antigen. In the genotypes ***I^Ai*** and ***I^Bi,*** the alleles ***I^A*** and ***I^B*** are fully ***dominant***, but they are ***codominant*** in the genotype ***I^AI^B.***

Table 2. ABO blood groups in human

Red blood cell phenotype (group)	***Genotype***	***Antigens in red blood cells***	***Antibodies in plasma***
O (I)	*ii*	None	*A and B*
A (II)	$I^A I^A$ *or* $I^A i$	*A*	*B*
B (III)	$I^B I^B$ *or* $I^B i$	*B*	*A*
AB (IV)	$I^A I^B$	*AB*	None

Two other blood polymorphisms human have in addition to the ABO system. Two alleles L^M and L^N determine the *M*, *N* and *MN* blood groups. The dominant allele *R* of a different gene causes a person to have the *Rh*+ (rhesus positive) phenotype whereas the homozygote for r is *Rh*- (rhesus negative).

Lethal alleles confer very interesting and some time confusing effects in organisms.

Lethal alleles (or in other words, lethals or lethal genes) are alleles causing the death of the organism, in which they are present. Lethal alleles are usually the result of mutations in genes that are essential to growth or development [2, 6]. They may be *recessive, dominant*, or *conditional* depending on the gene or genes involved. Lethals can cause death of an organism prenatally or in any time after birth, though they usually manifest early in development.

Dominant lethal examples. Lethal alleles were first discovered by Lucien Cuénot in 1905 while studying the inheritance of yellow coat colour in mice [3]. W.E.Castle and C.C.Little confirmed the findings of L.Cuénot in 1910. The lethal A^Y allele discovered by L.Cuénot is dominant. It controls 2 characters: color and survival. Such genes that have more than one distinct phenotypic effect are said to be ***pleiotropic***. It is entirely possible, however, that both effects of A^Y pleiotropic allele result from the same basic cause, each promotes yellowness of coat in a single dose and they are lethal in a double dose [6].

Huntington disease is a progressive brain damage that causes uncontrolled movements, emotional problems, and loss of thinking ability. Adult-onset of the disease, the most common form of this disorder, usually appears in a person's thirties or forties. It is caused by mutations in the *HTT* gene. The *HTT* gene codes production of a protein *huntingtin*. The gene has a trinucleotide repeat variable in length between individuals. This repeat may change in length between generations. When the length of this repeated section reaches a certain threshold, it results in production of the mutant huntingtin protein (*mHTT*). The presence of this gene in homozygotes and heterozygotes (*HH* and *Hh*) leads to Huntington's disease.

Early symptoms can include depression, irritability, small involuntary movements, poor coordination, and learning and making decisions problems. Ill individuals may have problems in walking, speaking, and swallowing. They usually live about 15 to 20 years after the first symptoms appear.

The "creeper" allele in chicken confers the legs to be short and stunted. Heterozygous chickens display the creeper phenotype. If these are crossed, the deviation from Mendelian ratio is observed: the offspring obtained is 2/3 creeper and 1/3 normal. This is because of homozygous creeper chicken embryos death.

Recessive lethals examples. The *IHH* gene encodes protein for bone growth and differentiation. A single mutated copy of this allele results in few deformations of skeletal bones (brachydactyly only) as one dose of functional *IHH* allele is almost enough to produce a required amount of a protein. When two *IHH* alleles are inherited, no protein essential for skeletal bone formation is produced and the embryo stops to develop and dies.

Cystic fibrosis is fatal to every homozygous recessive person by age 30. Sticky mucus accumulates in the lungs giving rise to frequent and chronic respiratory infections. It is caused by chloride ion channels malfunctioning in ducts.

Congenital ichthyosis. Children are born with crusted leathery skin with deep splits. These splits lead to bleeding, infection and death. The skin's natural shedding process is slowed or inhibited and in some types, skin cells are produced too rapidly. Most types of ichthyosis are treated by suppression of scale build up and humidification of the underlying skin.

Recessive or dominant, the lethal genes are commonly fatal only in the homozygous condition [9]. Heterozygotes may display a form of disease phenotype, as in the case of acatalasia [1] or achondroplasia [6, 9]. Some lethal alleles produce a recognizable phenotype in the heterozygote, as in the yellow mouse and Manx cat (see Problems 15 and 16 here). However, some lethal alleles are fully dominant, killing in one dose in the heterozygote and some confer no detectable effect in the heterozygote at all, and the lethality is recessive [6].

We see, the lethals differ in the developmental stage at which they manifested. Some are expressed as deaths in uterus, where they usually unnoticed, or regarded as spontaneous abortions. Others, such as those responsible for Duchenne muscular dystrophy, phenylketonuria, and cystic fibrosis, exert their effects in childhood. Huntington's disease leads to death in adulthood. The sum of all the deleterious and lethal genes that are present in individuals is called genetic load. It has been estimated that we each carry a small number of recessive lethals in our genomes [6].

Usually, lethal alleles kill, because of deficiency in essential chemical reaction or structural defect (e.g., Sickle-cell anemia, phenylketonuria, abnormal cartilage, etc.).

Whether an allele is lethal often depends on the environment in which the organism develops. However, certain alleles would be lethal in virtually any environment [6].

The favism is an example of a sex-linked ***conditional lethal***, conferring to carrier glucose-6-phosphate dehydrogenase deficiency that causes the organism to develop hemolytic anemia after eating of faba beans (*Vicia faba*). An estimated 400 million people on the globe have this lethal allele [7].

Sometimes, the lethal allele is said to be subvital, or semilethal, because the lethality is expressed in only some individuals. Thus, lethality may range from 0 to 100%, depending on the gene itself, the rest of the genome, and the environment [6].

Dominance

Problem 1. Normal pigmentation (*C*) in humans is dominant over albino (*c*). A diabetic man marries a normal woman whose mother is an albino and whose father is diabetic. What are the genotypes of the man and the woman? What proportion of their children would be expected to be both non-diabetic and have normal pigmentation?

Solution: We are looking at 2 traits in this question. The man is *ccdd*. The woman was normal (for both traits) so she must have at least 1 dominant allele for each character: *C_D_*. Her mother is albino, supplying her with the recessive allele, *c*.

Her father is diabetic, so he provided her recessive allele, *d*. Therefore her genotype, with respect to these two traits is *CcDd*. Let us regard mating ♀ *CcDd* × ♂*ccdd*:

♂G: / ♀G:	*cd*
CD, 1/4	*CcDd*
Cd, 1/4	*Ccdd*
cD, 1/4	*ccDd*
cd, 1/4	*Ccdd*

Answer: 1/4 of their children will be expected to be non-diabetic and have normal pigmentation.

Problem 2. A person with *Rh+* blood has a specific protein on red blood cells. Persons with *Rh-* blood do not have this particular protein in their blood. *Rh+* is dominant in respect to *Rh-*. Also, normal insulin production dominates over abnormal insulin

production. If two individuals are heterozygous for *Rh* factor and insulin production, what probable phenotypes might their children be?

Solution: The individuals in question are heterozygous for both traits, so here we can regard the mating: ♀+-*Ii* × ♂+-*Ii*

♂G: / ♀G:	+*I*, 1/4	+*i*, 1/4	-*I*, 1/4	-*i*, 1/4
+*I*, 1/4	++*II*, 1/16	++*Ii*, 1/16	+-*II*, 1/16	+-*Ii*, 1/16
+*I*, 1/4	++*Ii*, 1/16	++*ii*, 1/16	+-*Ii*, 1/16	+-*ii*, 1/16
- *I*, 1/4	+- *II*, 1/16	+- *Ii*, 1/16	-- *II*, 1/16	-- *Ii*, 1/16
- *I*, 1/4	+- *Ii*, 1/16	+- *ii*, 1/16	-- *Ii*, 1/16	-- *ii*, 1/16

Answer: 9 *Rh*+, normal insulin production; 3 *Rh*+, diabetic; 3*Rh*-, normal insulin production; 1 *Rh*-diabetic.

Incomplete dominance and codominance

Problem 3. What progeny is expected from the couple in which father is heterozygous on Sickle cell anemia and mother is normal on this trait?

Solution: Parents, *P*: ♀ Hb^AHb^A × ♂ Hb^SHb^A

Gametes, *G*: Hb^A Hb^S, Hb^A

Children, *F*: Hb^AHb^S – survive, mild anemia;

Hb^AHb^A –no anemia, 1 : 1.

Answer: Hb^AHb^S – no anemia, red blood cells sickle only under low oxygen concentration. Sickle cell disease is the result of incomplete dominance of Hb^A. However, in regard to hemoglobin we see codominance as both hemoglobins are produced. Those individuals who have the disease at low oxygen carry both: 50% normal (fast in gel-electrophoresis) and 50% changed (slow in gel-electrophoresis) hemoglobin.

Problem 4. Mather has A group of the blood, the father has B. They have two children. The boy has A and his sister O group of blood. What are the genotypes of this couple? Can they have a child with another group of blood?

Solution:The problem's information suggests the mating: female II(A)×male III (B)

Parents, *P*: ♀I^AI^O × ♂ I^BI^O or *P*: ♀I^AI^A × ♂I^BI^B

Gametes, *G*: I^A, I^O I^B, I^O *G*: I^A I^B

Children, *F*: I^OI^O*(I);* I^AI^O*(II);* I^BI^O*(III);* I^AI^B*(IV).* *F*: I^AI^B*(IV).*

Answer: They can have children with another groups of blood: I^OI^O*(I),* I^AI^B*(IV).*

Problem 5. A man with blood group *A* marries a woman with blood group *B*. Their child has O blood group. What are the genotypes of these individuals? What other genotypes and in what frequencies would you expect in offspring from this marriage?

Solution: Based on the information provided above, we can summarize:

	Genotype	Phenotype	Type of genes interaction
P:	♂ I^AI^A or I^AI^O	blood group A	*A* dominance over *O*
	♀ I^BI^B or I^BI^O	blood group B	*B* dominance over *O*

In order to have type O blood (genotype I^OI^O or in other symbols - *ii*) the children must have received one O allele from one parent and the other O allele from the another parent. In this marriage the father must be I^AI^O and the mother must be I^BI^O.

To determine the possible genotypes in the offspring of this couple, you can draw up the Punnett square:

♀ I^BI^O × ♂ I^AI^O

♂G: / ♀G:	I^A, 1/2	I^O, 1/2	*Answer*: We would expect all four groups with equal probability 25%: 1/4 I^AI^B, 1/4 I^AI^O, 1/4 I^BI^O, 1/4 I^OI^O.
I^B, 1/2	I^AI^B, 1/4	I^BI^O, 1/4	
I^O, 1/2	I^AI^O, 1/4	I^OI^O, 1/4	

Problem 6. In a maternity ward, four new born babies become accidentally mixed up. The ABO types of 4 babies are known to be O, A, B, and AB. The ABO types of the four sets of parents are determined. Which baby belongs to which set of parents: a) AB × O, b) A × O, c) A × AB, d) O × O [6]?

Solution: If we write the crosses for each set of parents, we can estimate the family for each child (table 3).

Table 3. Parents and their children

Babies	*Parents*
O	O × O
A	A × O
B	AB × O
AB	A × AB

Problem 7. Two men took a paternity dispute to court, each claiming three children to be his own. The blood groups of the men, the children, and their mother were as indicated in Table 4. From this evidence, can the paternity of the children be established [6]?

Table 4. Blood types of children and parents

Person	*Blood group*		
Husband	O	*M*	*Rh+*
Wife's lover	AB	*MN*	*Rh-*
Wife	A	*N*	*Rh+*
Child 1	O	*MN*	*Rh+*
Child 2	A	*N*	*Rh+*
Child 3	A	*MN*	*Rh-*

Solution: To estimate the paternity, we must take into account that *Rh+* dominates over *Rh-* ; alleles *MN* are codominant; alleles I^A and I^B are fully dominant in genotypes I^Ai and I^Bi , but codominant in genotype I^AI^B (AB blood group) and *ii* genotype determines O group of the blood, the inability to produce antigen; I^Ai or I^AI^A and I^Bi or I^BI^B are for the A and B groups, respectively.

On the basis of this information we can conclude the paternity of the children (Table 5).

Answer: Only 1st child belongs to husband and other two of children are from the lower.

Table 5. Paternity of the disputed children

Children	Blood group			Father
Child 1	O	*MN*	*Rh+*	Husband
Child 2	A	*N*	*Rh+*	Wife's lover
Child 3	A	*MN*	*Rh-*	Wife's lover

Problem 8. Sickle cell anemia is inherited as autosomal trait with incoplete dominance. Homozygous individuals generally die before puberty; heterozygous are viable, the anemia in them is often subclinical to mild. The malarial plasmodium cannot feed on hemoglobin of heterozygotes. The people with this form of hemoglobin can resist malaria. What is the likelihood of having children resistant to malaria in families where both parents are heterozygous on the gene of sickle cell anemia [1]?

Solution: Hb^A - fast hemoglobin, normal gene condition; Hb^S - slow, abnormal hemoglobin. Thus, Hb^AHb^A is healthy individual, Hb^AHb^S - mild anemia, resistance to malarial *Plasmodium* and Hb^SHb^S is severe anemia condition.

Parents, *P*: ♀Hb^AHb^S × ♂Hb^AHb^S

Gametes, *G*: Hb^A, Hb^S Hb^A, Hb^S

Children, *F*: *1 Hb^AHb^A : 2 HbAHbS : 1 HbSHbS*

Answer: The probability of children with resistance to malaria in the family is 50%.

Problem 9. Familial hypercholesterinemia is an abnormally high level of cholesterin in the blood. This disease is inherited as dominant autosomal character. In heterozygotes the disease appears as high cholesterin level in blood and homozygotes develop atherosclerosis and xanthomas (benign tumors) in skin and tendons. Determine the severity of possible hypercholesterinemia development in children if both parents have just high level of cholesterin in the blood [5].

Solution: *C* - dominant allele of familial hypercholesterinemia**;** c – recessive allele; *CC* - atherosclerosis and xanthomas; *Cc* - high cholesterin level in blood, Parents, *P*: ♀*Cc* × ♂*Cc* Gametes, *G*: *C, c* *C, c* Children, *F*: 1*CC,* 25% : 2*Cc*,50% : 1 *cc* 25%	*Answer*: The probability of healthy children is 25%, the probability of high cholecterol level in blood is 50% and the chance to have severe familial hypercholesterinemia, the atherosclerosis, xanthomas, is 25%.

Problem 10. Both grandfathers have just wavy hair and both grandmothers have curly hairs. The mother of the child has wavy hair, and the both: father and child have curling hair. Determine the genotypes of all individuals. What is the probability of the birth of the next child with wavy hair and with straight hair (curly hair gene dominates incompletely over the gene of straight hair) [6]?

Solution: C - curly hair gene**;** c – recessive allele. CC - curly hair; Cc - wavy hair; cc - straight hair. Grandparents, *GP*: ♀*CC* × ♂*Cc* Parents, *P*: ♀*Cc* × ♂*CC* Gametes, *G*: *C, c* *C* 1st Child *CC* Children, *F*: 1 *CC* (50%) :1 *Cc* (50%).	*Answer*: The probability of child birth with curled hair is 50% and it is not possible to have child with strait hair.

Problem 11. A is an autosomal gene with incomplete dominance. When in homozygous state, it causes severe deformation of limbs, but in heterozygotes only brachydactyly develops, the shortening of the fingers and toes due to unusually short bones. Identify possible phenotypes of children in the family if one parent is healthy, and another has brachydactyly [9].

Solution: *A* - the gene that determines the development of skeleton anomalies; *a* - gene for their absence; *AA* - deformation of extremities; *Aa* - shortening of the fingers; aa - normal skeleton. Parents, P: ♀*Aa* × ♂aa Gametes, G: *A, a a* Children, F: 1 *Aa* (50%) : 1 *aa* (50%).	*Answer*: Probability of children with normal skeleton in the family is 50% and of children with brachydactyly also 50%.

Problem 12. Acatalasia caused by a rare autosomal recessive gene. In heterozygote catalase level decreased slightly. A woman with a normal catalase activity has a

husband with low catalase activity. What is the probability of children without anomalies in the family, if grandparents from both sides have reduced activity of catalase [9]?

<u>*Solution*</u>: *A* - the gene that determines the normal activity of catalase; and - the gene that determines Acatalasia; *AA*- normal catalase activity *Aa*- reduced catalase activity *aa* acatalasia. Grandparents, *GP*: *Aa* × *Aa Aa* × *Aa* Parents, P *AA* × *Aa* Gametes, *G*: *A A, a* Children, *F*: *AA : Aa* or 1:1	<u>*Answer*</u>: The probability of healthy children is 50%.

Lethal alleles

Problem 13. Pelger-Huët anomaly involves an arrest of the segmentation of the nuclei of certain white cells. It was first reported in 1928 by Pelger, a Dutch hematologist, who described neutrophils with dumbbell-shaped bilobed nuclei, a reduced number of nuclear segments, and coarse clumping of the nuclear chromatin [10]. The nuclei of several types of white blood cells (neutrophils and eosinophils) have unusual shape (bilobed, peanut or dumbbell-shaped instead of the normal trilobed shape) and unusual structure (coarse and lumpy).

In 1931 Huët, a Dutch pediatrician, identified it as an inherited disorder. The disease was previously thought to be inherited in an autosomal dominant manner; however, co-dominant inheritance has been suggested also [2, 10]. In homozygotes on this basis segmentation of nuclei is completely absent and in heterozygotes it is unusual.

Determine the character of segmentation of nuclei in leukocytes of grandchildren, knowing that mother of children and her parents have unusual segmentation of leukocytes' nuclei and father of children and his parents are normal for this trait.

<u>*Solution*</u>: *P*- Pelger anomaly gene, problems with segmentation of leukocyte nuclei; *N*- gene normal segmentation of nuclei of leukocytes; *PP*- complete lack of segmentation in leukocyte nuclei; *PN*- unusual segmentation of nuclei of leukocytes; *NN* - normal segmentation of nuclei of leukocytes. *GP*: ♀PN × PN♂ ♀NN × ♂NN *P*: ♀*PN* × ♂*NN* *G*: *P, N* *N* *F*: *PN* - 50% unusual segmentation of nuclei of leukocytes *NN* - 50% normal segmentation of nuclei of leukocytes	<u>*Answer*</u>: The chances to have grandchildren with normal segmentation leukocyte nuclei is 50%, and with unusual segmentation - 50%.

Problem 14. Hans Nachtsheim investigated an inherited anomaly of the white blood cells of rabbits. This anomaly, termed the Pelger anomaly, involves an arrest of the segmentation of the nuclei of certain white cells. The disease does not appear to seriously inconvenience the rabbits [10].

a. When rabbits showing the typical Pelger anomaly were mated with rabbits from a true breeding normal stock, Nachtsheim counted 217 offspring showing the Pelger

anomaly and 237 normal progeny. What appears to be the genetic basis of the Pelger anomaly?

b. When rabbits with the Pelger anomaly were mated to each other, Nachtsheim found 223 normal progeny, 439 showing the Pelger anomaly, and 39 extremely abnormal progeny. These very abnormal progeny not only had defective white blood cells but also showed severe deformities of the skeletal system; almost all of them died soon after birth. In genetic terms, what do you suppose these extremely defective rabbits represented? Why do you suppose there were only 39 of them?

c. What additional experimental evidence might you collect to support or disprove your answers to part b?

d. In Berlin, about one human in 1000 shows a Pelger anomaly of white blood cells very similar to that described in rabbits. The anomaly is inherited as a simple dominant (or codominant [6], but the homozygous type has not been observed in humans. Can you suggest why, if you are permitted an analogy with the condition in rabbits?

e. Again by analogy with rabbits, what phenotypes and genotypes might be expected among the children of a man and woman who both show the Pelger anomaly [9]?

Solution:

a. 217 offspring showing the Pelger anomaly and 237 of young rabbits are normal progeny. Here is a relation close to 1:1 ratio. As the pure line was mated with Pelger anomaly animals, we conclude, the cross was *PN* × *NN*, where *P* is Pelger anomaly and *N* is for no disorder. Progeny: *PN* : *NN* as 1:1.

b. This is the result of heterozygotes cross, *PN* × *PN*; 439 are *PN*, the Pelger anomaly; 39 are *PP* - extremely abnormal progeny; 223 are normal progeny – *NN*.

c. We can analyze the cross of animals participating in mating (b) with pure healthy line (*NN*). If we observe a 1:1 ratio (healthy: Pelger anomaly) as a result, the animals in cross (b) are *PN*, the heterozygotes.

d. The zygotes with two copies of the *P* gene will be reabsorbed, or the fetus stillborn or die shortly after birth.

e. In this case we can presume the ratio *PP*: *PN*: *NN* as 1:2:1. However, *PP* is not alive, only *PN* and *NN* progeny viable [6].

Problem 15. In 1904, Lucien Cuenot studied mice having yellow coat color. The mating of yellow mouse to a gray mouse from a pure line resulted in 1:1 ratio (yellow : normal mice) in the progeny, suggesting that a single gene determines these phenotypes, that the yellow mouse was heterozygous for this gene, and that the allele for yellow is dominant to an allele for normal color. However, when Cuenot crossed yellow mice with one another the result was always the same:

Yellow × yellow → 1/3 normal agouti color, 2/3 yellow

When he studied uteri from pregnant females of the yellow × yellow cross; one-fourth of the embryos were found to be dead [3]. In 1910, W.E.Castle and C.C.Little confirmed Cuénot's work, further demonstrating that one quarter of the offspring were dying during embryonic development. How can L. Cuenot's results be explained?

Solution: Note first, the 2:1 ratio is a departure from Mendelian expectations. Second, because no cross of yellow × yellow ever produced all yellow progeny, as

there would be if either parent were a homozygote, it appeared that there were no homozygous yellow mice.

The heterozygotes cross would be expected to yield a genotypic ratio of 1:2:1. If all of the mice in one of the homozygous classes died in the uterus, the live births would then show a 2:1 ratio of heterozygotes to homozygotes. The allele A^Y for yellow might be dominant to the normal allele *A* in its effect on color, but it might act as a recessive lethal allele with respect to viability. Thus, A^YA^Y genotype mice die before birth and are not observed in progeny. All surviving yellow mice were heterozygous A^YA, so a cross between yellow mice will always yield the following results:

$$A^YA \times A^YA \rightarrow \begin{matrix} 1/4\ AA & \textit{normal color} \\ 1/2\ A^YA & \textit{yellow} \\ 1/4\ A^YA^Y & \textit{die before birth} \end{matrix}$$

The Mendelian ratio of 1:2:1 would be observed among the zygotes, but it is altered to a 2:1 ratio, because the A^YA^Y genotype progeny do not survive.

Problem 16. The tailless Manx phenotype (absence of tail in cats) is produced by an allele that is lethal in the homozygous state. A single dose of the Manx allele, M^L, severely interferes with normal spinal development, resulting in absence of a tail in the M^LM heterozygote. Two tailless Manx cats were crossed. What kind of progeny is expected?

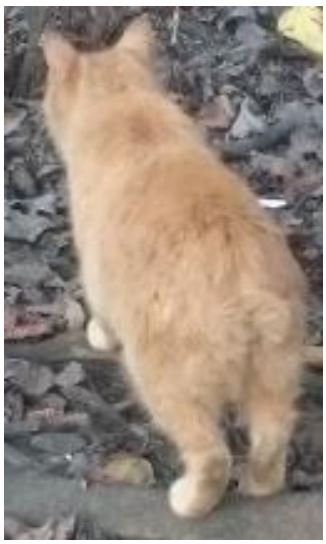


Fig. 1. Tailless Manx cat, a result of one lethal allele action in heterozygote.
By B.M. Sharga.

<u>*Solution*</u>: Parents, *P*: ♀ M^LM × ♂ M^LM
Gametes, *G*: M^L, M M^L, M
Kittens, *F1*: $1M^LM^L$ *(died in uterus);*
$2M^LM$ *(tailless)*
1MM (normal)

In M^LM^L homozygotes, the double dose of the gene produces such extreme developmental abnormality that the embryos do not survive [6].

Problem 17. The ratio of *"creeper"* to normal phenotype, 2 : 1 was observed in young of Courte-pattes pure breed chickens. The cock and hens were of "creeper" phenotype (chondrodystrophy, resulted in short stunted legs) also. Explain the mating result with genetic basis provided.

<u>Solution</u>. There are several breeds possessing the recessive lethal "creeper" allele, *Cp* particularly, Chabo, Krüper, Jitokko, Scots Dumpy, Luttehøns, Courte-pattes [4,8]. The gen is pleiotropic, *i.e.*, it codes for two charcters: lethality in homozygotic state and chondrodystrophy in heterozygotes. If heterozygotic parents ("creepers") are crossed, the deviation from Mendelian ratio is observed: the offspring obtained is 2/3 "creeper" and 1/3 normal.

Let us simplicity use *c* for "creeper" allele and *C* for normal allele of the gene. Thus the mating can be writted as:

Parents, *P*: ♀ *Cc* × ♂ *Cc*
Gametes, *G*: *C*, *c* *C*, *c*
Young, *F1*: *1cc (not hutched); 2Cc ("*creepers*"); 1CC (normal chickens)*

Answer. Heterozygotes *Cc* display the "*creeper"* phenotype. Homozygous for "*creeper" allele* (*cc*) die in eggs with no hatching. This results in deviation from normal Mendelian ratio 1:2:1 (1*CC*: 2*Cc*: 1*cc*). Instead we have ratio 1:2, *i.e.*, 1*CC* (normal) : 2*Cc* ("*creeper"*chickens). The genetic basis of the results is pleiotropic effect of recessive lethal "*creeper"* allele.

Problems for homework

Problem 18. Mother has IIIrd group of blood and father has IVrth. What blood groups can be in their children?

Problem 19. Father has AB NN blood group and mother has O MM group their children have A MN and B MN. Are these children adopted or natural?

Problem 20. At which genotypes of parents babies can't inherit their blood group?

Problem 21. The court hears the case of paternity. Mother has 1st group of blood, her baby has a 2nd group of blood. Can a man be the father of a child, if he has 3rd blood group. What blood group can be in the natural father of the child?

Problem 22. In one family both parents have hazel eyes. This character is inherited as autosomal and dominant in respect to blue eyes. Family has 4 children. 2 of them are blue eyed and have 1st and 2nd group of blood and other 2 have hazel eyes and 2nd and 3rd blood groups. Determine the chance for hazel eyes and 1st blood group in the next child.

Problem 23. The recessive gene of anencephaly causes fetal death in homozygotes, while in heterozygotes it manifests as *spina bifida* of different severity. *Spina bifida* is a birth defect where there is incomplete closing of the backbone and membranes around the spinal cord. The condition often leads to death. Young families need consulting, because two of their children were stillborn with anencephaly and one is alive, however has a severe case of *spina bifida*. What is the probability of a healthy child birth in this family? [1]

Literature

1. Барна І.В. Загальна біологія: збірник задач.- Тернопіль: підручники і посібники, 2013.- 736 с.
2. Borovik L., et al. //*American J. Med. Genet*. - 2013.- Vol. 161A.- N8.- P. 2066-2073.
3. Cuénot L. //Arch Zoo Exp Get.- 1904.- Vol. 2.- P. 45–56.
4. Dohner J.V. *The Encyclopedia of Historic and Endangered Livestock and Poultry Breeds*. - Yale University Press, New Haven, Connecticut, London, 2001.-514 p.
5. Gluecksohn-Waelsch S. // Science.- 1963.- Vol. 142 (3597).- p. 1269–76.
6. Griffits Anthony J.F. et al. An Introduction to genetic analysis.- 5th edition Freeman and Company. – 1993.- 840 p.
7. Glucose-6-phosphate dehydrogenase deficiency. Genetics Home Reference.- https://ghr.nlm.nih.gov/condition/glucose-6-phosphate-dehydrogenase-deficiency
8. Hutt F.B. Genetics of the fowl: the classic guide to chicken genetics and poultry breeding. McGraw-Hill Book Company, New York.- 1949.- 592 p.
9. Lobo I. //Nature Educat.- 2008.- Vol. 1.- N1.- P.138.
10. Nachtsheim H. //J. Hered.- 1950.- Vol.41.- P.131-137.

Practical 4. None-allelic genes interaction. Modifier genes. Epistasis. Complementation. Suppressor and Duplicate genes. Polygenic inheritance.

Theoretical background. Interactions of genes result in modified Mendel's ratios.

Mammalian coat color is produced by a set of interacting genes coding for pigment type, its distribution in the individual hairs and on the body, and pigment presence/absence [5]. The hair color inheritance was best studied on mice [18]. At least 5 major genes interact in mice color production: ***A, B, C, D*** and ***S*** [5, 18].

The A gene is for distribution of pigment in the hair. The wild *A* gives a wild 'agouti' phenotype, the gray "salt-and-pepper" color, due to the yellow band on the dark hair shaft. The allele ***a*** codes for dark hair with no band. The lethal A^Y allele is for yellow hair shafts. The allele $\boldsymbol{a^t}$ in mice causes a yellow belly and dark rest of the body.

The B gene determines the agouti color together with ***A***, but gives solid black with ***aa***. The genotype ***A_bb*** gives a streaked brown color, a cinnamon, and ***aabb*** provides solid brown (Fig. 1). Domestic horses, in contrast to wild horses, have no ***A*** allele. The brown color in horse (chestnut) is recessive to black [5].

AABB (agouti) × ***aabb*** (brown) or
AAbb (cinnamon) × ***aaBB*** (black)
F1: all ***AaBb*** (agouti)
AaBb (agouti) × ***AaBb*** (agouti)
***F2*: 9 *A- B-* (agouti) 9**
3 ***A- bb*** (cinnamon) 3
3 ***aaB-*** (black) 3
1 ***aabb*** (brown) **1**
Fig.1. *A* and *B* genes inheritance

Modifier gene D (MG) is for pigment intensity in mice. The genotypes ***DD*** and ***Dd*** give full color, but ***dd*** "dilutes" black, cinnamon and agouti fur to "milky" due to an uneven distribution of pigment in the hair shaft [5, 17]. If both parents are ***aaCC***, we can write the crosses as in Fig. 2. The MGs modulate the phenotype of individuals with monogenic and multigenic traits. One class of MGs act as protective alleles suppressing disease in otherwise susceptible people [16].

P: ***BBDD*** (black) × ***bbdd*** (dilute brown) or ***BBdd*** (dilute black) × ***bbDD*** (brown)
F1*: *BbDd* ×** ***BbDd (black all)
F2*: *9 B-D- (black) **9**
3 ***B-dd*** (dilute black) 3
3 ***bbD-*** (brown) 3
1 ***bbdd*** (dilute brown) 1
Fig. 2. Modifier gene action

The S gene. This gene controls the spot's presence/absence. The ***S*** results in no spots, and ***ss*** produces a piebald spotting in animals. The spotting can be visible on any of the colored coats. Human piebaldism is a result of *kit* gene mutations [20]. This is a rare autosomal dominant disorder of melanocyte development resulting in congenital white forelock and, usually, multiple symmetrical depigmented or hypopigmented macules. Brown, blond and black hair in humans is due to black and brown eumelanins, the hair pigments produced from pheomelanin under the *MC1R* gene control. The recessive mutant *MC1R* gene codes for an altered version of the *MC1R* protein resulting in red hair in people with 2 alleles of this gene [19].

The Fischer–Saller scale determine 8 main shades of human hair: *A* (very light blond), *B* to *E* (light blond), *F* to *L* (blond), *M* to *O* (dark blond), *P* to *T* (light brown to brown), U to Y (dark brown/black), I to IV (red) and V to VI (red blond) [13].

Epistasis (E) (= Greek "standing over") is non-allelic genes interaction in their effects on a trait [1]. Generally, E means any type of interaction, when the genotype at one locus affects the expression of the genotype at another locus. In a strict sense, E

refers to a situation in which the genotype at one locus determines the phenotype in such a way as to mask the effect of other locus genotype. The epistatic gene disallows the phenotypic manifestation of hypostatic gene [7]. For example, the human baldness gene is epistatic to genes for blond or red hairs [15]. The E is found among the genes of ontogenesis and genes regulating immunity. The E complicates the identification of risk loci for complex disorders. Localization of these interacting loci requires DNA sampling from many families with 2 or more disorder cases.

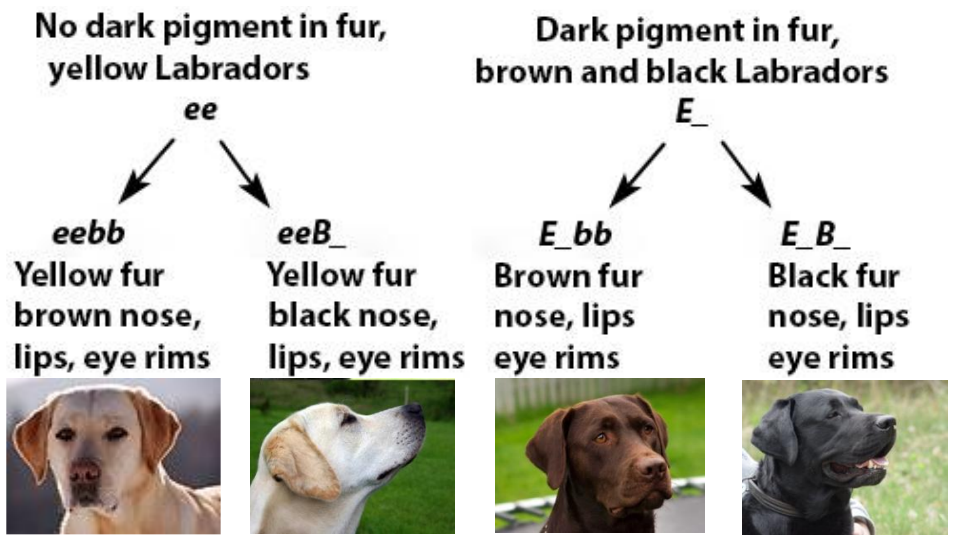
Recessive epistasis (RE). The wild-type allele ***C*** allows coat coloring, and allele ***c*** in homozygotes (***cc***) prevents coloring. This resuls in a 9:3:4 phenotypic ratio (Fig.3).

BB CC (black) × ***bbcc*** (albino)
or
BBcc (albino) × ***bbCC*** (brown)
F1: all ***BbCc*** (black)
BbCc (black) × ***BbCc*** (black)
F2: 9 ***B_C_*** (black) **9**
3 ***bbC_*** (brown) **3**
3 ***B_cc*** (albino)
1 bbcc (albino) } **4**

Fig. 3. Recessive epistasis

RE governs the colors of Labrador dogs also. As in mice, the alleles ***B*** and ***bb*** code for black and brown color, respectively. The homozygotes show RE upon the ***B_*** and ***bb*** combinations, providing golden fur (Fig.4). The black and brown dogs have the allele ***E***. Whether a golden dog is ***B_*** or ***bb*** is estimated by nose, lips and eye rims color, as RE acts mainly in the dog coat with tissue-specific expression of genes [5]. The ***E*** allele is necessary for coat coloring. The ***e*** is an inactive form of ***E***, and ***B*** or ***bb*** alleles are black and brown color determinants. In homozygotes ***ee*** no pigment is produced. Thus, in RE, the inhibition of color production occurs only in recessive homozygotes.

Dominant epistasis (DE). In fox gloves (*Digitalis purpurea*) gene ***M*** stands for ability and ***m*** is for inability of anthocyanin synthesis. In cross between the ***MMDDww*** and ***MMddWW*** genotypes the first parent is dark reddish, due to the ***D*** modifier allele allowing for anthocyanin large levels deposition. The ***d*** allows for small levels of the anthocyanin accumulation. The second parent is white with reddish spots as the plant is capable to synthesise pigment due to allele ***M***, however, ***W*** allele prevents deposition except in the flower throat spots. The 12:3:1 phenotypic ratio is produced (Fig. 5). The allele ***W*** in *F2* eliminates the alternatives coded by ***D_*** or ***dd***, dark or light reddish, and replaces them by white with



By Herwig Kavallar [9] *By John R. & Ph. Hound* [10] *By R. Hanson-Hershey* [8] *By Hanna Dworniczak* [12]

Fig. 4. Recessive epistasis in Labrador retriever dogs

reddish spots phenotype. In DE, the epistatic allele is the dominant allele. The inhibition occurs in homozygotes ***WW*** and heterozygotes ***Ww*** (Fig. 5).

P: *(MM)* ***DDww*** **×** *(MM)* ***ddWW***
(dark reddish) (white, reddish spots)
F1*: *DdWw* × *DdWw (white, reddish spots)
***F2*: 9 D_ W_** (white with reddish spots)
3 ddW_ (white, reddish spots) } **12**
3 D_ww (dark reddish) **3**
1 *dd ww* (light reddish) 1
Fig. 5. Dominant epistasis in fox gloves

In melon white color allele *W* suppresses the effect of yellow *Y* and green color alleles *yy*. Recessive combination ww allows the expression of *Y_* and *yy* coded skin colors and *W* allele is epistatic over these alleles (Fig. 6).

Always in E one gene is "upstream," in the chain of commands and it has effect on the genes lower in the hierarchy of command [5].

P: white ***WWyy*** × ***wwYY*** yellow ***F1*:** white all ***WwYy*** × ***WwYy***		
F2: 12 white	3 yellow	1 green
9 *W_Y_* + + 3 *_yy* = 12	**3 wwY_**	**1 wwyy**
		

Fig. 6. Dominant epistasis in melon. *By D.B. Pylypiv*

Complementation (C) is a phenomenon in which 2 recessive mutations with similar phenotypes in 2 pure lines result in a wild phenotype when both are combined in *F1* genotype. C means that the mutations are in different, complementary genes. If two genotypes coding similar recessive phenotypes fail to complement, they are alleles of the same gene [5; 7]. For example, two specific white-flowered pure lines of peas are crossed and *F1* have purple flowers. The *F2* from *F1* selfing have purple and white plants in ratio 9:7. Here two different genes have similar effects on petal color. Let's represent the alleles of these genes by ***A, a, B,*** and ***b*** (Fig. 7). Homozygosity for the recessive allele of either results in white petals. To have the purple flowers, at least 1 dominant allele of both genes is needed. When we have two white lines, it is necessary to know if these variants appear due to recessive alleles of the same gene or not. The complementation test is an effective test of allelism. If the 2 recessive phenotypes producing wild-type phenotype in *F1*, the parental genotypes have complemented each other, i.e., recessive alleles are of different genes. If the *F1* and the *F2* are all albino, the recessive alleles are of the same gene [5].

White line 1 **AAbb × aaBB** White line 2
F1*: *AaBb all are purple
AaBb (purple) **×** ***AaBb*** (purple)
F2*: 9 *A_B_ (purple)
3 *A_bb* (white)
3 *aaB_* (white)
1 *aabb* (white)
9 purple : **7** white
Fig. 7. Complementation of genes

The suppressor gene (SG). Malvidin synthesis in *Primula* plant is determined by a dominant allele ***K*** which may be suppressed by a separate dominant suppressor gene ***D*** (Fig. 8). A SG may have an associated phenotype or it may, as in the malvidin example, have no detectable phenotypic effect other than the suppression of the phenotypic expression of another gene.

malvidin ***KKdd*** × ***kkDD*** no malvidin
F1*:** all ***KkDd, no malvidin
♀***KkDd*** × ♂***KkDd***
F2*: 9 *K_D_ (no malvidin production)
3 *kkD_* (no malvidin production)
3 *K_dd* (production of malvidin)
1 *kkdd* (no malvidin production)
13 no malvidin : **3** malvidin
Fig. 8. The suppressor gene effect

Duplicate genes (DGs). The gene copies may be present in the genome. DGs control fruit shapes ("heart" or "narrow") in 2 lines of *Capsella bursa – pastoris* plant (Fig. 9). Both: A_1 or A_2 alleles code for "heart" fruit. A cross of 2 lines resulted in *F1* with "heart" fruits. The *F2* shows a 15:1 ratio of "heart" to "narrow". "Heart" fruits result from the presence of at least 1 dominant allele of one or another ***A*** gene (A_1 or A_2), which are identical in function.

$A_1A_1A_2A_2$ × $a_1a_1a_2a$
"heart" "narrow"
F1: $A_1a_1A_2a_2$ "heart"
F2: 9 $A_1\_\ A_2\_$ "heart"
3 $A_1\_\ a_2a_2$ "heart"
3 $a_1a_1A_2\_$ "heart"
1 $a_1a_1a_2a_2$ "narrow"
15 "heart" **: 1** "narrow"
Fig. 9. Duplicate genes

Polygenic inheritance (PI) is controlled by 2 or more genes. For example, human eye color, which was thought of as a single gene trait, has PI. At least 3 genes (*BEY1*, *BEY2* and *GEY*) with complicated patterns of expression are determining eye color. The genes *BEY1*, *BEY2* as dominant provide brown color to the iris and as recessive allele code for blue eyes. The *GEY* gene as dominant determines green eye color and as recessive conditioning blue eyes. The green allele is dominant to blue alleles, but it is recessive to all brown alleles (brown>green>blue). These genes do not code for all eye color variations, *e.g.*, changes in eye color over time, the continuous range of eye colors, and patterns of colors in iris. Still undiscovered genes affect eye color.

PI can cause a trait to have continuous gradual variation between 2 extremes, *e.g.*, human body weight, height, cleft lip, schizophrenia, diabetes mellitus, myopia.

Baldness genes are situated on X and 20th chrs [15]. Testosterone and other androgens can bind onto hair follicle cells and affect when, where, and how much a person's hair grows [15]. The androgen receptor gene is located on the X chr, which means that, for males, it was inherited from their mother. The male pattern baldness has PI with many genetic variants involved [6].

According to many textbooks, the human main skin color types are the result of at least 3-4 genes interaction. Each skin color gene has 2 forms: dominant and recessive, coding for high and low levels of melanin production, respectively. Melanin is a brown pigment that protects skin from harmful UV rays: the darker skin, the better protection. The skin color genes exhibit incomplete dominance, *i.e.*, recessive homozygote *aabbcc*, dominant homozygote *AABBCC* and heterozygous *AaBbCc* for 3 skin colour genes have 0, 6 and 3 units of pigment, respectively. Based on these 3 genes, 7 shades of darkness in people can be determined. Of course, more genes and skin types exist. Felix von Luschan's chromatic scale of not tanned human skin includes of 36 colors [4, 11] (Fig.10).

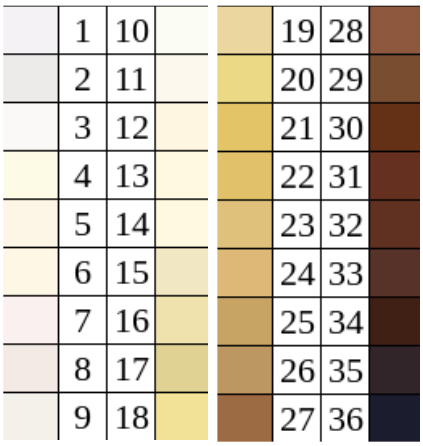


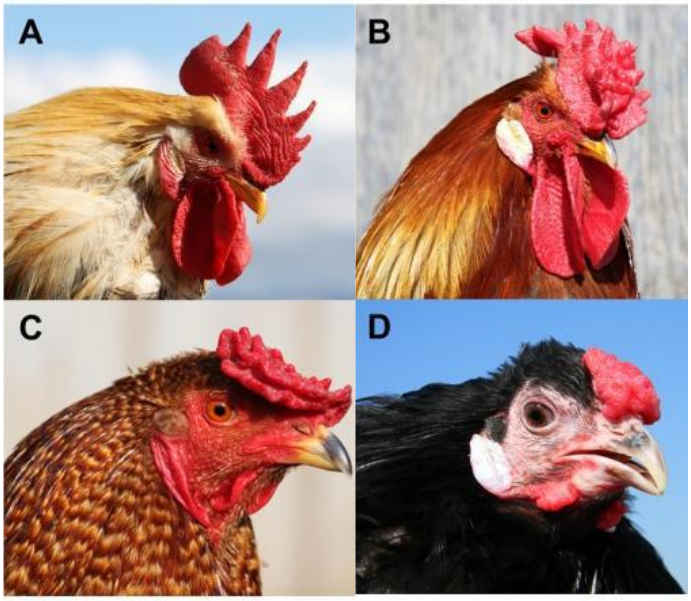
Fig. 10. F. Luschan's skin color scale [4, 11].

Beside humans, the PI is observed in animals, plants and other living things.

The comb types in roosters are another example of PI (Fig.11). Rose-comb inheritance was first described by W. Bateson [1]. The rose-comb and pea-comb mutated alleles are coding together for walnut-comb phenotype [2] (Fig.11). When 1 of dominant genes ***P*** or ***R*** is present, pea or rose comb is produced. The single

comb is in recessive homozygotes. Extensive rose-comb phenotypic variability [17] indicates that its morphogenesis is influenced by several genes and represents a good model to study the genes interactions during development [14]. Homozygotes ***RR*** (mutation ***R*** of wild type with an inversion and disruption of the *CCDC108* gene) show poor sperm motility. Heterozygotes ***Rr*** have good fertility and transmit ***R*** and ***r*** to progeny equally [3]. *CCDC108* is conserved in chicken and human, suggesting *CCDC108* as a gene for sperm motility disorders studies in man [14]. To find *a*, the number of pairs of alleles involved in PI from number of phenotypic forms *p* of the trait they condition, the formula $a = (p-1)/2$ is used. However, due to the multigenic nature of PI strict *p* estimation is often impossible, as the variation of phenotypes shows a continuum or the trait is affected by environment.

Single, wild type (rrpp) **Rose (*R_pp*)**



Pea (*rr P_*) **Walnut (*R_P_*)**

Fig. 11. Comb types in roosters
By F. Imsland (A–C) *and D. Gourichon* (D) [14].

Solved problems

Problem 1. The allele ***A*** (non-lethal in homozygous) causes yellow coats in rats. The allele ***R*** of another gene that assorts independently results in black fur. Together, ***A*** and ***R*** produce a gray, whereas ***a*** and ***r*** produce a white coat. A gray male is crossed with a yellow female, and the *F1* is 3/8 yellow, 3/8 gray, 1/8 black and 1/8 white. Estimate the genotypes of the parents and inheritance pattern [5].

Solution: ***A***- yellow coat; ***R***- black coat; ***A_R_*** - gray coat; ***aarr*** – white coat.

♀***Aarr*** × ♂***AaRr***

♂***G:*** / ♀***G:***	***AR***	***Ar***	***aR***	***ar***
r	***AARr***, *gray*	***AArr***, yellow	***AaRr***, gray	***Aarr***, yellow
ar	***AaRr***, gray	***Aarr***, yellow	***aaRr***, black	***Aarr***, white

Answer: Parents' genotypes are *Aarr* and *AaRr*. Genetic basis of this offsprings is complementary genes interaction.

Problem 2. The interferon production in humans is result of complementary interaction of two dominant genes: ***A*** and ***B***, localized onto chrs: ***A*** on 2nd and ***B*** on 5th. Estimate the probability of children with normal interferon production regarding 2 situations: 1) husband has suppressed ability to produce the interferon as gene ***B*** is lost 2) the couple has both genes in heterozygotic state. The wife and all her relatives are homozygotic for ***A*** and ***B*** genes.

Solution: 1) Parents, *P*: ♀ ***AABB*** × ♂ ***Aabb;*** *G*: ***AB Ab, ab;*** *F1*: ***AABb*; AaBb****

2) Parents, *P*: ♀***AaBb*** ×♂ ***AaBb***, let us build Punnett square for this mating:

♂**G**: / ♀**G**:	***AB***	***Ab***	***aB***	***ab***
AB	***AABB****	***AABb****	***AaBB****	****AaBb***
Ab	***AABb****	***AAbb***	***AaBb****	***Aabb***
aB	***AaBB****	***AaBb****	***aaBB***	***aaBb***
ab	***AaBb****	***Aabb***	***aaBb***	***aabb***

Note: *normal interferon production

Answer: 1) All children are normal in interferon production. 2) Probability of the children with interferon production is: 9/16 × 100 = 56.25%.

The genetic basis of the mating results is complementation of genes ***A*** and ***B*** providing phenotypic ratio producers: non-producers 9:7.

Problem 3. The pedigree brown and white dogs were mated continuously and all the *F1* young are white. The *F2* progeny from *F1*× *F1* crosses are: 125 white, 34 black, and 8 brown. Estimate the genetic basis for these results.

Solution: It is necessary to calculate the ratio of white, black and brown animals in *F2*. Because here is a dihybrid cross, we should regard the total number in *F2* (125 + + 34 + 8 = 167) as a multiple of 16. So, young white (125 × 16) : 167 = 11,98 ≈12; for black (34 × 16) : 167 = 3,26 ≈ 3; and for brown (8 × 16) : 167 = 0,73 ≈ 1. The true ratio is: 12 white: 3 black: 1 brown as in DE.

Let us regard ***W*** allele as epistatic over black ***B*** and brown ***bb*** alleles, and ***w*** as recessive allele allowing color manifestation. Than the combinations of the alleles ***W_B_*** and ***W_bb*** code for white, ***ww_ B*** for black, ***wwbb*** for brown dogs:

P: white ♀***WWBB*** × ♂***wwbb*** brown

F1*:** white♀ ***WwBb × ♂***WwBb*** white

F2*:** 9W_B_*** white, 3***ww_B*** black, 3***W_bb*** white, 1***wwbb*** brown

Answer: The DE of ***W*** upon ***B*** and ***b*** genes is a basis of the mating results.

Problem 4. Yellow Labrador dog was mated with black Labrador. The *F1* pups were as follows: 4 yellow, 2 black and 2 brown. Determine the genotypes of the parents and young and genetic basis of the results.

Solution: Here ***E_*** alleles allows to manifest dog color and ***ee*** prevent pigmentation of the hairsaft in black or brown.

P: yellow ♀***eebb*** × ♂ ***EeBb*** *black;*

G: eb EB, Eb, eB, eb

F1*:** black ***EeBb, brown ***Eebb***, yellow ***eeBb*** and ***eebb***

Answer: The basis of the mating results is RE of ***ee*** upon ***B*** and ***b*** genes.

Problem 5. Two *Primula* plants unable to produce the malvidin were crossed and the *F1* was evaluated for this chemical production. The malvidin synthesis was detected in 10 of 80 *F1* plants. Estimate all genotypes and genetic interaction mode.

Solution: ***K_***- malvidin production, ***kk*** – no malvidin production, ***D*** – suppressor gene, ***dd*** – no suppression of malvidin production.

Parents, *P*: ♀***kkDd*** × ♂***KkDd***

♂**G**: / ♀**G**:	***KD***	***Kd***	***kD***	***kd***	*Answer*: The basis of the cross results is the dominant **SG** effect.
kD	***KkDD*** no malvidin	***KkDd*** no malvidin	***kkDD*** no malvidin	***kkDd*** no malvidin	
kd	**KkDd** no malvidin	**Kkdd** *malvidin*	**kkDd** no malvidin	**kkdd** no malvidin	

Problem 6. The single comb in chicken is coded by recessive alleles *r* and *p* in homozygous state (*rrpp*), the genes combinations *R_pp* provide fowl a rose comb, *rr_P* give a pea comb, and *R_P_* give a walnut comb.

a) What comb types will appear in the *F1*, *F2* and in what proportion if single-combed birds are crossed with birds of a pure-breeding walnut line?

b)What are the genotypes of the parents in a walnut × rose mating from which the progeny are 3/8 rose, 3/8 walnut, 1/8 pea, and 1/8 single?

c) Write the genotypes of the parents in a walnut × rose cross from which all the progeny are walnut?

d) How many genotypes produce the walnut, rose and pea phenotype [5]?

Solution: **a) *P*: ♀ *rrpp* × ♂ *RRPP;***

G*: *rp* *RP

F1*: ♀ *RrPp* × ♂ *RrPp

F2*: 9 *R_P_ walnut comb, **3 *rr_P*** pea comb, **3 *R_pp*** rose comb, **1 *rrpp*** single comb:

♀***G*:** \ ♂***G*:**	***1/4 RP***	***1/4 rP***	***1/4 Rp***	***1/4 rp***
1/4 RP	***1/16 RRPP*** walnut comb	***1/16 RrPP*** walnut comb	***1/16 RRPp*** walnut comb	***1/16 RrPp*** walnut comb
1/4 rP	***1/16 RrPP*** walnut comb	***1/16 rrPP*** pea comb	***1/16 RrPp*** walnut comb	***1/16 rrPp*** pea comb
1/4 Rp	***1/16 RRPp*** walnut comb	***1/16 RrPp*** walnut comb	***1/16 RRpp*** rose comb	***1/16 Rrpp*** rose comb
1/4 rp	***1/16 RrPp*** walnut comb	***1/16 rrPp*** pea comb	***1/16 Rrpp*** rose comb	***1/16 rrpp*** single comb

b) ♀ ***RrPp*** × ♂ ***Rrpp*** 3/8 rose, 3/8 walnut, 1/8 pea, and 1/8 single:

♀ ***G*:** \ ♂***G*:**	***1/4 RP***	***1/4 rP***	***1/4 Rp***	***1/4 rp***
1/2 Rp	***1/8 RRPp*** walnut comb	***1/8 RrPp*** walnut comb	***1/8 RRpp*** rose comb	***1/8 Rrpp*** rose comb
1/2 rp	***1/8 RrPp*** walnut comb	***1/8 rrPp*** pea comb	***1/8 Rrpp*** rose comb	***1/8 rrpp*** single comb

c) *RRPP* × *R_pp.* One of the parents gives to zygote only one gamete kind ***RP***. Thus, all young must be with walnat comb only: ***R_Pp***.

d) 4 genotypes for walnut comb *RRPP*, *RrPP*, *RRPp, RrPp*; 2 genotypes for rose comb *RRpp*, *Rrpp*; 2 genotypes for pea comb *PPrr*, *Pprr*.

Problem 7. The man with extremely dark skin has a wife with extremely light skin. What kind of skin they may expect in children?

Solution: The extremely dark skin is due to ***A, B, C*** alleles in homozygotic state. The ***a, b*** and ***c*** alleles in homozygotes code for extremely white skin. For this couple:

P*: ♀*aabbcc* × ♂*AABBCC ***G*: abc ABC** ***F*: *AaBbCc***	*Answer* : The expected skin color in children is intermediate.

Problems for homework

Problem 1. Two strains of *Drosophila* flies are white eyed due to autosomal recessive mutations. How to test if these mutations interrupt different steps in a single red pigment-producing metabolic pathway or they are mutants of one gene?

Problem 2. The human height is determined at least by 3 pairs of not linked genes. Each dominant allele adds about 5 cm of height. The recessive homozygotes' height is ≈ 150 cm. The man with height of 180 cm has a wife with height of 150 cm. Predict the height of their future children.

Problem 3. Two rose combed roosters were crossed with single combed hens. One male has low sperm motility and another is normal by this trait. Write the genotypes of all these birds. What comb types are expected in young?

Problem 4. There are many variations in hue of blue and brown eye color in humans. What kind of inheritance may be involved in eye color production?

Problem 5. 2 agouti mice mated several times. The phenotypes of young are: 48 agouti, 18 black, 25 albino. What genotypes and kind of inheritance are in action here?

Literature

1. Bateson W. // Rep. Evol. Com. Roy. Soc.-1902.-Vol.1.- P.87–124.
2. Bateson W., Punnett R.C. // Rep. Evol. Comm. Roy. Soc. - 1908.- Vol. 4.- P.18–35.
3. Crawford R.D, Merritt E. // Can J. Genet. Cytol.- 1963.- Vol. 5.- P.89–95.
4. Felix von Luschan Völker, Rassen, Sprachen: Anthropolog. Betrachtungen.- Broschiert Berlin, 1927. - 383 S.
5. Griffiths A.J.F. et al. An introduction to Genetic Analysis.-W.H.Freeman.-N.Y., 2000
6. Hagenaars S.P. et al. //PLoS Genet.- 2017.- Vol.13(2): e1006594. https://doi.org/10.1371/journal.pgen.1006594
7. Hartl D.L., Jones E.W. Genetics: Principles and Analysis.- 4th Ed.- Jones and Bartlett Publishers, Sudbury, MA.-USA – 1998.- 844 p.
8. https://commons.wikimedia.org/w/index.php?curid=32111029
9. https://commons.wikimedia.org/wiki/File:Afra_013.jpg
10. https://wikimedia.org/wiki/File:American_and_English_Labrador_heads.jpg
11. https://wikimedia.org/wiki/File:Felix_von_Luschan_Skin_Color_chart.svg
12. https://commons.wikimedia.org/wiki/File:Labrador_retriever_bulaj1.jpg
13. https://en.wikipedia.org/wiki/Fischer%E2%80%93Saller_scale
14. Imsland F. et al. //PLoS Genetics.– 2012.- Vol.8(6):e1002775.doi:10.1371/journal.pgen.1002775.
15. Lolli F. et al. // Endocrine.- 2017.- Vol. 57, N1. - P. 9-
16. Nadeau J.H. //Curr Opin Genet. Dev.- 2003 .- Vol.13, N3.- P. 290-295.
17. Punnett RC. Heredity in Poultry. London: Mac Millan and Co., Ltd; 1923.
18. Silvers WK. The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction. Springer Verlag; New York: 1979.
19. Valverde P. et al. // Nature Genetics.- 1995. – Vol. 11, N3.- P. 328–30.
20. Ward K.A., Moss C., Sanders D.S. //Br. J. Derm. -1995.- Vol. 132, N6- P.929-935.

Practical 5. Sex determination and sex linkage.

Theoretical background. The temperature was regarded as factor of human sex determination (SD) since antiquity, *e.g.*, Aristotle (335 B.C.E.) naively proposed that the heat of the male partner during intercourse determines the gender of child [13].

Temperature sex determination (TSD) was found in crocodiles and turtles and in fishes [14, 30]. Nearly all eggs of *Alligator mississippiensis* nested at 33°C, produce males. The development at 30°C resulted in nearly all hatching as females [10]. TSD occurs in species with undifferentiated chromosome (chr) Y.

The developing gonads in vertebrates have the bipotential genital ridges: the cortex and the medulla. Ovaries develop from growing cortex, while testes develop from the medulla with an antagonism between two alternative processes [28].

Aromatase regulating gonadal estrogen level is proposed as the main target of a putative thermosensitive factor in TSD. The estrogen levels may influence SD. Yolk steroids and steroids from the embryonic nervous system are sources of hormones for TSD. The TSD animals have different SD modes. They have thermosensitive genes: in *Emydidae—sox9*, in *Testudinae— sox9*, *sf1*, *wt1*, and in *Emydidae—dax1*. In TSD a genes chain *amh-sox9* affects the appearance of testes. The chain *sry-sox9-amh* is used for genetic sex determination (GSD) in mammals [28, 49].

Teleost fishes (>30 000 species) are variable in sexuality: *gonochorism*, *synchronous/sequential hermaphrodite*, or *unisexual* reproduction. Fishes have GSD or environmental SD [19]. The amazon mollies (*Poecilia formosa*) is a unisexual fish.

In *protandrous hermaphrodite* clownfish (*Amphiprion*) the males can turn into females due to behavioral changes after *α*-female death. Both clownfish sexes are of the same karyotype. The aggressiveness of *α*-female suppresses an area of the brain in the other clownfish that is responsible for female hormones production and no new females form. When *α*-female dies, estradiol level in the *α*-male is increased and testosterone is decreased. The female organs grow and the male genitalia degenerate. The *α*-male fish becomes *α*-female [47]. If a clownfish is left alone (no suppression) it will develop as female directly.

There are fish species with *XX/XY* and *ZW/ZZ* (most common) SD systems and the swordtail has a *WXZ* system [19]. About 10% of fish species have heteromorphic sex chrs, but most of them are at an early stage of differentiation. Birds and mammals have *GSD only* by distinct sex chrs [13]. In *ZW* system of birds, insects and fishes, the male is *ZZ* (*homogametic*) and female is *ZW* (*heterogametic*) sex.

McClung C. discovered the “accessory chromosome” in grasshoppers [26]. He concluded, that females have two *X* chrs combination (*XX*), while males have one *X* chr only (*XO*), proving that chrs carry genetic information that determines phenotype.

The majority of insects have dimorphic sex chrs. In moths and butterflies (*Lepidoptera*) females are *XO*, and males have *X* chrs pair (*XX*) [40].

T.H.Morgan explained the *XY* system of SD. He was awarded the Nobel Prize in Physiology or Medicine (1933) for his discoveries concerning the role played by the chrs in heredity. White eyes gene in *Drosophila melanogaster* fruit fly [29] and "lacticolor" gene in moth *Abraxas grossulariata* [8] were the first sex-linked recessive genes found. To illustrate the *X*-linked inheritance of white-eyes in fruit flies,

T.H.Morgan performed an experiment in *reciprocal* crosses [29]. These are crosses in which the sexes and phenotypes of parents are reversed in mating combinations. Difference in reciprocal crosses proved the *X*-linkage of white eye gene, *w* (Table 1).

Table 1. Reciprocal crosses performed by T.H.Morgan

Fist cross: Parents, P: Red-eyed female ♀ $X^{w+}X^{w+}$ × ♂ $X^{w}Y$ White eyed male Gametes, G: X^{w+} X^{w}, Y Offspring, F1: All red eyed ♀ $X^{w+}X^{w}$ × ♂ $X^{w+}Y$ Gametes, G: X^{w+}, X^{w} X^{w+},Y Offspring, F2: $X^{w+}X^{w+}$; $X^{w+}X^{w}$; $X^{w+}Y$; $X^{w}Y$ ***Result:*** All females are red eyed. Red eyed males : white eyed males, 1 : 1
Second cross: Parents, P: White eyed female ♀ $X^{w}X^{w}$ × ♂ $X^{w+}Y$ Red-eyed male Gametes, G: X^{w} X^{w+},Y Offspring, F1: Red-eyed female ♀ $X^{w+}X^{w}$ × ♂ $X^{w}Y$ White eyed male Gametes, G: X^{w+}, X^{w} X^{w},Y Offspring, F2: $X^{w+}X^{w}$; $X^{w}X^{w}$; $X^{w+}Y$; $X^{w}Y$ ***Result:*** Red eyed females : white eyed females, 1 : 1. Red eyed males : white eyed males, 1 : 1.

The *Hymenoptera* (ants, bees, wasps and sawflies) are lack of sex chrs, as do thrips and some smaller clades. Many of them have a *haplodiploid* system of SD in which haploid males develop from unfertilized eggs and diploid females develop from fertilized eggs [4]. In honeybees, *e.g.*, females, the worker and queen, are grown from fertilized (2n = 32) eggs. The drones develope from unfertilized haploid (n = 16) eggs.

Paternal genome elimination was found in *Liposcelis* booklice and some other insects [4, 16]. Both sexes start from fertilized 2n eggs. The paternal chrs are eliminated early during development in males. Females transmit a recombined genome to offspring, but males pass only the maternal copy.

In *D. melanogaster*, the male is *XY* and the female is *XX*. Unlike in mammals, the Y in the fruit fly does not determine maleness (Table 2). The ratio *X* chrs to the number of autosomal sets (*X : A*) provides SD. If a fly has 1 *X* and is diploid for the autosomes (2n), the *X:A* ratio is 1:2 = 0.5, this means that fly is male. The males are *XX*/4n also. To be a female, 2n fly should have 2 *X* chrs, the *X:A* = 2:2 = 1.0. Metafemale genetic constitutions is *XXX*/2n, (*X:A* > 1.0). The metamales appear at combination *XY*/3n and *X0*/3n (*X:A* < 0.5). The latter is sterile. The combinations of *XX*/3n, *XXX*/4n provide intersex fly (0.5< *X:A* < 1.0).

Table 2. Chromosomal sex determination in fruit fly and humans

Chromosomes		***Gender phenotype***	
Sex	*Autosomal*	*D. melanogaster*	*Humans*
XX	2 n	Female (*X:A* = 1.0)	Female
XY	2 n	Male (*X:A* = 0.5)	Male
XO	2 n	Male, sterile (*X:A* = 0.5)	Female, Turner syndrome
XXY	2 n	Female (*X:A* = 1.0)	Male, Klinefelter syndrome
XYY	2 n	Male (*X:A* = 0.5)	Male
XXYY	2 n	Female (*X:A* = 1.0)	Male, Klinefelter syndrome
XXX	2 n	Metafemale (*X:A* >1.0)	Female, triple-*X* syndrome

In mammals, e.g., humans, females, usually, have two *X* chrs (*homogametic* sex), while males have *XY* chrs (*heterogametic* sex). The *SRY* gene on *Y* chr causes maleness by activating and regulating an autosomal gene *Sox9*. If the *Sox9* becomes active in an *XX* embryo, it produces male gonads, no ovaries, and it develops into an anatomical male. If the *Sox9* does not turn on in an *XY* embryo, the ovaries develop, and the individual becomes an anatomical female. This happens for up to 1 in 20 000 *XY* embryos [44]. After transgenosis *Sox9* induces testis formation in *XX* mice [50] also.

R-spondins are a family of growth factors. The R-spondin 1 (*RSPO1*) gene disruption (recessive mutation) results in *XX* sex reversal, palmoplantar hyperkeratosis and predisposition to skin squamous cell carcinoma. The mutation leads to female-to-male sex change in the absence of the testis-determining gene, *Sry* [35]. As a rule, in females *Sox9* is switched off by *RSPO1*, which, via other genes in the cascade, leads to ovaries production. The mutated *RSPO1* is unable to switch-off, leaving *Sox9* on and leading to male with *XX* chrs [34]. *XX* mice with *Sox9* expressed form testes [50].

Some animals have sex chrs series, e.g., the platypus karyotype (2 n = 52) consists of 21 autosomes and 10 sex chrs (5*X* and 5*Y* chrs in male and 5 *X*-pairs in female) [12].

The majority of plants are *hermaphroditic, i.e.*, have both sex organs on the same flower (*e.g.*, *Rosaceae*), other are *monoecious* as they have them in separate flowers on the same plant (*e.g.*, corn). The *dioecious species* (~ 6% of species) [51] have male or female flowers on separate plants, e.g., hollies, ginkgo (Fig.1).

Fig. 1.The holly (*Ilex aquifolium* L.), left, Maidenhair tree (*Ginkgo biloba* L.), right.
By D.B. Pylypiv.

Morphologically distinct sex chrs have only few species, *e.g.*, ginkgo [21], liverwort [33], sorrel and white campion [51]. Of these, heteromorphic sex chrs have been best studied in 2 model species - *Silene latifolia* and *Rumex acetosa* [51].

During meiosis some parts of *X* and *Y* chrs (*homologous regions*) are paired and recombined and other parts are not (*differential regions*) [44]. The *X* and *Y* chrs contain short regions of homology with same genes, the *pseudoautosomal* genes, *e.g.*, *Mic2* gene, encoding a cell surface antigen, is found on short arms of both the *X* and *Y* chrs. It has *pseudoautosomal inheritance*. Genes in *differential regions* are called *hemizygous* in males. The genes in differential regions of *X* or *Y* chrs are said to be *X-linked* or *Y-linked* (*holandric*) genes. The genes in homology regions are *X-and-Y linked*. The genes on *X* and *Y* chrs in general are *sex-linked* [11]. Evolutionary suppression of recombination between *Y* and *X* chrs resulted in their current different

gene numbers [39], ~1 100 and <200 genes on the human *X* and *Y* chrs, respectively [39, 44]. The genes are more numerous on *X* chr, and *X*-linked traits are more common.

X-linked recessive inheritance is concluded from pedigree by next clues: 1) more males, than females affected 2) none of the offspring of an affected male is affected, all his daughters are carriers 3) 50% of sons from these daughters are affected 4) sons of an affected male are free of the mutated allele.

X-linked recessive trait is expressed in all males and in homozygous for trait allele females. The *incidence* of recessive *X*-linked phenotypes in females is the square of that in males: *e.g.*, if 1 : 30 males is affected, then expected females with condition are: 1/30×1/30 = 1/900. *X*-linked recessive trait is inherited from carrier mother or from an affected father. Each son of a carrier mother has a 50% probability of inheriting the trait. A daughter of an affected father and a non-carrier mother will be a carrier, Fig. 2.

There are many *X*-linked recessive disorders in human, such as adrenoleukodystrophy, Becker's muscular dystrophy, creatine transporter defect, Duchenne muscular dystrophy, endocardial fibroelastosis, Fabry disease, Wieacker, spinal and bulbar muscular atrophy, lysosomal storage disease, Menkes disease, Norrie disease, ocular albinism, ocular albinism type 1, ornithine transcarbamylase deficiency, *X*-linked agammaglobulinemia, *X*-linked dystonia parkinsonism, *X*-linked intellectual disability, *X*-linked recessive chondrodysplasia punctata, *X*-linked spinal muscular atrophy type 2, Pelizaeus-Merzbacher disease, *XMEN* disease and syndromes, such as of occipital horn, Renpenning, Say-Meyer, *FG*, Simpson-Golabi-Behmel, *MASA*, *McLeod*, nasodigitoacoustic, oculocerebrorenal, Smith-Fineman-Myers, etc.

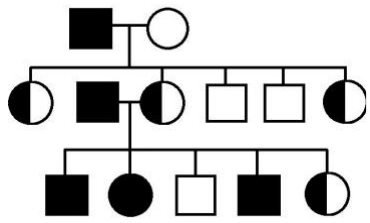


Fig. 2. *X*-linked recessive trait pedigree. *By D.B. Pylypiv.*

Hemophilia, the life threatening inability of blood to clot, is more common in males, as males are hemizygous and express 1 mutant allele. To be hemophilic, a female must inherit 2 mutant alleles, a less frequent event since the mutant allele is rare in the humans. It has an *X*-linked recessive pattern of inheritance (Table. 3). This allele was passed from UK queen Victoria to her son Leopold and, by intermarriage through her daughters, Alice and Beatrice, to many royals in Europe [39].

Table 3. Inheritance of hemophilia, *X*-linked recessive disease

Parents, P: carrier ♀X^hX × ♂XY, non-carrier, healthy Gametes, G: X^h, X X, Y Children, F: daughters X^hX, XX, sons X^hY, XY	<u>*Probabilities of disease*</u>: Daughters are healthy, 50% of them are healthy carriers, 50% of sons may be diseased.

Duchenne muscular dystrophy (DMD) was first described by G. Duchenne in the 1860s. Diseased people may show muscle weakness as early as 3 years of life. The DMD gradually weakens the skeletal muscles and eventually affects the myocard and breathing muscles. Patients rarely survive beyond their early 30s. These are due to recessive mutation in the gene of *dystrophin*, a protein needed as structural support into myocytes for anchoring elements of the internal cytoskeleton to the plasma membrane.

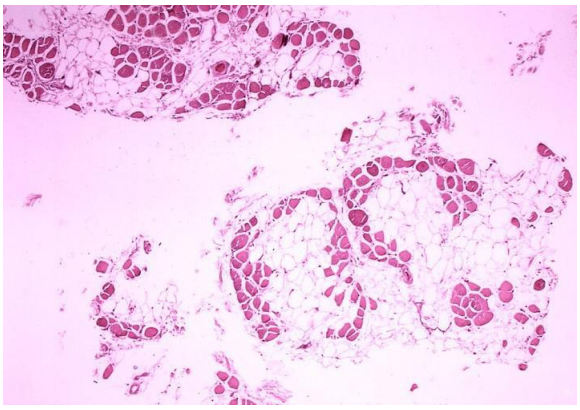


Fig. 3. Cross section of gastrocnemius muscle in patient, who died of pseudo-hypertrophic DMD: extensive replacement of muscle fibers by adipose cells. *By E.P. Ewing, Jr.* [18].

Without dystrophin, the plasma membrane becomes permeable and may rupture. In pseudohypertrophic DMD the damaged myocytes are gradually substituted by fat cells, Fig. 3.

Similar to DMD in human, *X*-linked muscular dystrophy is found among in dogs [1, 2, 3, 42]: the mutation in the dystrophin gene results in devastating muscle atrophy. It starts at about 6 to 8 weeks of age. Ill dogs, usually, die within the 1st year of life, however, some can reach age 3 to 5 and mate. Using these dogs example (Table 4), we can see again, how reciprocal crosses are performed to test the trait for *X*-linkage. From the reciprocal crosses yielded different results, we can conclude inheritance of *X*-linked gene.

Table 4. Muscular dystrophy inheritance in dog

1. Parents, *P*: diseased female ♀X^dX^d × ♂X^DY healthy male
   Gametes, *G*: X^d X^D, Y
   Offsprings, *F*: ♀X^DX^d, ♂X^dY, all females are healthy carriers, all males are diseased
2. Parents, *P*: healthy females X^DX^D × X^dY diseased males
   Gametes, *G*: X^D X^d,Y
   Offsprings, *F*: X^DX^d, X^DY 100% healthy carriers females and healthy males.

X-linked dominant inheritance is recognized through the following:
1) condition is passed from father to daughters only
2) females mated to unaffected males pass the trait to half of their sons or daughters.

Each child of a mother affected with an *X*-linked dominant trait has a 50% chance of inheriting the trait and being affected with the disorder. If only the father is affected, 100% of the daughters will be affected, since they inherit their father's disease coding *X* chr, and 0% of the sons will be affected, since they inherit their father's *Y* chr, Fig. 4.

Males are normally hemizygous for the *X* chr, having only one copy of mutated gene. As a result, *X*-linked dominant disorders usually show higher expressivity in males than females.

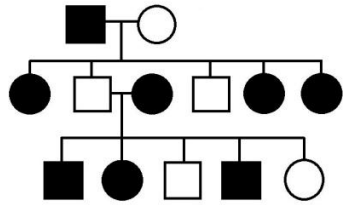


Fig. 4. *X*-linked dominant trait pedigree. *By D.B. Pylypiv.*

Among *X*-linked dominant human disorders are: idiopathic hypopara-thyroidism, incontinentia pigmenti, ornithine carbamoyltransferase deficiency, vitamin D-resistant rickets and syndromes: Alport's, Aarskog-Scott, Rett, Coffin-Lowry, fragile *X*.

X-linked vitamin D-resistant rickets (or *X*-linked hypophosphatemia, XLH, or *X*-linked dominant hypophosphatemic rickets) is a form of osteomalacia due to ineffective D vitamin ingestion because of *PHEX* gene mutation resulting in

PHEX protein inactivity [41]. It leads to bone deformity, short stature and genu varum (bow leggedness), Fig. 5 (treated with Ilizarov frames and by surgery).

The disease prevalence is 1 : 20 000 [7]. The *PHEX* protein regulates protein *FGF*23, a fibroblast growth factor 23 coded by *FGF*23 gene. *FGF*23 inhibits the kidneys' ability to reabsorb phosphate into the bloodstream. Mutated *PHEX* regulates *FGF23* incorrectly. The resulting overactivity of *FGF-23* reduces vitamin D 1α-hydroxylation and phosphate reabsorption by the kidneys, leading to hypophosphatemia and to the symptoms of hypophosphatemic rickets. Also in the disease, where *PHEX* enzymatic activity is absent or reduced, *osteopontin*, a mineralization-inhibiting secreted substrate protein found in the extracellular matrix of bone, accumulates in bones, e.g., teeth, and contributes to the osteomalacia (odontomalacia)[5]. Biochemically disease is recognized by hypophosphatemia and low level of calcitriol (1,25-$(OH)_2$ vitamin D3).

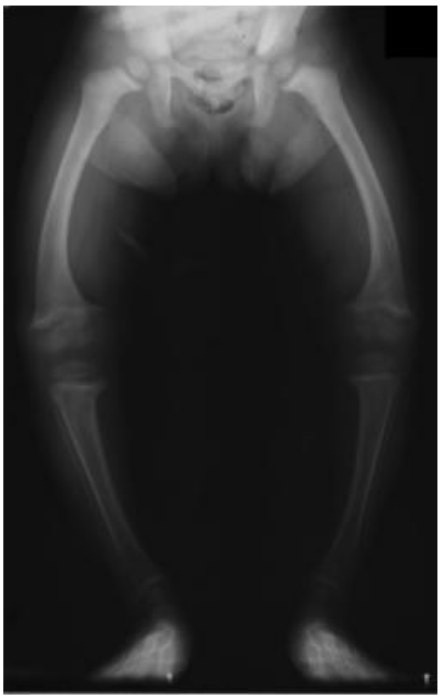


Fig. 5. Anteroposterior view of the 2 y. o. rickets child legs. *By M.L.Richardson* [17].

Y-linked inheritance* or *holandric inheritance is observed for genes located on the *Y* chr; these are inherited by sons from their father (Fig. 6). The *Y* linkage has 3 clues: 1) occurs in males only
2) it is passed to all sons of affected males
3) daughters of affected males are healthy and have healthy offspring.

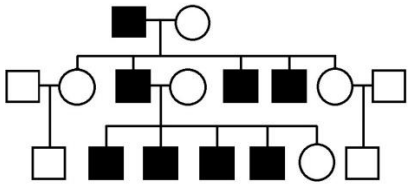


Fig. 6. The *Y*-linked trait pedigree. *By D.B. Pylypiv.*

Stern C. (1957) suggested *Y*-linkage for several genes [45]. In the 1950-60s using human pedigrees, many genes were recognized as *Y*-linked. New advanced techniques and statistical analysis showed that this was done incorrectly for many of them, particularly, for hairy ear rims [20, 34].

In guppies, *Y*-linked genes help determine sex selection due to traits that allow the male fish to appear more attractive to a female [37]. Four sexual activity measures in guppies are *Y*-linked [9].

The *Y* chr has been almost entirely mapped. The *Y*-linkage is proved for many important genes, *e.g.*, *ASMTY* (acetylserotonin methyltransferase), *TSPY* (testis specific protein), *IL3RAY* (interleukin 3 receptor), *SRY* (sex-determining region), *TDF* (testis determining factor), *ZFY* (zinc finger protein), *PRKY* (protein kinase, *Y*-linked), *AMGL* (amelogenin), *CSF2RY* (granulocyte-macrophage, colony stimulating factor receptor, alpha subunit of *Y* ch), *ANT3Y* (adenine nucleotide translocator-3 on the *Y*, *SOX21*(cause of baldness), *AZF2* (azoospermia factor 2), *BPY2* (basic protein on the *Y* chr), *AZF1* (azoospermia factor 1), *DAZ* (Spermatogenes is deleted in azoospermia),

RBM1 (RNA binding motif protein, *Y* ch, family1, member A1), *RBM*2 (RNA binding motif protein 2), *UTY* (ubiquitously transcribed *TPR* gene on *Y* chr), *USP9Y* and *AMELY*. *Y* chr deletions are frequent cause of male infertility.

Dosage compensation (DC) is the phenomenon of similar expression of many genes on the sex chrs (*e.g.*, the *X* chr) in both sexes, even though males and females differ in sex chrs complement. The term was suggested by H.Muller in 1932 [31] to explain the effects of eye color mutations in *Drosophila*: 1 apricot eye color allele in male produces the same effect as 2 of these alleles in female. The female with deletion of 1 of 2 alleles on *X* chrs has eyes of paler color, *i.e.*, 1 allele in female is not equivalent to 1 copy of the allele in the male. Two copies in females versus one copy in males is being DC at the level of gene expression. In *Drosophila*, the male doubles the expression of most genes on the *X* ch.

To equalize the *XX*, in hermaphrodite with *XO* in male the *XX Caenorhabditis elegans* decreases both *X* chrs genes expression to ~ 50%. In birds, males are *ZZ* and females are *ZW*. Only some *Z*-linked genes may be in *DC*. M.Lyon (1961) found that DC in mammals occurs by inactivation of 1 of 2 *X* chrs in female [24].

K.Patau with co-workers identified and studied the *X* chr inactivation centre (*Xic*) [46]. The counting of *X* chrs by human cell is done by estimation of the *Xics* number. If 1 of the 2 *X chrs* in a female is missing its *Xic* due to mutation, a cell counts only one *Xic* and *X* inactivation not happens. This leads to embryo death.

The specific *Xist* (*X-inactive specific transcript*) gene expression within the *Xic* is needed for the *X* chr compaction into a Barr body (Bb) [6]. While most other genes on the inactivated *X* chr are silenced, the *Xist* gene is expressed in *Xist* RNA which coats and inactivates the *X* chr. After coating, proteins associate with the *Xist* RNA and promote chromosomal compaction into a Bb.

The *X* inactivation process can be divided into 3 phases: *initiation*, *spreading*, and *maintenance*. During initiation one of the *X* chrs is chosen. During the spreading, this chr is inactivated. *X* chrs that lack the *Xist* gene cannot be inactivated [36]. The transfer and expressing the *Xist* gene on another chr leads to its silencing [15, 23]. Prior to inactivation, both *X* chrs weakly express *Xist* RNA from the *Xist* gene. Then, active *X* chr ceases *Xist* expression, whereas the future inactive *X* chr highly increases it. The *Xist* RNA coats the future inactive *X* chr only and recruits compaction proteins [32]. The inactivation spreads from *Xic* in both directions on the *X* chr [15]. The silencing of genes on the inactivating chr occurs soon after coating. *Xist* RNA is involved in Bb movement to the nuclear periphery. *Xist* initiates *X* inactivation *X* genes and maintains it by methylation. The *maintenance* phase continues from the embryonic stage through adulthood. When cell divide, the Bb is replicated, and both copies remain compacted. Some genes are expressed in Bb of adult female, e.g., *Xist*.

In humans, up to 25% of the *X* chr genes may escape inactivation. Many of them occur in clusters, *e.g.*, *pseudoautosomal* genes. Their inactivation is not necessary as they are located on both: the *X* and *Y* chrs. In mice, the paternal *X* chr is silenced. In rabbits, maternal or paternal selection occurs downstream of *Xist* expression.

The *Tsix* RNA is the *antisense* to *Xist*. The *Tsix* gene overlaps the *Xist* gene and is transcribed on the opposite strand of the *Xist* gene DNA [22]. *Tsix* is a negative regulator of *Xist*; *X* chrs without *Tsix* expression (high levels of *Xist* transcription) are

inactivated much more frequently than active chrs. Like *Xist*, prior to inactivation, both *X* chrs weakly express *Tsix* gene. At start of *X*-inactivation, the future inactive *X* ch ceases to express *Tsix* RNA (and increases *Xist* expression), whereas active *X* chr continues to express *Tsix* for several days.

Rep A is another long non coding RNA working with *Xist* RNA in *X* chr inactivation. *Rep A* inhibits *Tsix* and eliminates expression of *Xite*. *Rep A* promotes *Tsix* region methylation by attracting PRC2, *i.e.*, inactivation of one of the *X* chrs [27]. Thus, *X*-linked long non-coding RNAs are key players in *X* chr inactivation [25].

Another form of X inactivation occurs only in male meiotic spermatogenesis, *meiotic sex chromosome inactivation* (MSCI). MSCI is the process of transcriptional silencing of the X and Y chrs [48].

Solved problems

Problem 1. Wife is red colour blindness carrier, husband is healthy. What proportion of their female and male progeny will show the trait? What are the genotypes of parents and progeny?

<u>*Solution*</u>: *P*: wife, carrier, ♀ X^rX × ♂XY, healthy husband *G*: X^r, X; X,Y *F*: girls - X^rX, XX; boys - X^rY, XY	<u>*Answer*</u>: The trait is shown in 50% sons and not in daughters. 50% of daughters are carriers (X^rX). The parents' genotypes X^rX; XY.

Problem 2. A bent tail in mice is caused by mutant allele. Six pairs of mice were crossed. Their phenotypes and those of their progeny are given below [11]. *N* is normal, *B* is bent phenotype. Estimate the inheritance way of this phenomenon.

<u>*Solution*</u>:

Parents		Progeny		Cross and progeny	<u>*Answer*</u>:
females	males	females	males		
N	B	All B	All *N*	$X^NX^N \times X^BY \rightarrow X^NX^B; X^NY.$	The genetic basis of these results is *X*-linked dominant mutation.
B	N	1/2 B, 1/2 N	1/2 B, 1/2 N	$X^NX^B \times X^NY \rightarrow X^NX^B; X^NX^N; X^BY; X^NY.$	
B	N	All B	All B	$X^BX^B \times X^NY \rightarrow X^NX^B; X^BY.$	
N	N	All N	All N	$X^NX^N \times X^NY \rightarrow X^NX^N; X^NY.$	
B	B	All B	All B	$X^BX^B \times X^BY \rightarrow X^BX^B; X^BY.$	
B	B	All B	1/2 B, 1/2 N	$X^NX^B \times X^BY \rightarrow X^BX^B; X^NX^B; X^BY; X^NY.$	

Problem 3. Who of any female's grandparents can't contribute any of *X*-linked genes to her genotype?

<u>*Solution*</u>: Mother side: Father side: Grandparents, *GP*: $XX \times XY$ and $XX \times \underline{\boldsymbol{XY}}$ Parents, *P*: $XX \times XY$ Female, *F*: XX	<u>*Answer*</u>: The grandfather from her father side can't contribute *X*-linked genes.

Problem 4. How many Barr bodies form in human somatic cells with next compositions of sex chrs: 1) *XO*2) *XXX* 3) *XYY* 4) *XX*Y?

Solution: The number of Barr bodies is estimated from equation: *Number of Bb = X – 1*, where *X* is the number of *X* chrs. *Answer*: 1) 0 2) 2 3) 0 4) 1.

Problem 5. A man with hemophilia and a healthy woman have a son and daughter with hemophilia. What are the genotypes of this couple?

Solution: *h*-hemophilia allele. Mother must be heterozygous carrier if daughter has disease. *Answer*: ♀X^hX × ♂ X^hY.

Problems for homework

Problem 1. A man is heterozygous *Aa* for one autosomal gene, and he carries a recessive *X*-linked allele *d*. What proportion of his sperm will be *ad*?

Problem 2. Deduce the inheritance pattern for trait in pedigree on Fig.7.

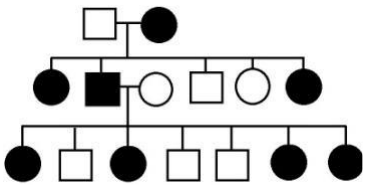


Fig. 7.
By Diana B. Pylypiv

Problem 3. Select the correct answer. In order to be a male a human: 1) must have one *X* chr 2) must have two *X* chrs 3) must have at least one functional *Y* chr 4) can't have any of *X* chrs.

Problem 4. Humans and *Drosophila* both have *X* and *Y* chrs, however, SD differs in these species. How?

Problem 5. Describe the phenotype of *XY* person with *SRY* gene deletion.

Problem 6. Explain why heterozygotic females may have Hunter syndrome?

Problem 7. *Case study*. A baby with fuzzy, sparse hair, fair complexion, chubby cheeks and irritability was subjected to *X*-rays examination, which demonstrated abnormal skull and skeleton bones development. Microscopic examination of hair revealed a classical sign of *pili torti*. The urine homovanillic acid/vanillylmandelic acid ratio is 56.1. Molecular biology analysis revealed *ATP7A* gene mutation. Estimate the diagnosis and explain the inheritance of the disease.

Problem 8. A blood smear of Tunisian lady showed half RBC number parasitized by *Plasmodium*. These cells have normal activity of G6PD. The rest of cells have weak activity of the enzyme, however, resistant to this parasite. Explain, why?

Literature

1. Baltzer W.I., et al. // J.Am.Anim.Assoc.- 2007.- Vol. 43, N4.- P.227-232.
2. Beltran E., et al. //J.Small Anim. Pract.- 2015.- Vol.56, N5.- P.351-354.
3. Bergman R.L., et al. // J. Am. Hosp. Assoc.- 2002.-Vol. 38, N3.- P. 255-261.
4. Beukeboom L.W. // Genetics.- 2017.- Vol. 206.- P.751–753.
5. Boukpessi T., et al. // Bone. - 2016.- Vol. 95.- P. 151–161.
6. Brown C.J., et al. // Nature.- 1991.- Vol. 349.- P. 38-44.
7. Carpenter T.O. // Pediatr. Clin. North Am.- 1997.- Vol. 44, N2.- P. 443–466.
8. Doncaster L. , Raynor G.H. // Proc. Zool. Soc. London.- 1906.- Vol. 1. - P. 125-133.
9. Farr J. // Evol.- 1983.- Vol. 37. - P.1193–1209.
10. Ferguson M.W.J., Joanen T. // Nature.- 1982.-Vol.296, P. 850–853.

11. Griffiths A.J.F., et al. Introduction to Genetic Analysis.- 11th Edition.-New York, W.H. Freeman & Company.- 2015.- 868 p.
12. Gruetzner F., et al. // Chromosoma.- 2006.- Vol.115, P. 75-88.
13. Hake L., O'Connor C. // Nat. Edc.- 2008.- Vol.1, N1.- P.25
14. Hawkes L.A., et al. // Glob. Change Biol.- 2007.- Vol.13.- P.923–932.
15. Herzing L.B., et al. *Xist* has properties of the *X*-chromosome inactivation centre // Nature.- 1997.- Vol. 386, N6622.- P.272–275.
16. Hodson C. N., et al., // Genetics.- 2017.- Vol.206.- P. 1091–1100.
17. https://commons.wikimedia.org/w/index.php?curid=59299
18. https://phil.cdc.gov/default.aspx
19. Kobayashi Y., et al. // Sex. Dev. – 2013.- Vol. 7, N 1-3.- P.115-25.
20. Lee A. // Eur. J. Hum. Genet.- 2004.- Vol. 112.- P:1077–1079.
21. Lee C.L. // Am. J. Bot.- 1954.- Vol.41, N7.- P. 545-549.
22. Lee J.T., et al. // Nature Genetics.- 1999.- Vol.21, N4.- P.400–404.
23. Lee J.T., Jaenisch R. // Nature. 1997.- Vol. 386, N6622.- P.275–279.
24. Lyon M. F. // Nature. -1961.- Vol.190.- P.372–373.
25. Maclary E., et al. //Chromosome Res.- 2013.- Vol. 21(0) .- P. 601–614.
26. McClung C. E. // Anat. Anz. – 1901.- Vol.20.- P. 220–226.
27. Mercer, T.R., et al. // Nat. Rev. Genet.- 2009 .- N10.- P. 155-159.
28. Merchant-Larios H., et al. // Sex.Devel. - 2013.- Vol. 7, P. 95–103.
29. Morgan T.H. // Science.- 1910.- Vol. 32.- P.120-122.
30. Mork L., et al // Devel. Biol.- 2014.- Vol. 386, N 1. - P. 264-271.
31. Muller H.J., Further studies on the nature and causes of gene mutations.- Proceedings of the Sixth International Congress of Genetics, Ithaca, NY.- 1932.- 1: 213–255.
32. Ng K., et al. // EMBOReports. – 2007.- Vol. 8, N1.- P. 34–39.
33. Okada S, et al. // Proc. Natl. Acad. Sci. USA.- 2001.- Vol. 98. – P. 9454–9459.
34. Ott J. //Am. J. Hum. Genet.- 1986.-Vol. 38.- P.891–897.
35. Parma P., et al. // Nat. Genet.- 2006.- Vol. 38, N11.- p.1304-1309.
36. Penny G.D., et al. // Nature. -1996.- Vol. 379, N6561.- P. 131–137.
37. Postma E. // Soc. Study Evol. 2011. – Vol.65.- P. 2145–2156.
38. Price M. //ScienceNOW Daily News. AAAS. (8 October 2009) http://sciencenow.sciencemag.org/cgi/content/full/2009/1008/2
39. Ross M.T., et al. //Nature- 2005.- Vol. 434: 325-337.
40. Saccone G., et al. // Genet.- 2002.- Vol.116.- P.15–23.
41. Saito T., et al. // Eur. J. Endocr.- 2009.- Vol. **161, N**4.- P.647–651.
42. Sánchez L., et al. // PLoS One. 2018 Feb 23; 13(2):e0193372.
43. Sekido R., Lowell-Badge R. //Nature 2008.- Vol. 453.- P. 930–934.
44. Skaletsky H., et al. // Nature.- 2003.- Vol. 423.- P.825–837.
45. Stern C. // Am. J. Hum. Gen.- 1957.- Vol.9,N3.- P. 147–166.
46. Therman E., et al. // Chromosoma.- 1974.- Vol. 44,N4.- P. 361–366.
47. Todd E.V., et al. // Sex. Dev.- 2016.- Vol.10.- P. 223–241.
48. Turner J.M. et al. // Devel.- 2007.- Vol.134.- P.1823-1831.
49. Valenzuela N., et al. //Devel. Dynam., 2013.- Vol. 242, P. 307–319.
50. Vidal V.P.I., et al. // Nat. Genet.- 2001.-Vol. 28, P. 216–217.
51. Vyskot B., Hobza R. //Pl. Sci.-2015.-Vol.236.-P.126-135.

Practical 6. Genetic linkage and mapping.

Theoretical background. According to Mendel's 2nd law, the alleles of different genes, located on different chromosomes (chrs) have independent assortment (IA). The genes on the same chromosome (chr) may show no IA, if they are close together or ***linked***. They tend to stay together in meiosis (M) and pass to the same gamete (*G*).

T.H. Morgan tracked this issue in *Drosophila melanogaster* [16]. He found that *white eye* (*w*) and *miniature wing* (*m*) genes on *X* chr tend to be inherited together as linked. The linkage (L) was not complete. He discovered in *F1* from the cross ♀♀*wm/wm* ×♂♂ *w+m+/Y* that all females were of wild type (*w+m+/wm*) and all males were with white eyes and miniature wings (*wm/Y*). When female and male flies of *F1* were crossed, most frequent phenotypes in their progeny were grandparents' phenotypes: white eyes, miniature wings and wild type. These are coded by original genotypes of grandparents and called *parental genotypes*, *parental classes (types)* or just *parentals* (Pr). 33% of the cross progeny comprises flies with genes combinations that differ from Prs. They had white eyes and normal wings or red eyes and miniature wings. He called them *recombinant classes* (Rc). To explain the data, T. H. Morgan suggested genes exchange during M between homology *XX* chrs in *F1* females, as no exchange is observed in M between the non-homology *Y* and *X* chrs in males [18].

In a similar experiment the pure line white-eyed, yellow bodied females (*wy*, recessive traits) were crossed with pure line wild (red-eyed, gray-bodied *w+y+*) males. Then, wild traits *F1* females were mated with recessive traits *F1* males. The progeny was obtained with only 1% of Rcs, the red-eyed, yellow bodied and white-eyed, gray-bodied flies. From these results T.Morgan concluded more close linkage for eye color and body color genes, than for eye color and wing size. The traits inherited together to a higher extent, the closer coding genes are on the chr [18].

The Rc/Rc and Prs/Prs ratios were close to 1 in these experiments, as the classes were reciprocal products of one exchange event. T.H.Morgan and E. Cattell introduced the term ***'crossing-over'*** for this exchange [17]. The *crossing-over* or *crossover* (CO) is a process of exchange of gene loci between *non-sister chromatids* of homologous chrs which may result in *recombination* (R), i. e., production of Rcs in *late prophase* of M I. CO breaks up the L between genes. In M II the genes that were linked will then assort independently and finally appear in different *G*s.

The probability (P) of CO and thus, the P of genetic R increases with increase of distance (D) between 2 linked genes. The P of R serves as a measure of D between genes and allows the construction of *genetic linkage map*, showing relative position of the genes and Ds between them. This idea was first proposed by Alfred Henry Sturtevant, the undergraduate student, in 1911, when he and his teacher, T.H. Morgan, were discussing IA. During the following night A.H. Sturtevant built the first L map [21].

The *recombination fraction*, *r* (or *q*) between genes *w* and *y* in the above study is: *r = 0.01*. The genes are linked as $r < 1/2$. If being $r = 1/2$, the traits are in IA (and Mendel's 2nd law holds true). *Recombination frequency*, RF (or θ) is the genetic L measure used in the L maps construction. It is the frequency of a single crossing over (SCO) between 2 marker genes. The RF is a measure of recombination distance (D)

between two genes. In the example above 1% of RF (r = 0.01) was observed. It means that D between genes *w* and *y* is 1 map units (m. u.) or 1 cM. A centimorgan (cM) is a unit describing a RF of 1% [6]. The map unit 1 centimorgan (cM) is used in honor of T.H. Morgan, 1 morgan = 100 m. u. = 100 cM.

The linked genes belong to the same L group. It is important to know which alleles on chrs are linked. We can depict the genes on homological chrs in coupled (*cis-)* or in repulsive (*trans*-) positions as shown in Fig. 1.

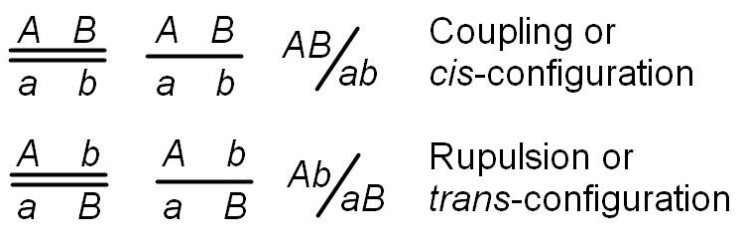


Fig. 1. The ways to show alternative positions of genes on chromosomes pair. *By D.B. Pylypiv*.

The *G*s produced in M have alleles in Pr and Rc arrangement. If Pr configuration is *cis*-, so Rcs chrs must be in *trans*- position and *vice versa*. The RF between 2 mutant alleles is independent of whether they are on same chr (*cis*-) or if they are separated on different homologous chrs (*trans*-configuration). This was shown, particularly, in Morgan's study of *D. melanogaster X* chr linked white eyes (*w*) and miniature wing (*m*) genes (Fig. 2). In Cross 1 he allowed white eyed, normal-wing (*w* +) females and red-eyed, miniature wing (+ *m*) males to mate. Then, *F1* trans-configuration females (*w* +/+ *m*) were crossed with males having both mutant alleles on *X* chr (*wm/Y*). The Prs and Rc classes were observed (Fig. 2, A).

The Cross 2 was done between *wm* homozygous females and wild type males. Then, *F1* females with 2 mutant alleles on the same chr were test-crossed. The Rc and Pr classes % were in quantities close to those in Cross 1. The difference between the classes was in the range of variation. The results of crosses 1 and 2 are not consistent with IA ratio 1:1:1:1. The 33.5 and 37.7% of *X* chr Rcs in Crosses 1 and 2 are RFs and these RFs < 50%. The Rc *X* chrs *wm* and ++ (Cross 1) or +*m* and *w*+ (Cross 2) appeared in *F1* females due to CO in M. These resulted in above calculated RFs for genes *w* and *m*. Thus, no matter, how mutant alleles are arranged: in *cis*- or *trans*- position the RF between them is the same [8].

The RFs are variable and depend on Ds between linked genes allowing CO. So, the genes with RF < 50% are linked. No-linkage means 50% of COs. If genes are on different chrs or separated by large D on the same chr, they segregate as not linked, RF = 50%. A RF > 50% can't exist [3, 23]. Unlike most living things, the males of *D. melanogaster* have no CO and R. So, genes of same chr location show complete L and they are inherited always as a group without R. For example, *cn* and *bw* genes for cinnabar or brown eyes are linked on chr 2. They are so distant that in females show 50% RF. The cross of female *cnbw/++* and male *cnbw/cnbw* results in offspring with Rc (*cn+/cnbw; +bw/cnbw*) and Pr (*cnbw/cnbw; cnbw/++*) traits in 1:1:1:1 ratio.

In reciprocal cross produced Prs only, as there is no CO in male [8]:

Parents, *P*: ♀♀ ***cnbw/cnbw*** **×** ♂♂ ***cnbw/ ++***
Gametes, *G*: ***cnbw,*** ***cnbw, ++***
Offsprings, *F1* ***cnbw/cnbw; cnbw/ ++* (1:1).**

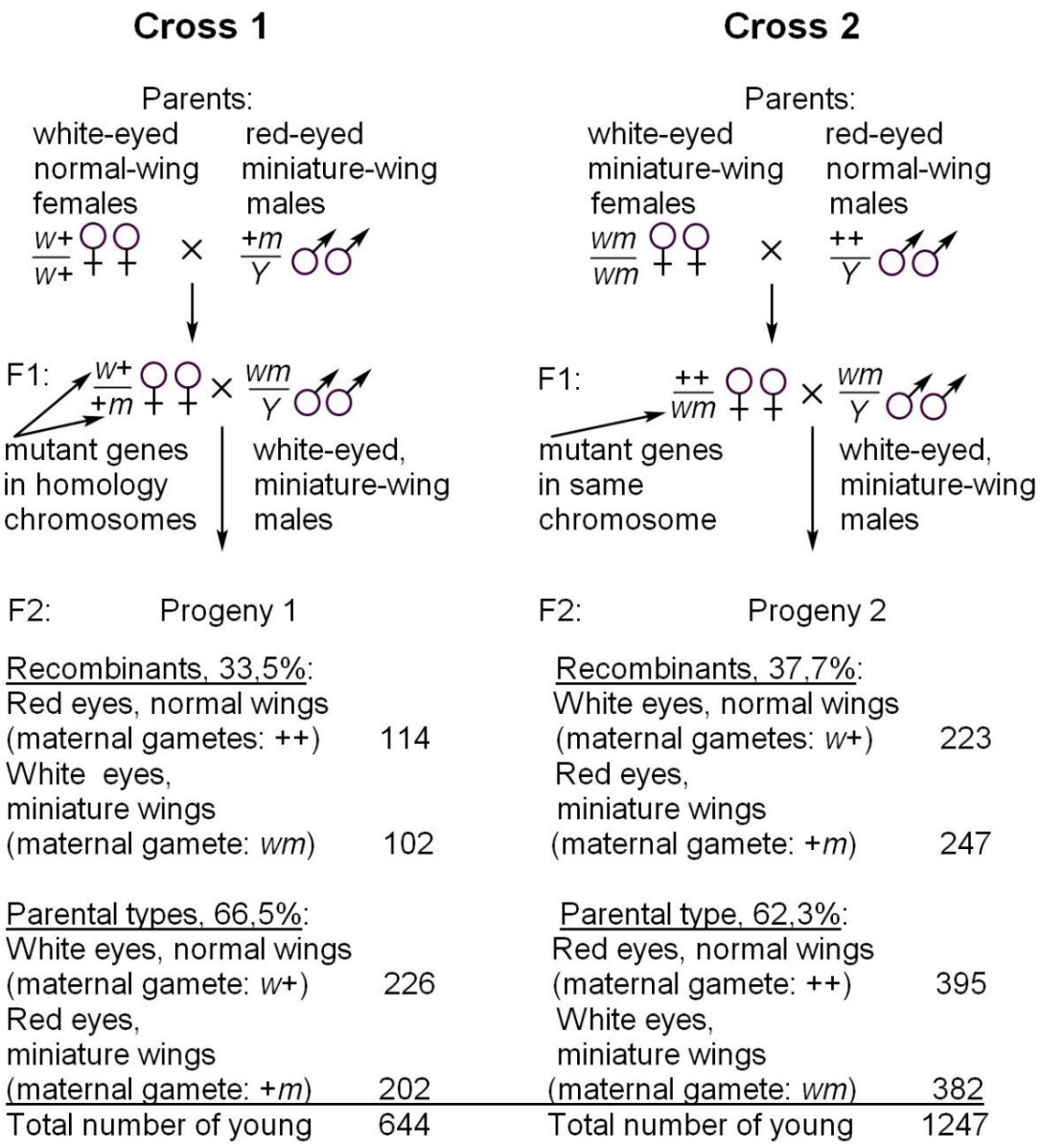


Fig. 2. Morgan's crosses show that number of recombinants is independent from mutant genes configuration on chromosomes pair. *By D.B. Pylypiv*

The COs are manifested by cross-like structures, the *chiasmata*, in prophase I of M. They were first found by F. Janssens at the Leuven University (Belgium) in 1909 [1]. The chiasmata (in Fig. 3 as X) are produced from breakage and rejoining of chromatids, but only in non-sister chromatids they may result in R, as COs in sister chromatids exchange identical parts. When CO occurs, the parts of chromatids are exchanged, providing alleles R (Fig. 3, A). If one CO is followed by another CO between the same chromatids, there is no R for marker genes, as second CO returns the markers to the previous place (Fig. 4, A). If loci of the genes are very close (< 7 cM), a double CO (DCO) is very rare [5]. The higher Ds increase the likelihood of a DCO. If the D between *A* and *B* is long enough, it allows more than two COs. When chiasma takes place outside the *A*-*B* region, e. g., between the gene *A* and centromere, the exchange by chr parts does not result in R, the genes remain in the same place (Fig. 4, *B*). During COs, the chromatids for exchange are joined by chiasmata randomly. No

Rcs are produced in DCO involving 2 chromatids (2-strand DCO); 2 Rcs are produced when 2 exchanges have 1 chromatid in common (3-strand DCO); 4 Rcs are produced, when after 1st CO the 2nd CO involves the chromatids, which were not participating in 1st CO (4-strand DCO).

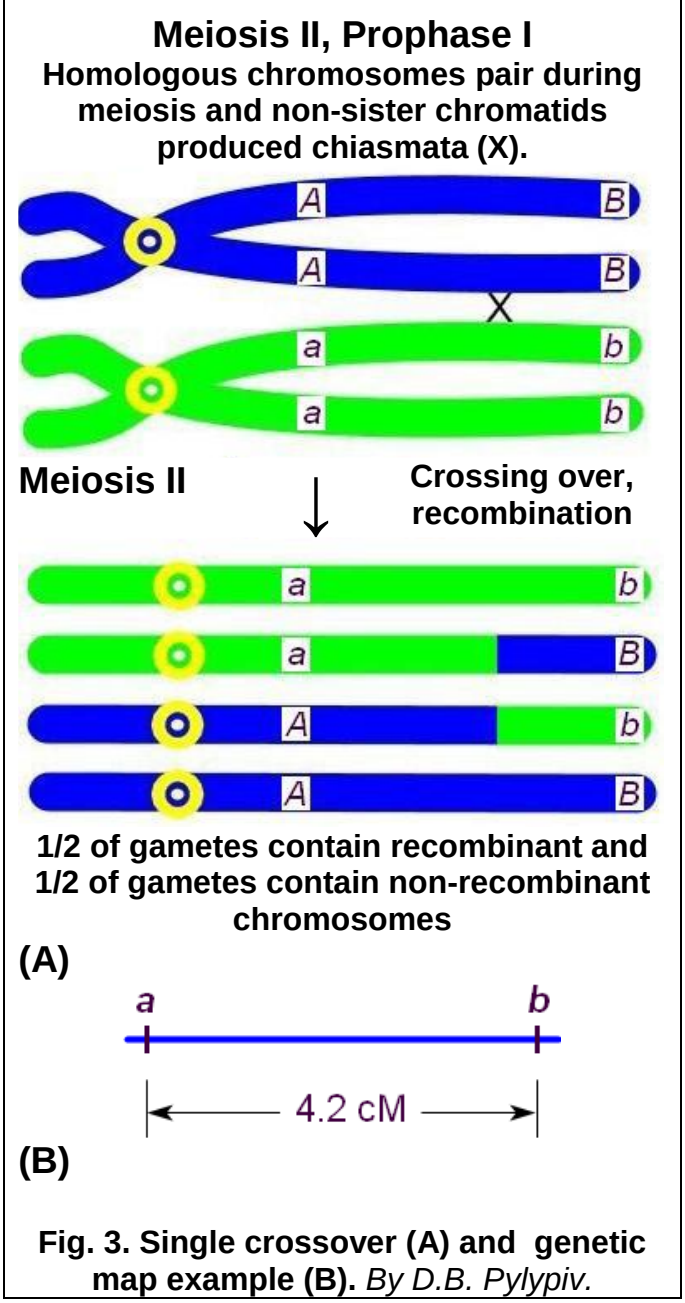


Fig. 3. Single crossover (A) and genetic map example (B). *By D.B. Pylypiv.*

The probabilities of 2-strand, 3- strand and 4-strand DCOs are 1/4, 1/2 and 1/4, respectively. The average of Rcs is: 1/4 × 0 + 1/2 × 2 + 1/4 × 4 = 2, i. e., on average 2 Rcs are produced from 2 chrs undergone DCO during M. As DCOs are undetected because of no R for particular marker genes, there is a difference in D measured in m. u. and by RF. The map D between two genes is a measure of CO. It equals half of the average number of COs that take place between 2 marker genes during M. The RF between the genes tells how much of Rs were detected, it is a measure of R. The difference between map D and RF appears due to DCOs that do not yield Rs, so do not contribute to RF, but do contribute to map D. The difference is important only if D between 2 markers is large enough to provide the possibility of DCOs. When D is too short to allow more than one CO, the RF and map units are the same, because there are no DCOs. On this basis 1 m.u. regarded as equal to 1% RF, because each CO at D < 7 cM results in R.

The 1 m.u. is a length of chr in which on average 1 CO happens per every 50 cells undergoing M. If 1 of 50 meiotic cells has a CO, the frequency of CO is 1/50 × 100% = 2%. It is in correspondence with 1% of RF, because only half of chromatids in each cell with COs are actually Rcs. The *CO frequency* of 2% means that 2 out of 200 chrs produced during M in 50 cells are Rc (Fig. 5). The 49 cells without COs produced 98 *ab* and 98 *AB* non-Rc chrs.

One cell with CO yields 2 Rc chrs (*aB* and *Ab*) and 2 chrs of Pr class (*AB* and *ab*). We can calculate the *r* as [8]:

$$r = \frac{1+1}{49+49+49+49} = \frac{2}{196} \approx 0.01 \approx 1\ map\ unit\ (m.u.) = 1\ centimorgan\ (cM).$$

The RF = 0.01 × 100% = 1%. Thus, 1% of RF means that 1 of 50 meiotic cells has a CO between the genes *A* and *B*. Independently from D between two genes, the maximum RF between them is 50% and this is found for genes located on largest Ds.

The 50% RF is observed in genes situated on non-homologous chrs that have IA also.

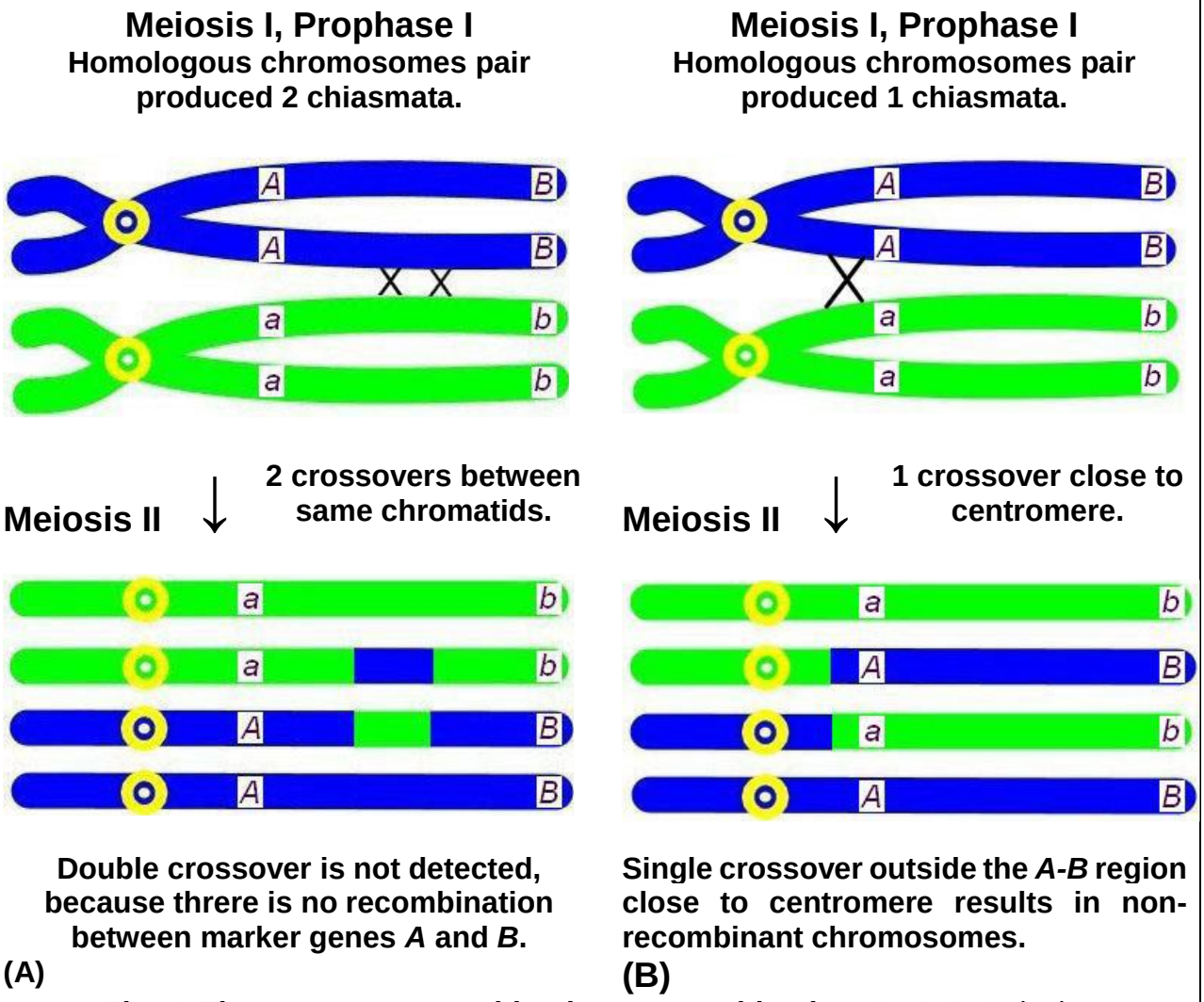


Fig. 4. The crossovers resulting in no recombination. *By D.B. Pylypiv.*

The P of multiply CO increases with increase of D between genes and this complicates the mapping, because some of the multiply COs do not result in R and go undetected. Thus, the true frequency of CO is higher than detected by Rcs number.

The Ds estimated on the basis of RF are additive approximately. For instance, if D between *a* and *b* is 4.2 m. u., the *b*-*c* region is 8 m. u., and the D *a*-*c* is 12.2 m.u., the gene *b* location must be between genes *a* and *c*.

To avoid the multiply COs effect, we must build the map on RF data for closely linked (≤ 10 m. u. apart) genes. If the Rc and Pr classes are 50%, the D = 50 cM. The correlation between physical distance and D is not strict. Physically chrs and orders of the genes on them are same, but the map Ds in chrs of two sexes may differ. The minimum RF is 0%. The D = 0 between any of two genes on chr of fruit fly males,

as there is no CO and R in this sex. These genes are in IA for the fly males.

Meiotic R differs in two sexes of most mammals [7, 9, 15, 19], e.g., humans. The genome-wide R levels are higher in females than in males, and the placement of exchanges varies between the sexes. The R differences in human sexes may derive from chromatin differences established prior to the onset of the R pathway [7].

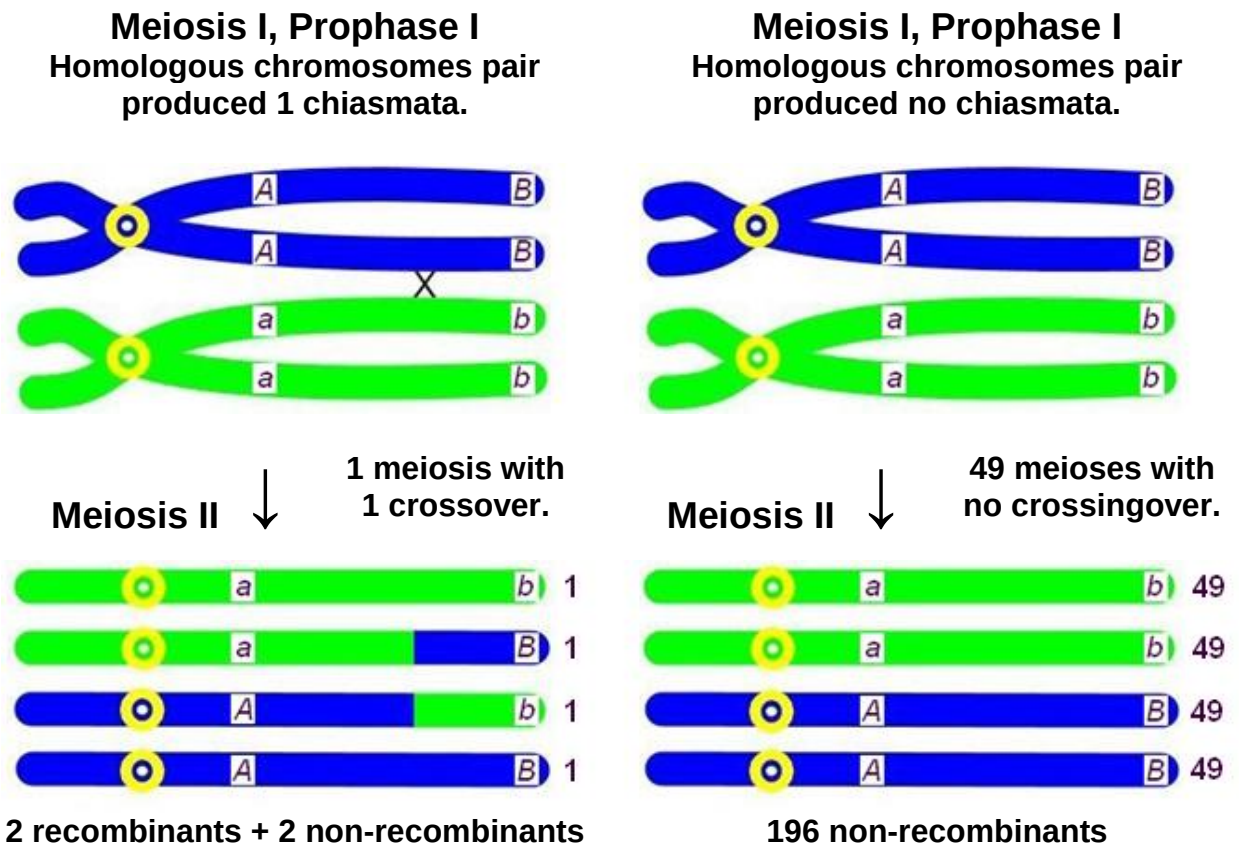


Fig. 5. Chromosomes outcome in 50 meiotic cells with 1 of them with single crossover. *By D.B. Pylypiv.*

The heterochromatin on flanks of centromere is poor for genes and has low frequencies of CO or R. The large physical distance here may correspond to 10 times smaller D in L map. The euchromatin has high CO and RFs, these parts of chr physical distances correlate better with Ds on L map. Note, even COs number decrease the D [8].

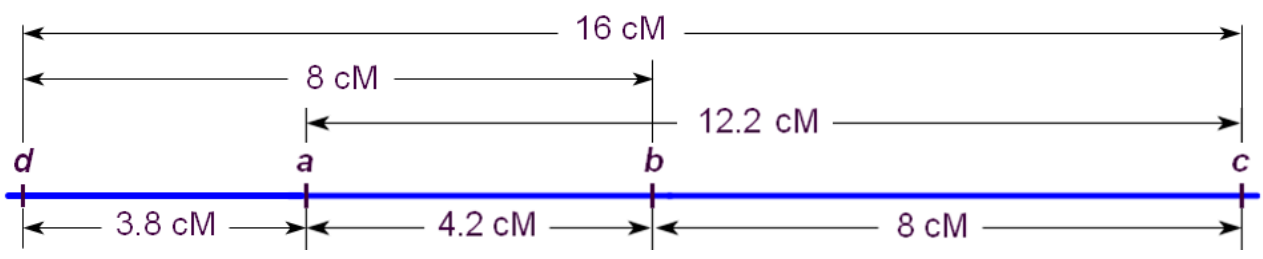


Fig. 6. Linkage map of *a*, *b*, *c* and *d* genes. *By D.B. Pylypiv.*

The L map on Fig. 6, shows only genes of interest. It is a line with 3 marks for *a*, *b* and *c* genes separated by particular Ds, e.g., 4.2 cM for *a*-*b*; 8 cM for *b*-*c* and 12.2 cM for *a*-*c*. The maps with *a* on the left, *b* in the middle and *c* on the right or in opposite orientation are right for these data. Having RF for an additional gene *d* we can find the true *a* and *c* sites. If RF between genes are 3,8 for *a*-*d*, 8 for *b*-*d*, 16 cM for *c*-*d*, the true genes order *dabc*. RF-based L map is not precise at large Ds.

Table 1. Two-point test-cross

Gene loci in test-cross	R frequency (%)
a and *b*, *a* and *c*, *a* and *d*	50
b and *c*	15
b and *d*	13
c and *d*	26

Two-point test-cross, the test-cross between 2 genes, allowing construction of L maps on the basis of their RF. Presume that two-point crosses for genes *a*, *b*, *c*, *d* yield the RFs shown in Table 1. The RF for each pair of genes *a*-*b*, *a*-*c*, *a*-*d* is 50%.

These pairs may therefore be on different chrs or on a large distance on the same chr. We must place them in separate L groups. The genes *b* and *c* or *b* and *d* are linked through the regions of 15 and 13 m. u. (Fig. 7), as they have RFs 15 and 13%, respectively.

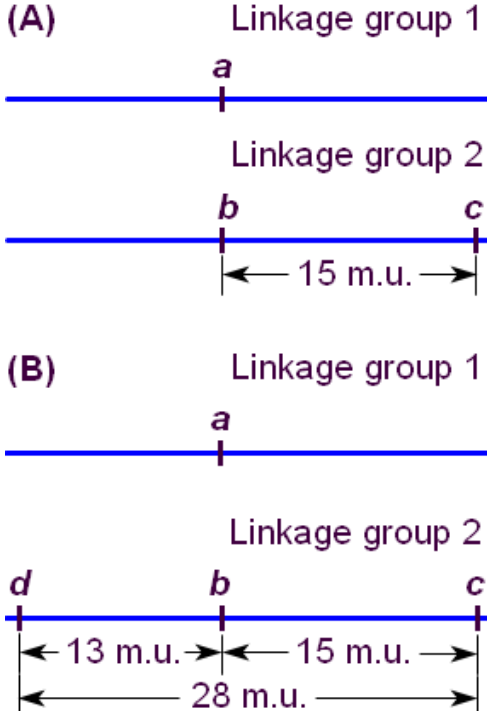


Fig. 7. Map construction.
By D.B. Pylypiv.

The *a* and *d* genes belong to different L groups, as their RF is 50%. To estimate, whether the *d* is 13 m.u. to the right or left of gene *b*, we need regard the *c*-*d* distance. If gene d is 13 m.u. to the right the *b*-*c* map fragment must be 2 m.u. This is not in compliance with the cross RF data. Thus, the gene *d* is 13 m.u. to the left of gene *b*, and genes *d* and *c* is 15 + 13 = 28 m.u. apart. The RF obtained for these 2 genes from the test cross is 26 m.u. The difference is due to DCOs without R between these genes. As they are not counted, the RF is underestimated. The rule is true: *RF is approximately additive*. The map from the two-point cross is not precise, as some DCOs are missed.

Three-point test-cross is a test-cross between three genes for mapping. The positions of the three genes are estimated in offspring, when SCOs and some DCOs are indicated, allowing more precise map Ds estimation. The cross *AABBCC* × *aabbcc* was performed. Next, the *F1 AaBbCc* female was test-crossed to an *aabbcc* male. Three CO variants are possible for *ABC* genes with Rc chrs production: SCOs (between *A* - *B* or *B* - *C*) and a DCO (between *A* - *B* and *B* - *C*), Fig. 8, *A*. The *G* composition of *F1* female reflects the progeny classes (Table 2), as male in test-cross is complete recessive homozygote. How to estimate the Ds and genes order?

First, determine the Prs, they are most frequent in progeny. Here the *ABC* and *abc* are Prs, as they are most numerous.

Second, find DCOs. They are always in the lowest number. Here *ABc* and *abC* types are in the lowest number. Remember, that in DCO progeny only the middle gene differs from non-Rcs. From Pr and DCO classes comparison (*ABc* and *ABC* or *abC* and *abc*), the *c* is "switched" as middle gene. The *acb* is true genes order and Fig. 8, A can be rearranged as Fig. 8, B.

Third, determine the L Ds *A-C* and *C-B* by dividing the total number of Rc *G*s onto the total number of *G*s including DCOs. The DCOs frequencies are used in the calculations of both Ds. So, the D between *A* and *C* is (68+70+3+2)/1000 × 100 = 14.3 cM and the D between C and B is (27+25+3+2)/1000 × 100 = 5.7 cM. Now, the L map can be constructed, that shows the Ds between the genes (Fig. 8, C). But, their precise position on chr and which chr they are on remains unknown.

Using cytogenetic analyses [20], the scientists correlate the L maps with particular chrs and with specific regions on them. We will learn these methods later.

Usually, the first CO affects the P of the next CO in the adjusted region. This interaction is called ***interference*** (*I*). If the COs in *A-C* and *C-B* regions are independent, the P of double Rs is a product of RFs in these regions. The *r* for d *A-C* is 0,143, and *r* value for *C-B* region is 0.057, so if DCO are independent, they are expected at the frequency: 0.143 × 0.057 = 0.0082. In the offspring of 1000 individuals 8 double Rs are expected: 0.0082 × 1000 = 8.2≈ 8. In progeny we have only 5 DCO Rs suggesting that two regions are not independent, they are interfering.

Table 2. Three-point test-cross results

Genotype	Observed	Total	True order	Gametes Type
ABC	403	1000	*ACB*	Pr
abc	402		*acb*	Pr
AbC	25		*ACb*	Single crossover between *C* and *B*
aBc	27		*acB*	Single crossover between *C* and *B*
ABc	2		*AcB*	Double- crossover
abC	3		*aCb*	Double- crossover
Abc	70		*Acb*	Single crossover between *A* and *C*
aBC	68		*aCB*	Single crossover between *A* and *C*

The interference (*I*) is evaluated by subtracting the coefficient of coincidence (*c.o.c.*) from 1:

$$Interference\ (I) = 1 - c.o.c.$$

The *c.o.c.* is a ratio of observed to expected DCO Rcs or ratio of their frequencies. For this experiment:

$$Interference\ (I) = 1 - \frac{Observed\ DCO\ number\ or\ RF}{Expected\ DCO\ number\ or\ RF} = 1 - \frac{5}{8} = 0.375 \text{ or } 37.5\%.$$

For regions, where never DCOs are observed, the c. o. c. = 0, thus *I* = 1 or 100%. In most cases the *I* values are between 0 and 1. However, in some studies observed DCO Rcs are more numerous than expected, and $I < 0$.

Human genes mapping is complicated by a small number of progeny in families and by the inability to have needed mating. Thus, data from several pedigrees are usually combined for analysis.

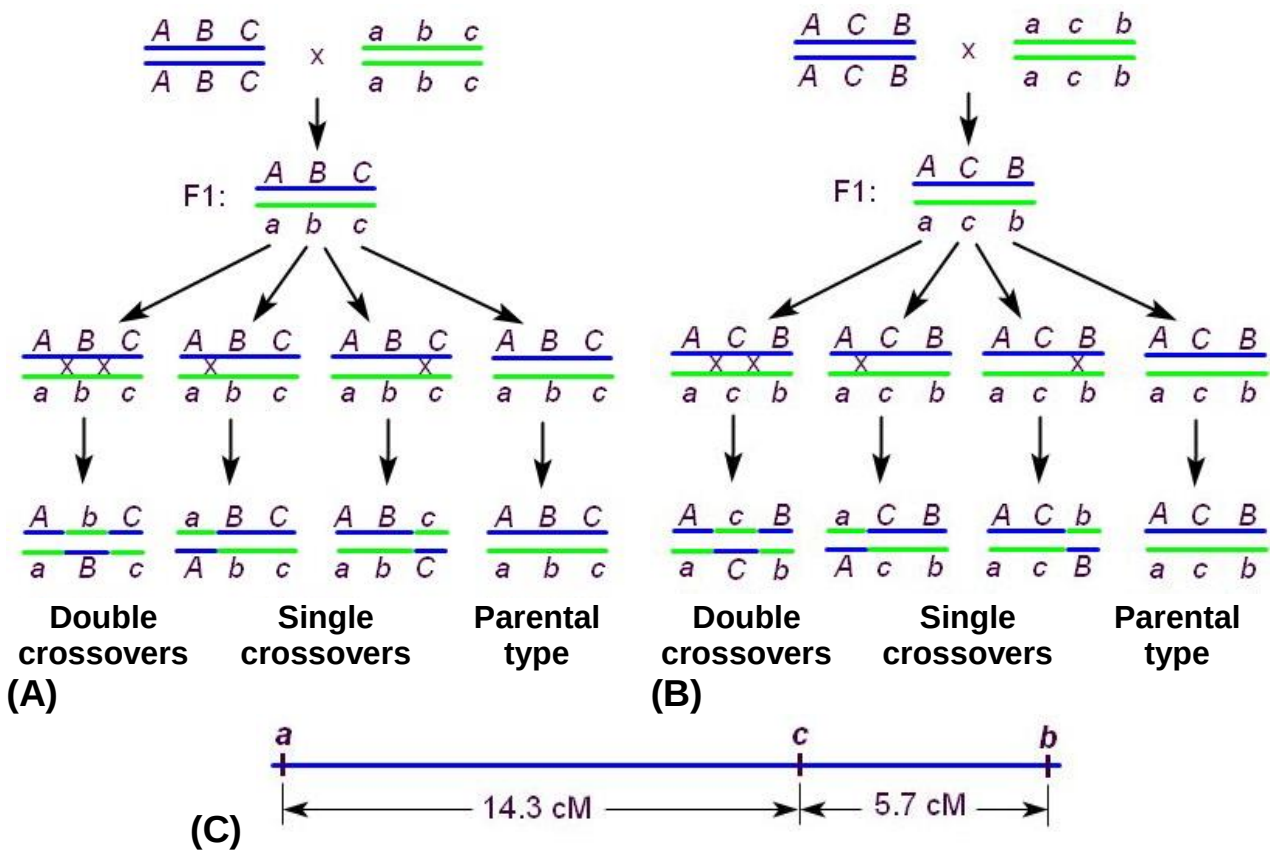


Fig. 8. Gametes at expected (A) and true order of genes (B) and L map derived in three point cross after true order of genes was estimated (C). *By D.B. Pylypiv.*

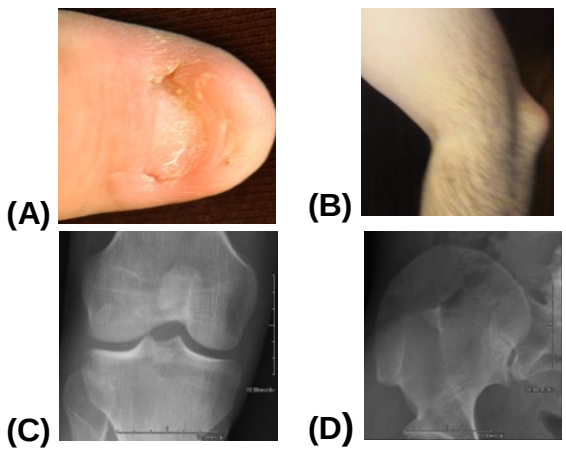


Fig. 9. Symptoms of nail-patella syndrome: Thumbnail (A), elbow (B) and AP radiographs of hypoplastic patella (C) and iliac crest with a bony exostosis or posterior iliac horn (D). *(A) By J.Wijten [9], (B) By J.Line [11], (C) and (D) by Jto410 [12,13].*

The L between the nail–patella syndrome (NPS) and ABO blood groups loci was among the first studied in humans. NPS is a rare dominant disease (2/100,000 births). The key features of NPS are poorly developed nails, patellae (kneecaps) and the presence of iliac horns (Fig. 9). NPS results from the loss of function or mutations in the *LMX1B* gene. This may result in dorsalising signals reduction, causing poor dorsal structures (e.g., nails and patellae) development [24]. Other abnormalities are: elbow deformities, abnormal shape of pelvic (hip) bones and kidney disease.

The ABO blood groups and NPS loci L were studied in pedigree with both traits

segregation. NPS is manifested, when at least 1 mutant allele N is present (Fig.10). The parent I1 is heterozygote (Nn). All diseased persons in generation II have either blood type B or O. In generation III sons 2 and 5 have a blood group A and NPS genotype and persons III1, III3 and III4 are healthy Rcs. So, there are 3 Rcs among 7 children in row III. This gives us $r = 3/7 = 0.4286$ and a D of 42.86 cM.

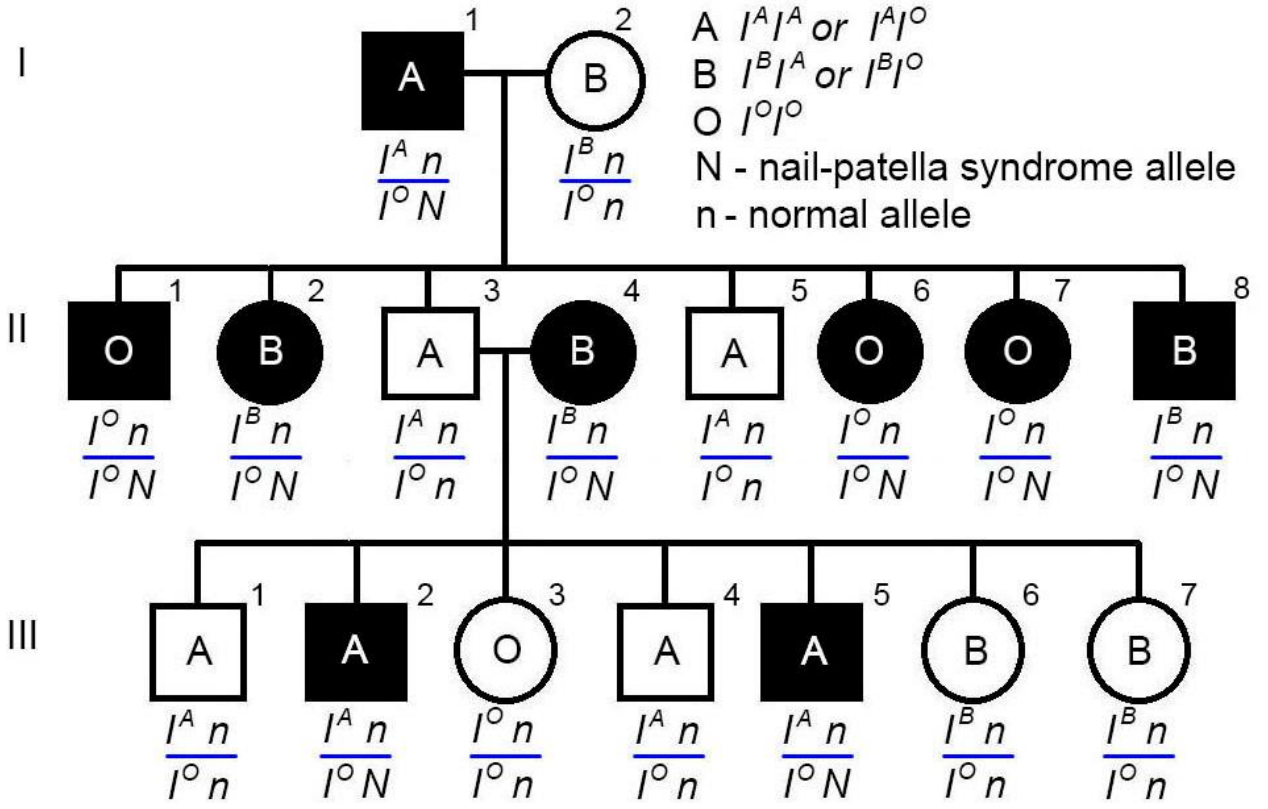


Fig. 10. ABO blood types and nail–patella syndrome L
ABO blood type and genotype are indicated inside and below each circle or square, respectively. The individuals 1, 3, and 4 in row III are recombinants. *By D.B. Pylypiv.*

We might conclude, that the loci for NPS and blood types are linked with the RF = 42.86% < 50% for IA. However, we must regard the possibility that the genes are in IA also. To verify their L, we can calculate the log_{10} of odds (*lod*) score. *First*, it is necessary to estimate the ratio of the P to have the observed results with L to the likelihood to have them with IA. *Second*, calculate the *log*10 of this ratio, the *lod score* [5]. The P of 2 Rcs types: 0.4286/2 = 0.2143. The P of 2 Pr types: (1- 0.4286)/2 = 0.2857. The P of a given birth (4 Prs + 3 Rcs) is the product of each of the independent events Ps: $(0.2857)4(0.\ 2143)3 = 6.6625 \times 10^{-3} \times 9.8416 \times 10^{-3} = 6.557 \times 10^{-5}$. If two loci are unlinked, the $r = 0.5$ and P of any genotype would be 0.25. The P of the birth sequence based on no linkage would be $(0.25)^7 = 6.1035 \times 10^{-5}$. Now, the ratio of the L likelihood and the non-L P is: $6.557 \times 10^{-5}/6.1035 \times 10^{-5} = 1.0743$ and the *lod* score is: $log_{10}1.0743 = 0.0311$. How to interpret the *lod* score? *Positive lod score supports a linkage. A lod score ≥ 3 is evidence for L.*

The not informative is *lod* = 0. The *lod* value ≤ - 2.0 is non-L evidence. The *lod* score >-2 and < 3, like in the case (Fig. 10), is inconclusive and more data is needed from additional families. On the lod score 0.0311 we can't decide, if NPS and ABO blood groups loci are linked or not. Usually, several pedigrees are used to get the estimates for *r*. *Lod* scores are additive across several pedigrees. The largest *lod* score is correspondent to one of *r* estimates and to most likely D.

Presume, the P of our results in L is 0.1 and the P of these with IA is 0.0001. So, the ratio 0.1/0.0001 = 1000 and the *lod* score = $log_{10}1000 = 3$. This tells, that is 10^3 times less likely to obtain these data in IA than from L.

The Ds are not additive in many cases. To avoid redoing the L map each time when new loci are found, the Ds are mapped using a *mapping function* (MF). The MF are *I*-dependent. With complete *I* or small Ds, a MF is: D = *r*. With no *I*, the Haldane MF is used, $D = - 1/2 \ln(1-2r)$, and given the map D, the $r = 1/2 (1-e^{-2D})$. Kosambi's MF allows some *I*: $D = 1/4 \ln[(1+2r)/(1-2r)]$ [22]. MFs of Haldane, Kosambi, Carter-Falconer or Morgan help to estimate the D in cM from *r* data more accurately [1, 2, 3, 4, 14, 22]. Precise Ds are important for the construction of large-scale L maps. Softwares, such as QTL Cartographer, Linkage, Mapmaker, etc., enable us to calculate different MFs and provide comparative analysis while producing L maps.

The genes-markers are coding for easily detectable phenotypic traits. The shortage of these traits in humans is among main obstacles for mapping.

Molecular markers mapping utilizes molecular biology methods, particularly, restriction fragment length polymorphisms (RFLPs), variable number of tandem repeats (VNTRs), single nucleotide polymorphisms (SNPs) and modern sequencing to provide wide variety of molecular markers and use them in L analysis similarly to use of traditional markers. About these and other molecular tools we will learn later.

Solved problems

Problem 1. Two homozygotes are crossed and the *F1* heterozygote is crossed back with the double recessive individual. The progeny was as follows: 510 *a+b+*, 490 *ab*, 45 *a+b* and 55 *ab+*. Estimate L between *a* and *b*. Draw the *a-b* L map.

<u>*Solution:*</u> The *a+b+* is wild, *ab* is mutant classes. Parents: *a+b+/a+b+* × *ab/ab*. *F1* back cross: *a+b+/ab* × *ab/ab*. The progeny of back cross: 410 *a+b+*, 490 *ab*, 45 *a+b*, 55 *ab+*. Prs: *a+b+* 410 + *ab* 490 = 900. COs: *a+b* 45 + *ab+* 55 = 100. Total offspring: 410 +4 90 + 45 + 55 = 1000. % of CO 100 /1000 × 100% = 10 %. L map for genes *a* and *b*: <u>*a* 10% *b*</u>.

Problem 2. White endosperm in corn (*p*) is recessive to purple (*P*) and shrunken (*f*) is recessive to the full (*F*) kernel. A pure white full plant was crossed to a pure purple shrunken. The *F1* is then test-crossed and the progeny was as follows: white full –557, purple shrunken – 443, white shrunken – 55, purple full – 45. Calculate the D between *p* and *f*. Draw a L map.

<u>*Solution:*</u> It is a two point test cross. Parents of the cross are *Pf* × *pF*. *Pr* – purple shrunken = 443, white full – 457. Rcs – white shrunken – 55, purple full – 45. Total offspring – 1000. % of CO between *p* and *f* = (55 + 45) ×100 /1000 = 10 %. L map = <u>*p* 10 % *f*</u> .

Problem 3. Forked bristles (*f*), miniature wings (*m*) and white eyes (*w*) are sex linked in *D. melanogaster* and recessive to the wild traits, the straight bristles (*f*+), long wings (*m*+), red eyes (*w*+). *F1* females from a cross *wfm/wfm* × *w*+*f*+*m*+ were mated with *wfm* males and gave the young, %: red, straight, miniature - 2.5; white, forked, long – 2.5; white, straight, miniature – 7.2; red, forked, long – 7.2; white straight long – 15,5; red, forked, miniature –15,5; white, forked, miniature – 25; red, straight, long – 25. Estimate Pr, SCO and DCO types. Verify the gene order. Determine the % of CO between the genes, the c.o.c. and *I*. Construct the L map.

Solution: Pr types are, %: white, forked, miniature *wfm* – 25 and red, straight, long, *w*+*f*+*m*+ – 25. The least numerous types are DCOs, %: white, forked, long, *wfm*+– 2.5 and red, straight, miniature, *w*+*f*+*m* - 2.5. From comparison of Pr and DCO types, the *wfm* and *wfm*+ or *w*+*f*+*m*+ and *w*+*f*+*m* we can see that *m* gene is 'switched' as a middle gene. So, the true position of genes is *wmf*. White long straight, *wm*+*f*+ - 15,5%. Red, miniature, forked, *w*+*mf* - 15,5%. % of SCO between white and miniature: 15.5 +15.5+ 2.5+ 2.5= 36. White, miniature, straight, *wmf*+ - 7.2%. Red, long, forked, *w*+*m*+*f* – 7.2 %. The % of SCO between miniature and forked: 7.2 + 7.2 + 2.5 + 2.5 = 19.4. Observed DCO = 5%. Expected DCO = 36/100 × 19.4/100 × 100 = 0.36 × 0.194 × 100 = 6.984%. C.o.c. = observed DCO /expected DCO = 5/6.984 × 100 = 71,59 ≈ 72%. Interference, *I* = 1- 0.72 = 0.28 = 28%, i.e., we noted 72% of DCOs that were expected and 28% of expected DCOs did not form due to *I*. L map: *w* 36% *m* 19.4% *f*.

Problem 4. The genes order *d–e–f* has *lod* = 0 and order *Ad–Ae–Af* has a negative *lod* score of -2. What does it mean?

Solution: That means that it is 10^2 times more likely to obtain the observed data from the first order of genes than from their second order.

Problem 5. A testcross *AaBb* × *aabb* produced in total 200 offspring of the following types: *AaBb* - 64, *aabb* - 76, *Aabb* - 29 and *aaBb* - 31. Provide conclusions concerning the L or IA of the genes.

Solution: To test for IA or L between two genes, we need to state the null hypothesis "*Luck of L*" which yields the ratio 1:1:1:1. Then, calculate a series of three *chi-square tests* (X^2 *test*) and estimate the probability *p*, that allows accept or reject the *null hypothesis* (H_0). From separate examination of each locus we can see that the observed numbers *(O)* differ from the expected numbers *(E)*. For locus *A* the cross *Aa* × *aa* was expected to yield 1/2 *Aa* and 1/2 *aa*. Instead for these we observed 64 + 29 = 93 of *Aa* and 76+31=107of *aa*. For locus *B* the cross *Bb* × *bb* was expected to yield 1/2 *Bb* and 1/2 *bb*. The *Bb* were produced less than expected 64+31= 95 and *bb* were observed in a larger quantity, than expected 76+29 = 105.

Applying the X^2 *test* to these *O* and *E* data, we calculate for locus *A*:

$$X^2=\sum\frac{(O-E)^2}{E}=\frac{(93-100)^2}{100}+\frac{(107-100)^2}{100}=\frac{49+49}{100}=0.98$$ and for the locus *B*:

$$X^2=\sum\frac{(O-E)^2}{E}=\frac{(95-100)^2}{100}+\frac{(105-100)^2}{100}=\frac{25+25}{100}=0.5.$$

The degree of freedom (df) for loci A the B is n - 1= 2 - 1 =1, where n, the number of classes equals 2, because 2 classes of progeny are expected for either of loci. Looking up chi-square values 0.98 and 0.5 in Table 3, we found that the calculated probability *p* associated with these X^2 values is above 0.05 (a critical probability for rejecting the null hypothesis) and calculated X^2 (0.98 and 0.5) are below the critical X^2 value 3.841 at the degree of freedom 1. So we can conclude that there is no significant difference between the 1:1 ratio that we expect in the offspring of the test-cross and the ratio that we observed.

Now we can test the independent assortment of genes at the two loci. The total number of progeny is 200 and 4 classes are produced, thus 50 individuals expected in each. The *O* and *E* numbers of progeny can now be compared in chi-square test:

$$X^2=\sum\frac{(O-E)^2}{E} = \frac{(64-50)^2}{50} + \frac{(76-50)^2}{50} + \frac{(29-50)^2}{50} + \frac{(31-50)^2}{50} = 33.48$$

Here, we have 4 classes of phenotypes; so the df = n-1 = 4-1 = 3. Calculated X^2 value 33.48 is above the X^2 value 7.815 at critical $p = 0.05$ and df = 3 (see Table 3). The *p* corresponding to calculated X^2 is much lower than 0.05. So, reject the null hypothesis and conclude that genes are not in IA and must be in L.

Table 3. Critical values of the X^2 distribution

p / df	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278

Problem 6. You are a medical geneticist in research on 3 rare recessive human mutations (*s, c* and *g*) which are also present in mice. One student has produced a genetic map of these genes (*s* 6.8% *c* 4.2% *g*) based on his cross of wild pure line animals with homozygous recessive for these traits. Thinking that the student was not accurate in his work, you have repeated his experiment. The progeny of your cross is *SCG* - 656, *scg* - 620, *Scg* - 83, *sCG* - 85, *scG* - 72, *SCg* - 68, *sCg* - 7, *ScG* - 9 (in total 1600). Is his map the same as yours?

Solution: Compare data of the studies and calculate the expected data based on the student's map. Your data are used as observed values in a X^2 test. Of 1600 progeny, 6.8% are Rcs between *S* and *C*: 1600 × 0.068 = 108.8 SCOs + DCO Rcs. The 4.2% are Rbs between *C* and *G*, thus 1600 × 0.042 = 67.2 SCOs + DCO Rcs. The DCO Rcs are: 1600 × 0.068 × 0.042= 2.5792 ≈ 4.6. Thus, *S-C* SCO Rcs are 108.8 – 4.6 = 104.2; *C-G* SCO Rcs are 67.2 – 4.6 = 62.6 and non-Rcs are: 1600 - 104.2 - 62.6 - 4.6 = 1428.6.

Table 4. Chi-square test result

Phenotype	O	Total	E	O-E	$(O-E)^2$	$(O-E)^2/E$
SCG	656	1600	714.3	- 58.3	3398.89	4.76
scg	620		714.3	- 94.3	8892.49	12.45
Scg	83		52.1	30.9	954.81	18.33
sCG	85		52.1	32.9	1082.41	20.77
scG	72		31.3	40.7	1656.49	52.92
SCg	68		31.3	36.7	1346.89	43.03
sCg	7		2.3	4.7	22.09	9.6
ScG	9		2.3	6.7	44.89	19.52
$X^2 = \Sigma(O-E)^2/E = 181.38$. Table 3: the critical $X^2 = 14.067$ at df 7 and $p = 0.05$. $X^2 = 181.38 > 14.067$ and $p < 0.05$. H_o is rejected.						

The calculated classes are 4 pairs of expected reciprocals. So, divide these numbers by 2. Now, use the X^2 test to see whether the calculated numbers are consistent with your data (Table 4). The null hypothesis (H_o) is: "*no significant difference between observed and expected values*". From your data, the SCOs between *S* and *C* loci are: *Scg* - 83 and *sCG* - 85. The SCOs between *C* and *G* loci are: *scG* - 72 and *SCg* – 68. The DCOs from the cross are: *sCg* – 7 and *ScG* – 9 animals. % of CO between *S* and *C*: (83+85+7+9)/1600×100% = 11.5%. % of CO between *C* and *G*: (72+68+7+9)/1600×100% = 9.75%. The L map for your data is: s 11.5% *c* 9.75% *g*. It is quite different from the student's map.

Problems for homework

Problem 1. The homozygous *Drosophila* line has the autosomal recessive genes *a*, *b*, *c* linked in that order. The *abc/abc* females were crossed with *a+b+c+/Y* males. Then the *F1* males were crossed with their *abc/abc* sisters with production of the F2 progeny: *a+b+c+* 555, *abc* 445, *abc+* 57, *a+b+c* 43, *ab+c+* 47, *a+bc* 44, *ab+c* 5, *a+bc+* 4. Give the RF between *a, b* and *c*, the c. o. c., *I* and a L map for *a, b* and *c*.

Problem 2. What are the genotypes in the pedigree with NPS, Fig.11? Calculate the *lod* scores and estimate the linkage relationship between NPS and ABO blood group loci.

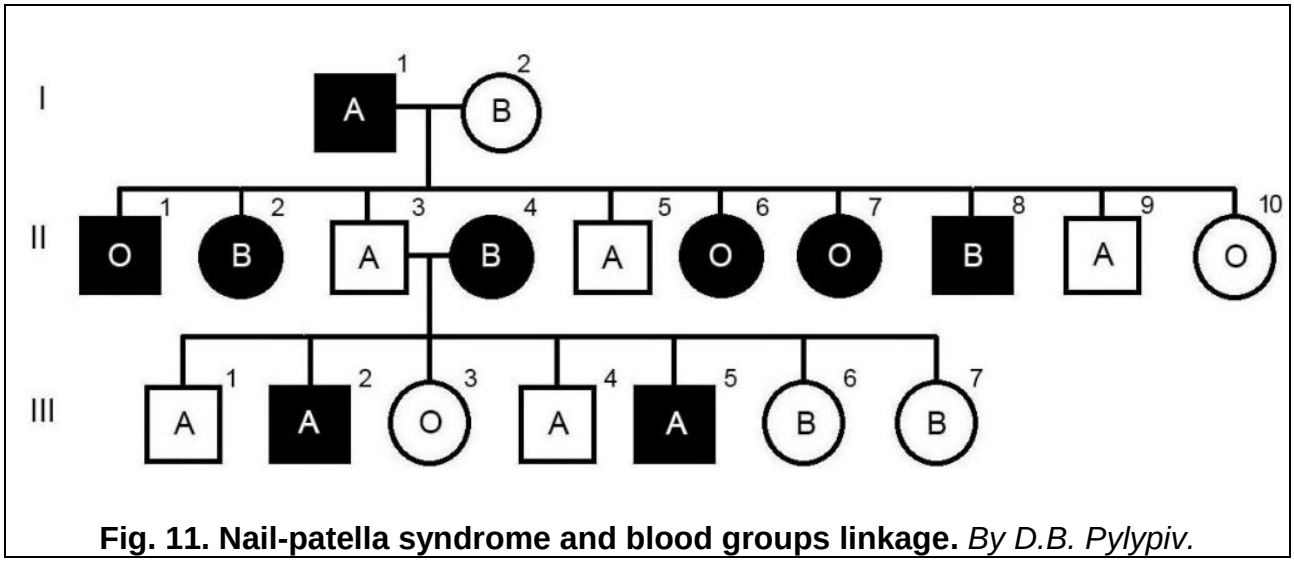


Fig. 11. Nail-patella syndrome and blood groups linkage. *By D.B. Pylypiv.*

Problem 3. A pure line wild fruit fly was crossed with a homozygous (*cu,* up curved wings), claret (*ca,* claret eye colour), fluted (*fl*, creased wings) fruit fly. *F1* females were test-crossed with production of offspring: fluted – 5, claret curled – 5, curled – 45, fluted claret – 55, claret – 182, fluted curled -178, fluted claret curled – 395, wild type – 405. Give the true genes order, c.o.c., *I*, map Ds between the genes.

Problem 4. In *B. mori* white-banded wing, *wb* and red eyes, *re* genes are recessive to wild *re*+ and *wb*+ genes, which are on the same chr. The insects *wb re* and *wb*+*re*+ are crossed. All *F1* have normal eyes and wings. The *F1* are test-crossed with *wb re* moths. The progeny is: 459 *wb*+*re*+, 14 *wb*+ *re*, 16 *wb re*+, 441*wb re*. What is the map D between *wb* and *re*? What proportions classes would be expected if the genes *wb* and *re* were located on different chrs?

Literature

1. Carter T.C. Falconer D.S. // J. Genet.- 1951.- Vol. 50.- P. 307–323.
2. Casares P.A. // Genet.- 2007.- Vol. 129. - N3.- P. 333-338.
3. Castle W. E. // Proc. Nat. Acad. Sci. USA, 1919.- Vol. 5, N2.- P.25–32.
4. Chhatre V.E. //http://www.crypticlineage.net/lib/Kosambi.pdf
5. Gogoi J., Tazid A. // IJIRSET.- 2015.- Vol. 4, N 8.- P. 6768-6772.
6. Griffiths A.J.F., et al. Introduction to Genetic Analysis.- 11th Ed.- N.Y., W.H. Freeman & Company, 2015.- 868 p.
7. Gruhn J.R. et al. //Plos One.-2013.-Vol. 8,N12–P.1.
8. Hartl D.L. Essential genetics. A genomics perspective. 6th ed.- Jones & Bartlett Learning, LLC.- New York.- 2014.- 572 p.
9. Hassold T. et al. //Cytogen. Gen. Res. - 2004.- Vol. 107, N3-4.- P. 249–55.
10. http://www.janwijten.com
11. https://commons.wikimedia.org/wiki/File:Nail-patella_syndrome_(NPS) Elbow1.JPG
12. https://en.wikipedia.org/wiki/File:Patella_radiograph_in_Nail_Patella_Syndr. jpg
13. https://en.wikipedia.org/wiki/File:Posterior_iliac_horn.jpg
14. Kosambi D.D. The estimation of the map distance from recombination values//Ann. Eugen.- 1944.- Vol. 12.- P.172–175.
15. Lenormand T. //Genet.-2003.-Vol.163.-P. 811.
16. Morgan T. H. // Z. Indukt. Abstam-mungs. Vererbungsl.- 1912.- N7.- P.323-345.
17. Morgan T.H. // *J. Exp. Zool*.- 1912.- Vol. 13.- P.79.
18. Morgan T.H., et al. The mechanism of mendelian heredity.- Henry Holt & Co, N.Y., 1915.-262 p.
19. Petko P.M., et al. //Trends Genet.- 2007.- Vol.23.- P.539.
20. Ruddle F.H., Kucherlapati R.S. //Sci. Amer.-1974.-Vol.231,N1.- P.36 –44.
21. Sturtevant A.H. //J. Exp. Zool.- 1913. – Vol.14.- P.43–59.
22. Tan Y. D. , Fornage M. // Genet.- 2008.- Vol.133.- P. 235-246.
23. van der Werf J. Ch 5. Basics of linkage and gene mapping.– P.45-54. https://jvanderw.une.edu.
24. Wright M.J. et al. // J. Med. Genet.- 2000.- Vol. 37, N9.- P.25.

Practical 7. Hardy–Weinberg equilibrium.

Theoretical background. Genetic variation is constantly observed in species natural populations, including human. The variation is rearranged in new patterns in each new generation due to sexual reproduction, in which the chromosomes (chrs) from parents recombine during gametogenesis and make new genotypes in fertilization for offspring. However, heredity itself does not change gene frequencies [12].

The German doctor W.Weinberg, and British mathematician G.H.Hardy published a ***principle of genetic equilibrium*** independently in 1908. It is also known as the *Hardy–Weinberg equilibrium* (HWE), *law, model, principle* or *theorem*, the cornerstone of population genetics [5, 7, 8, 12, 13]. It states: allele and genotype frequencies in a population are constant from one generation to next in the absence of evolutionary pressure. Such evolutionary influences as *natural selection, mate choice, sexual selection, assortative mating, disassortative mating, genetic drift, founder effect, inbreeding, population bottleneck, gene flow, genetic hitchhiking, mutation, meiotic drive* change the allele and genotype frequencies. As we are learning population genetics now, let us describe shortly all of these influences.

Natural selection is the differential survival and reproduction of individuals due to differences in genotype. It was well described by Charles Darwin in 1859 [4], however, without needed genetic substantiation yet.

Sexual selection is a kind of ***natural selection*** in which members of one biological sex choose mates of the other sex to mate with (*intersexual selection*), and compete with members of the same sex for access to members of the opposite sex (*intrasexual selection*). The theory of sexual selection was first launched by C. Darwin also. It found math-proof in fruit flies mating experiments carried out by A.J.Bateman [2].

Mate choice is a kind of natural selection when an animal can evaluate a potential mate for quality and respond by behavior [3].

The ***assortative mating*** (*homogamy* or *positive assortative mating*) is a mating pattern and form of sexual selection in which mating between individuals with similar phenotypes is more frequent than expected under a random pattern [1, 15, 16]. As a rule, people prefer assortative mating (marriage within the same class, race, ethnic and religion group, similar body size, etc.). It *increases* genetic relatedness. As opposed to this, ***disassortative mating*** (*heterogamy* or *negative assortative mating*) in which mating between individuals with different phenotypes (*e.g.*, by human body size, race, ethnic group) is more frequent than would be expected under random mating. It *increases* genetic diversity [1].

Genetic drift (GD), the *Sewall Wright effect* or *genetic sampling error*, a change in the *gene pool of a small population* that takes place strictly by chance. GD can result in genetic traits being lost from a population or becoming widespread in a population *(change in allele frequency)* without respect to the survival or reproductive value of the alleles [10]. GD may cause alleles to disappear and thereby reduce genetic variation or can increase the frequency of rare alleles and allow them to be even fixed [22]. GD occurs only in small, isolated populations in which the gene pool is small enough that chance events can change its makeup significantly. In large populations, each allele is

present in so many individuals that it is almost certain to be transmitted unless it is biologically unfavourable [10].

Founder effect (FE) is the genetic variation loss when a new population is established by a very small number of individuals from a larger population. It was first fully described by E. Mayr in 1942 [17], based mainly on work by S. Wright [23]. The FE may result in new populations genotypically and phenotypically different from original or in extremum cases, leads to new species development [14].

Gene flow (GF) (*gene migration or allele flow*) prevents the founder effect from occurring. The GF is the transfer of genes from one population to another. If the rate of GF is high, then 2 populations will have equivalent allele frequencies and can be considered a single effective population. It is enough "one migrant per generation" to prevent populations from diverging [7]. Populations can diverge due to selection even when they are exchanging alleles, if the selection pressure is strong enough [9, 21].

Mutation is a change in DNA sequence of the genome of an organism, virus or extrachromosomal DNA. Most of the mutations are not useful [12].

Population bottleneck or *genetic bottleneck* is a drastic decrease in a population size due to disaster (*e.g.*, epidemy, droughts, floods, fires, earthquakes) or human activities. These reduce the variation in the population gene pool. The smaller population, with reduced genetic diversity, pass on genes to future generations [24].

Meiotic drive is any process which causes some alleles to be over-represented in the gametes during meiosis. According to Mendelian equal segregation of alleles into gametes, approximately half of progeny inherit one of the alleles and the other half the another allele of the gene. Some times this rule is violated, and one of the alleles is found in more than half the offspring. The segregation distorter gene of *D. melanogaster* is an example [18].

Genetic hitchhiking, *genetic draft* or the *hitchhiking effect* **[**20**]**, is the changes in allele frequency not due to natural selection, but because it is situated on the same DNA chain near another gene that is undergoing a selective sweep. Any other nearby polymorphisms (gene variants) that are in linkage disequilibrium (non-random association of alleles at different loci) will tend to change their allele frequencies too [8]. Selective sweeps happen when newly appeared (and hence still rare) mutations are advantageous and increase in frequency. Neutral or even slightly deleterious alleles that happen to be close by on the chr 'hitchhike' along with the sweep.

Effects on a neutral locus due to linkage disequilibrium with newly appeared deleterious mutations are called ***background selection***. Both genetic hitchhiking and background selection are random evolutionary forces, like genetic drift [11].

Inbreeding is the production of progeny from the mating of genetically closely related organisms. The term is used in breeding of animals or plants and in human genetics, when harmful effects arising from expression of recessive traits in progeny from incest and consanquinity marriages are regarded [12]. Relative (1st cousins) marriages increase the frequency of diseased homozygotes. Gipsy study in Slovakia reports very high incidence of phenylketonuria, 1: 40 births, as result of inbreeding [6].

Hardy–Weinberg law states that in “population in equilibrium”, *i.e.*, with no evolutionary changes noted above, the genotypes present in frequencies that are a

simple function of the frequencies of alleles: the square expansion of the sum of the allelic frequencies [5, 12].

For alleles *B* and *b* at ***b*** gene locus, 3 genotypes are possible: *BB*, *Bb*, and *bb*. If *p* and *q* are frequencies of the *B* and *b*, respectively, the equilibrium frequencies of the 3 genotypes are: $(p + q)^2 = p^2 + 2pq + q^2$ for *BB*, *Bb*, and *bb*, respectively. Using square expansion of the sum of the allelic frequencies, the genotype equilibrium frequencies for any number of alleles can be calculated. Table 1 demonstrates how the principle functions with 2 alleles: *B* and *b*. Here *p(B)* and *q(b*) are the frequencies of 2 alleles *B* and *b* of parental generation.

Table 1. A Hardy-Weinberg low applied on 2 alleles

Gametic frequencies in parents	*p(B)*	*q(b)*
p(B)	p^2*(BB)*	*pq(Bb)*
q(b)	*pq(Bb)*	q^2*(bb)*

Probabilities of the 3 possible genotypes in the following generation are products of the probabilities of the corresponding alleles in the parents: p^2*(BB), 2pq(Bb),* q^2*(bb).*

The allele frequencies in equilibrium populations are the same through generations. The frequency of allele *B* in next generation is a sum of frequency of *BB* genotype and ½ of the genotype *Bb*, because only half of the alleles in these individuals is B: $p^2 + pq = p(p + q) = p$ (because $p + q = 1$). By analogy, the frequency of allele *b* in progeny is $q^2 + pq = q(q + p) = q$. These are exactly the same as frequencies of the alleles in the parents. The random mating takes place in HWE population. The probability of particular mating is the same as the frequency of the genotypes of the individuals in pairs. For instance, the probability of mating $BB \times bb$ is a product of p^2 (the frequency of *BB*) and q^2 (the frequency of *bb*). Even if the mates are chosen by particular traits, by all the rest of alleles the mating is random. This is true for the human population also. We select our partners by personality, sexual attraction, etc., however, concerning most genes, people mate randomly.

We need 2 formulas for solving a HWE problems: $p^2 + 2pq + q^2 = 1$ and $p + q = 1$, where p^2 and q^2 are the dominant and recessive homozygotes frequencies, *p* and *q* are the dominant and recessive alleles frequencies, respectively. The *2pq* is the heterozygous genotype individuals frequency.

If we know the frequency of homozygous dominant or recessive alleles in the population, it is possible to calculate the gene frequency and the gene distribution into 3 variants of possible genotypes. In a population with *equilibrium* the genes frequencies are not significantly altered from one generation to next. Thus, knowing the genes frequencies of one generation, we can predict the frequencies of the next. The equation, $p^2 + 2pq + q^2$, is based on the G. Mendel's ratio 1:2:1. Similarly, for 3 alleles, B_1, B_2, and B_3, with frequencies *p*, *q*, and *r*, the equilibrium frequencies corresponding to the 6 possible genotypes (shown in brackets) will be calculated as:

$$(p + q + r)^2 = p^2(B_1B_1) + q^2(B_2B_2) + r^2(B_3B_3) + 2pq(B_1B_2) + 2qr(B_2B_3) + 2pr(B_1B_3).$$

HWE presumes that the population is large enough, the frequencies of genes remain constant from one generation to the next, because there are no evolutionary effects, which may change them. However, note, such a population does not exist in nature, because evolution is always present, everywhere, including human population.

The effects of evolutionary processes onto gene frequencies can be measured starting from HWE as a baseline.

Solved problems

Problem 1. According to Scriver and Kaufman 2001 [19], the incidence of rare recessive autosomal disease phenylketonuria (PKU) in Australia is 1 in 10 000 births. Calculate the relative frequencies of genotypes and connect the rareness of the disease with the presence of carriers and healthy homozygotes in the population of Australia.

Solution: Relative frequencies of genotypes in a population can be obtained by assuming that the population is in a HWE ($p^2 + 2pq + q^2 = 1$). Let us now use the symbol "*a*" for PKU allele and "*A*" for healthy allele in analysis of 3 possible genotypes. We can presume p^2 for *AA*, *2pq* for *Aa* and q^2 for *aa*. From the proportion of FKU incidence above, frequency q for recessive PKU allele is $\sqrt{0.0001}=1/100$ and frequency of p, the dominant allele is 1-1/100 = 99/100. The heterozygotes frequency, $2pq = 2 \times 99/100 \times 1/100 = 0.0198 = 198/10\,000$. Thus, healthy carriers of FKU genes (the heterozygotes *Aa*) are 198 times more frequent among Australians, than people with disease, the recessive homozygotes *aa* for FKU genes hence, proportion of healthy homozygotes p^2 can be calculated as 1- (0.0001+0.0198) = 0,9801. Now we can write proportions of healthy homozygotes, heterozygous carriers and PKU people genotypes among Australians as follows: 98.01% p^2 (*AA*) + 1.98% *2pq* (*Aa*) + 0.01% q^2 (*aa*).

Problem 2. In one generation of the population on remote islands the frequency of allele for recessive disease is $q = 0.7$ and frequency for dominant allele $p = 0.3$. In the next generation of 100 people 51 are healthy individuals and the rest are diseased. Is this population in HWE?

Solution: The frequencies of alleles and genotypes remain the same through generations in HWE. If frequency remains the same for one of the alleles, it remains the same for all other alleles. The genotype proportions will remain constant also. Hence, there is enough to estimate the same frequency in one of the alleles, to conclude that the population is in the equilibrium. The number of diseased individuals is 100 – 51 = 49, thus $q^2 = 0.49$ and $q = \sqrt{0.49} = 0.7$, the same as in previous generation. The population is in HWE.

Problem 3. Consider a village population in which 25% are unable to roll the tongue (recessive trait). Estimate the frequencies of non-rollers, carrier-rollers and homozygotic rollers.

Solution: We deal here with recessive trait, and 25% of villagers are recessive homozygotes. Let us mark their genotype as *rr*. The task is to calculate the *R* and *r* alleles frequencies and then estimate the proportion of the genotypes *RR* (homozygotic rollers) and *Rr* (rollers-carriers) in this population.

Let us use the p to symbolize the frequency of the *R* allele, and the q to indicate the proportion of the *r* allele. For these alleles we can write: $p + q = 1$. If 25 people out of 100 are recessive homozygotes unable to roll the tongue, their frequency is 25/100 = 0.25. Presuming in HWE the q^2 as *rr*, we can write $q^2 = 0.25$. Hence, the $\sqrt{0.25} = 0.5$ is a frequency of the q, $q = r = 0.5$. Because the frequencies of 2 alternative alleles always add up to 1, the proportion of allele *R* is: $p = 1 - q = 0.5$. From here, the

population contains 50% of dominant allele *R* and 50% of recessive allele *r*. Table 2 shows how these alleles combine in possible genotypes, the rollers, rollers-carriers and non-rollers.

Alternatively, using the Hardy-Weinberg law equation it is now possible to calculate the proportion for all genotypes:

Table 2. The proportions of possible genotypes for population

Parental allele frequency	$p\boldsymbol{R} = 0.5$	$q\boldsymbol{r} = 0.5$
$\boldsymbol{R} = 0.5$	$p^2\,\boldsymbol{RR} = 0.25$, homozygous rollers	$pq\,\boldsymbol{Rr} = 0.25$, heterozygous rollers
$q\boldsymbol{r} = 0.5$	$pq\,\boldsymbol{Rr} = 0.25$, heterozygous rollers	$q^2\,\boldsymbol{rr} = 0.25$, homozygous non-rollers

$p^2 + 2pq + q^2 = 1$; 0.25 *RR*+ 0.50 *Rr* + 0.25 *rr* = 1 or 25% *RR* + 50% *Rr*+ 25% *rr*.

All things considered, the population consists on 25% of dominant homozygotic tongue rollers, on 50% heterozygotic tongue rollers and on 25% of homozygous recessive non-rollers, with a gene frequencies of 50% dominant and 50% recessive alleles for the gene locus controlling the ability to roll the tongue.

Problem 4. The ability to taste phenylthiocarbamide (PTC) is a dominant trait inherited as a mendelian character. The population has 36% of people unable to taste this chemical. Calculate the frequencies of the dominant and recessive alleles for this trate and the proportions for all possible genotypes.

Solution: Let us use the symbol *q* to indicate the proportion of the *t* allele for inability to taste the PTC and letter *p* for the frequency of the *T* allele, conferring to people the ability to taste the PTC. Thus, the proportion of non-tasters (*tt*) is 0.36 and frequency of the allele *t* is: $q = \sqrt{0.36} = 0.6$. The frequency of the allele *T* is: $p = 1 - 0.6 = 0.4$. Using the HWE $p^2 + 2pq + q^2 = 1$, we calculate the proportions of genotypes as 0.16 *TT* + 0.48*Tt* + 0.36*tt* = 1 (Table 3). Hence, regarded population comprised by 16% of dominant tasters-homozygotes, 48% of tasters-heterozygotes and 36% of homozygous recessive non-tasters, with the frequency of 40% for dominant and 60% for recessive alleles of the gene regulating the ability to taste PTC.

Table 3. The proportions of possible genotypes

Parental allele frequency	$p\boldsymbol{T} = 0.4$	$q\boldsymbol{t} = 0.6$
$p\boldsymbol{T} = 0.4$	$p^2\,\boldsymbol{TT} = 0.16$, homozygous tasters	$pq\boldsymbol{Tt} = 0.24$, heterozygous tasters
$q\boldsymbol{t} = 0.6$	$pq\boldsymbol{Tt} = 0.24$ heterozygous tasters	$q^2\,\boldsymbol{rr} = 0.36$ homozygous non-tasters

Problem 5. During one study in West Europe the following ABO blood group phenotypes were observed within the population of 193311 people: O - 90123, A-80016, B-17160, AB - 6012. Analyze these data with use of HWE equations.

Solution: Here for a gene locus we have three alleles O, A and B, which determine the presence or absence of particular antigens on the surfaces of erythrocytes and antibodies into plasma. For three alleles we can use trinomial square expansion:
$(p + q + r)^2 = p^2 + q^2 + r^2 + 2\,pq + 2\,pr + 2\,qr$ and $p + q + r = 1$, where the *p*, *q* and *r* are allele frequencies of I^A, I^B, I^O, respectively. Thus, the genotype frequencies are: p^2 for I^AI^A, q^2 for I^BI^B, r^2 for I^OI^O, 2 *pq* for I^AI^B, 2 *pr* for I^AI^O and 2 *qr* for I^BI^O.

Table 4. Results of genotype proportions calculation

Phenotype	*Genotype*	*Frequency*	*Genotype proportion*
O (I)	I^OI^O	r^2	90123 : 193311 = 0.4662
A (II)	I^AI^A and I^AI^O	$p^2 + 2\,pr$	80016 : 193311 = 0.4139
B (III)	I^BI^B and I^BI^O	$q^2 + 2\,qr$	17160 : 193311 = 0.0888
AB (IV)	I^AI^B	$2\,pq$	6012 : 193311 = 0.0311
Total phenotypes observed:			193311

Now we can start with frequency of I^O allele: $r = \sqrt{0.4662} = 0.6828$. Then, $(p+r)^2 = p^2 + 2\,pr + r^2$. The $p^2 + 2\,pr = 0{,}4139$ and $r^2 = 0.4662$, hence, $(p+r)^2 = 0.4139 + 0.4662 = 0{,}8801$ and $p + r = \sqrt{0.8801} = 0.9381$. Now $p = (p + r) - r = 0.9381 - 0.6828 = 0.2553$. Finally, q can be calculated as a mathematical difference: $q = 1 - (p + r) = 1 - 0.9381 = 0.0619$. Now we can calculate all genotype proportions (Table 5).

Table 5. Results of frequencies calculation

Genotype	*Algebraic frequency*	*Calculation*	*Genotype frequency*
I^OI^O	r^2	observed	0.4662
I^AI^A	p^2	$(0.2553)^2$	0.0652
I^BI^B	q^2	$(0.0619)^2$	0.0038
I^AI^O	$2\,pr$	$2 \times 0.2553 \times 0.6828$	0.3486
I^BI^O	$2\,qr$	$2 \times 0.0619 \times 0.6828$	0.0845
I^AI^B	$2\,pq$	$2 \times 0.2553 \times 0.0619$	0.0316

Problem 6. The blood type study in one population among 741 people revealed the next blood phenotypes: *M* – 620, *MN* – 111 and *N* – 10 individuals. Are these data in Hardy-Weinberg equilibrium?

Solution: Let us first calculate the allele frequencies at the *MN* locus in population:

$$p = M = \frac{(620\times2) + 111}{741\times2} = 0.9116, \quad q = N = \frac{(10\times2)+111}{741\times2} = 0.0883.$$

Now the genotype frequencies can be calculated and expected numbers of phenotypes (people with a particular blood group) in population of 741 individuals can be determined:

$p^2 = MM = (0.9116)^2 = 0.8310$, then $0.8310 \times 741 = 615.77$

$2pq = MN = 2 \times 0.9116 \times 0.0883 = 0.1610$, then $0.1610 \times 741 = 119.3$

$q^2 = NN = (0.0883)^2 = 0.0078$, then $0.0078 \times 741 = 5.7798$.

Now we can use these figures for Chi square goodness of fit test.

Table 6. Calculation of Chi-square for MN blood groups in the population

Phenotypes	*Observed (O)*	*Expected (E)*	*Residual (O - E)*	*(O - E)²*	*Component (O - E)²/E*
MM	620	615.77	4.23	17,29	0.0281
MN	111	119.3	- 8,3	68,89	0.5774
NN	10	5.7798	4.2202	17,81	3.0814
$\chi^2 = \Sigma(o - e)^2/e = 3.6869$					

The degree of freedom (DF) = number of categories - 1. We have in the task 3 phenotypes and 2 alleles, hence, the degree of freedom (DF) is 3 - 2 =1. The critical χ^2 value at the 5% level of significance at DF = 1 is 3.84146 (Table 7).

Table 7. Critical value for χ^2 test

Area / *DF*	0.900	0.750	0.500	0.250	0.100	**0.05**
1	0.01579	0.10153	0.45494	1.32330	2.70554	**3.84146**
2	0.21072	0.57536	1.38629	2.77259	4.60517	5.99146
3	0.58437	1.21253	2.36597	4.10834	6.25139	7.81473
4	1.06362	1.92256	3.35669	5.38527	7.77944	9.48773
5	1.61031	2.67460	4.35146	6.62568	9.23636	11.07050

The chi square value χ^2 of 3.6869 calculated for this population does not exceed the critical value yet. The deviations between observed and expected values are not significant, the null hypothesis (H_0) is true. Thus, *MN* blood group alleles and genotypes are in HWE in this population.

Problems for homework

Problem 7. The X-linked recessive trait is manifested in 25% of all the males. Analyse this information with HWE.

Problem 8. In the study of remote island isolated by ocean the following MN blood types were found in total 200 of inhabitants: M- 121 MN-43 N-36 What is the frequency of M and N allele in this population?

Problem 9. Albinism is a recessive condition for no melamine production into skin and hair. In a community of 100 people 16 are albinos. How many are healthy homozygotes and carriers?

Problem 10. In certain community the frequency of recessive disease is 1/100. Suppose 6000 marriages take place on an average each year. How many couples will have the potential of producing offspring with this disease?

Problem 11. Presume that the population of the town is isolated from the rest of the world and consists on 70% of the white and 30% of black-skinned people. Expecting random mating in this equilibrium population, predict the percentage of interracial and same race marriages.

Literature

1. Assortative mating. Encyclopaedia Britannica.- https://www.britannica.com/science/assortative-mating.
2. Bateman A.J. // J. Hered. 1948.- Vol.2 (Pt. 3).- P. 349–68.
3. Bateson P. P. G. Mate Choice. - Cambridge University Press, 1985.- 462 p.
4. Darwin C. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. – John Murray, London, 1859.
5. Dynamics of genetic change. Genetic equilibrium: the Hardy-Weinberg law.- https://www.britannica.com/science/evolution-scientific-theory/Dynamics-of-genetic-change
6. Ferák V., et al. // Bratislavské Lekárske Listy.- 1987.- Vol. 87, N2.- P.168–175.

7. Frankham R., Briscoe D.A., Ballou J.D. Introduction to Conservation Genetics.- Cambridge University Press. - 2002.- 650 p.
8. Futuyma D.J. Evolution: 3rd Ed. - Sinauer Associates, Inc: Sunderland, MA. - 2013.- 700 p.
9. Gemmell M.R., et al. // Biol. J. Linnean Soc. - 2018.- Vol.125.- N 4.- P. 827–843.
10. Genetic drift.- https://www.britannica.com/science/genetic-drift
11. *Gillespie J.H. // Evolution.- 2001.- Vol. 55, N11, P. 2161–2169.*
12. Griffiths A.J.F., Doebley J., Peichel C., Wassarman D.A. Introduction to Genetic Analysis Twelfth Edition.- W.H. Freeman, 12th ed., NY, 2020.- 816 p.
13. Hecht M.K., Wallace B., Prance C.T. (eds.) //Evolutionary Biology 1987.- Vol. 21. Plenum Press, NY.- 434 p.
14. Joly E. // Biology Direct.- 2011.- Vol.6, N 62. doi:10.1186/1745-6150-6-62.
15. Leftwich P., Chapman T. // Bio-Protocol.- 2018.-Vol. 8, N20.
16. Loyau A, et al. // PLOS ONE.- 2012 Vol.7. N12.- e51293.
17. Provine W.B. //Genetics.- 2004.- Vol. 167, N3.-P. 1041–1046.
18. Ridley M. Evolution.- 3rd Ed.- Blackwell Sci. Ltd., Oxford, Carlton.- 2004.- 751 p.
19. Scriver CR, Kaufman S. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Scriver CR, et al. editors. The Metabolic and Molecular Bases of Inherited Disease.- NY: McGraw-Hill, 2001.- Vol.8. - P. 1667–1724.
20. Smith J.M., Haigh J. // Genet. Res.- 1974.- Vol. 23, №1.- P. 23–35.
21. Stankowski S. //Molec. Ecol.- 2013.- Vol. 22, N10.- P. 2726–2741.
22. *Star B., Spencer H.G. // Genetics.- 2013.- Vol. 194, N1.- P. 235–244.*
23. Templeton A.R. //Genetics. -1980.- Vol.94, N4.- P.1011–1038.
24. William R. Catton, Jr. Bottleneck: Humanity's Impending Impasse.- Xlibris Corporation, 2009. - 290 p.

Practical 8. Numerical variations in chromosomes and their effects

Theoretical background. Regarding aberrations (numerical or structural changes) of the chromosomes (chrs) on this and next lesson, let us learn first their general structure. The chr have a centromere (*c*), short arm (p arm) and long arm (q arm). The *c* contains the kinetochore for correct spindle attachment and sister chromatids segregation to opposite poles during cell division. Sister chromatids remain joined by cohesin at the para-centric heterochromatin until anaphase, when cohesin removal makes them free. This protein facilitates DNA replication, repair and transcription, regulates chrs condensation, pairing and the orientation of sister kinetochores in meiosis I, non-homologous *cs* coupling, chr structure, *etc*. [47].

The pictures of the chrs showing their relative size, homologous groups and landmarks are called *idiograms*. By convention, the p-arm is always at the top of them. The arms are nearly of the same length in *metacentric* chr; the chr is said *sub-metacentric*, if one arm is shorter a little; when it is very short, the chr is said *acrocentric*, when it is almost invisible, the chr is *telocentric* (Fig.1). For example, in human karyotype, chr pairs 13, 14, 15, 21, 22 are *acrocentric* [52]. The p-arms of these carry nucleolar organising regions (NORs), containing genes coding for rRNA [33]. The Y chr is also acrocentric [52].

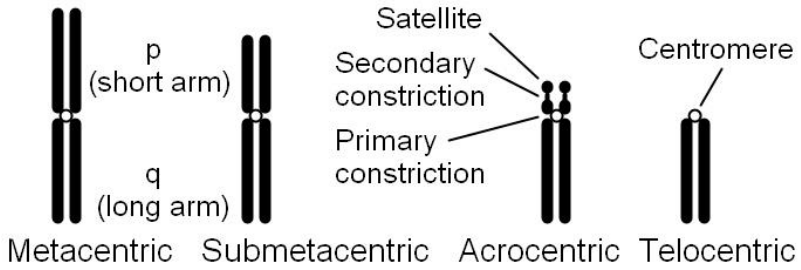


Fig.1. Classification of chromosomes based on short arm length. *By D.B. Pylypiv.*

Arms terminus, the *telomere* (ptel, qtel), is a highly conserved repetitive sequence that prevents end-end chr fusion, and is important for attachments of chr ends to the nuclear envelope, particularly, in meiosis. The telomere shortening is associated with cell ageing. The notation 3ptel means the telomere of the short arm of chr 3.

Several staining methods cause the chrs to take on a banded appearance. Patterns of the bands are specific for each chr, allowing gene mapping and structural changes recording. The number of bands depends on chr contraction, *i.e.*, prophase chrs have more bands than metaphase ones [33]. The Cytogenetic Banding Nomenclature for positional mapping was standardised to allow cytogenetic information flow and storage. Numbering starts from the *c* and continues to the end of each arm. The arms are divided into a number of regions by "land-mark" bands, detected in microscopy. They are numbered sequentially within each region. The cytogenetic bands are labeled p1, p2, p3, q1, q2, q3, etc. At higher microscopic resolutions, sub-bands and sub-sub-bands can be seen (Fig.2) and numbered from the *c* out toward the telomere. *E. g.*, the cytogenetic map location of the CFTR gene is 7q31.2, which indicates it is on chr 7, q arm, band 3, sub-band 1, and sub-sub-band 2 [28].

The normal number of chrs varies between species. Some organisms have very low numbers of chrs, *e.g.*, the plant *Haplopappus gracilis* [37], the equine parasite

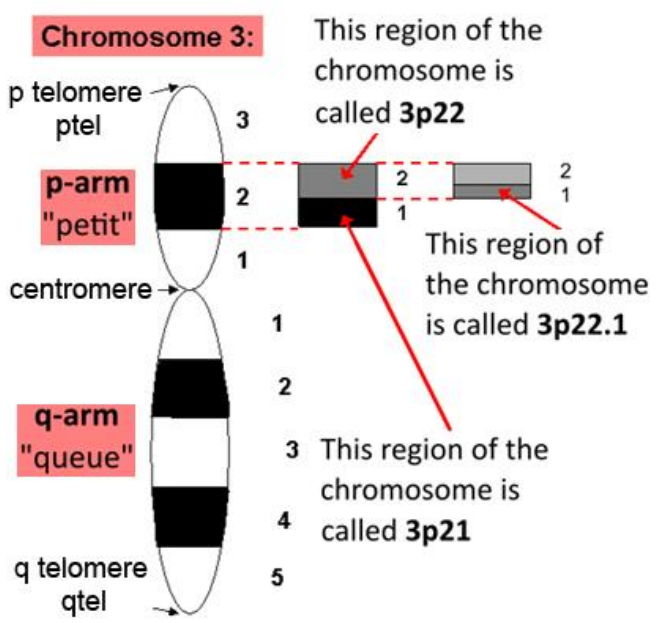


Fig. 2. Cytogenetic Banding Nomenclature. *Adapted from Geer R.C., Messersmith D.J.* [28].

(*Parascaris equorum*) [39], two species of water mites (*Eylais rimosa* and *Eylais setosa*) [62] and the venomous ant (*Myrmecia pilosula*) have one pair of chrs [16]. The male ant is haploid and has just one chr. The cells of some organisms can contain hundreds of them, *e.g.*, the atlas blue butterfly (*Polyommatus atlantica*) [44], black mulberry (*Morus nigra*) [6] or even to thousands, however, nano-size chrs, like in ciliate *Sterkiella histriomuscorum*, that has 16 000 chrs in macronucleus [67]. Almost all bacteria have 1 chr, a naked circular DNA molecule [29].

Most species are diploids (*2n*), *i.e.*, have 2 sets of homologous chrs. Some species are monoploids or haploids (*1n*), with only 1 set of chrs. Eggs or sperm cells of *2n* organisms are produced by meiosis and have *1n* set also. The true number of chrs in humans was estimated by J.H. Tjio [68] The *2n* humans karyotype consists of 46 chrs: 22 pairs of *autosomes*, numbered from 1 to 22 by order of size decrease, and a pair of sex chrs, or *gonosomes*: XX (female) and XY (male).

The term ***ploidy*** describes the number of copies (*n*) of the basic chrs set (the collection of the different chrs in a cell) and also tells the copy number of each of the different nuclear DNA molecules. The DNA content of a single chrs set (*n*) is marked as *C*. Animal body cells are, usually, **diploid** (*2n* or 2*C*), with nuclei containing 2 copies of each of chr, 1 from either of paternt. Human sperm and egg cells (gametes) are **haploid** *(n),* containing only 1 of each kind of chr (1*C*), so *n* = 23. Some human cells (erythrocytes, platelets, differentiated keratinocytes) lack nucleus or crhs and are nulliploid (0*C*). Others with ***polyploidy*** (*P*) are ***polyploids*** (*Ps*), *i.e.*, DNA content > 2*C*. *P* can appear by 2 ways. The DNA might undergo multiple rounds of replication without cell division, *e.g.*, large megakaryocytes in blood with 16 to 64 copies of each chr in large multilobed nucleus. Alternatively, *P* originates by cell fusion to give cells with multiple nuclei, *e.g.*, muscle fiber cells [65]. Changes in chrs number can cause ***homogeneous numerical anomalies***, when all somatic cells of the body contain the same chr(s) change, and ***mosaicism***, when the body is composed of 2 or more chromosomally different cell populations.

Homogeneous anomalies result from meiotic *non-disjunction* or from abnormal fertilization. For autosomes, non-disjunction in meiosis 1 results in 4 abnormal gametes and non-disjunction in meiosis 2 results 2 normal and 2 unbalanced gametes (Fig. 3).

Fusion of normal gamete and gametes with extra chr produce not viable trisomic zygotes. The exceptions are trisomies of chrs 8, 13, 18 and 21, that are partially compatible with life.

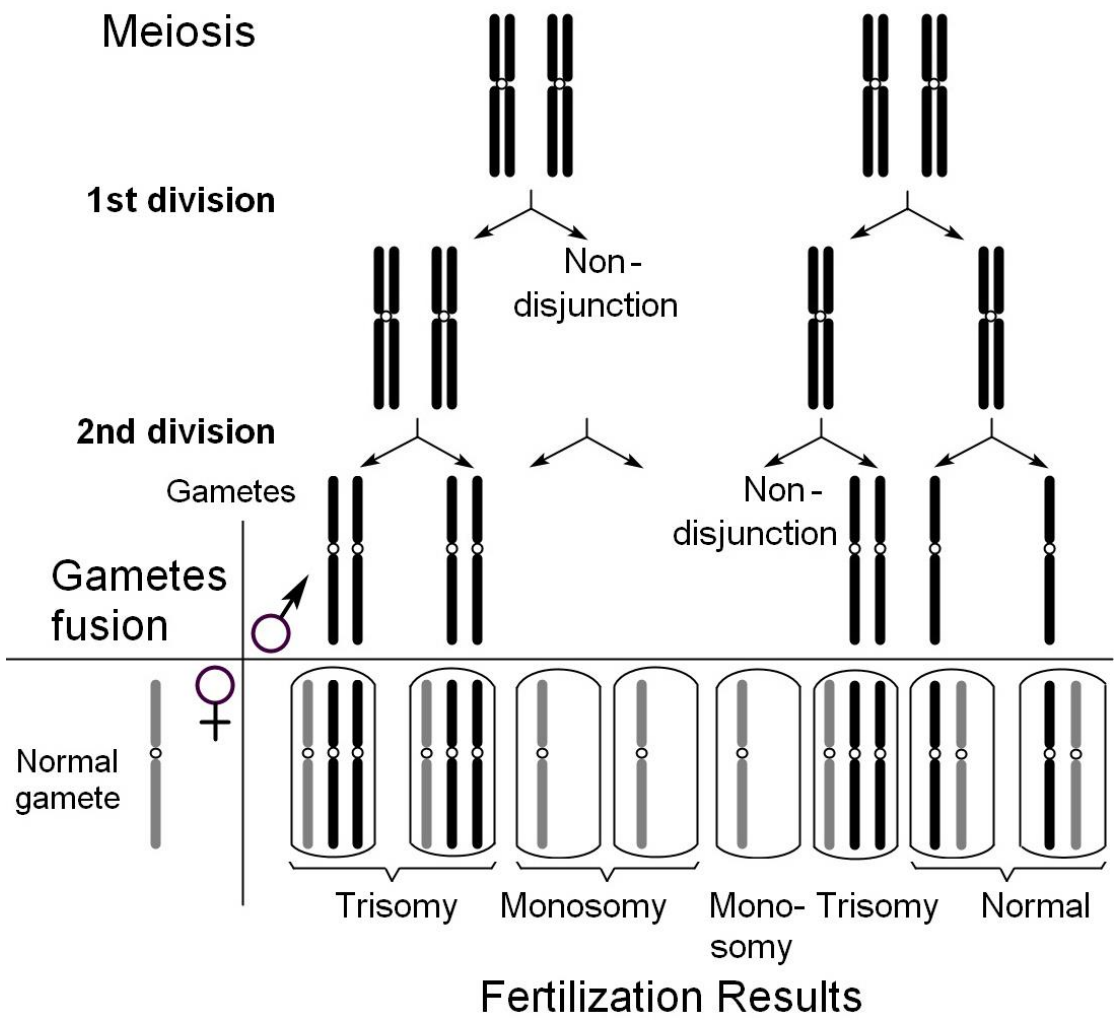


Fig. 3. Meiotic non-disjunction effect on gametes quality and fertilization results. *By D.B. Pylypiv.*

Participation of gametes with deficiency for one chr in fertilization results in monosomic zygotes, that lead to early abortions. Actually, they are not rare, however most of them are eliminated within unbalanced conceptuses so early that their losses remain not noticed. Non-disjunction can involve any pair of the chrs. More than one non-disjunction most often observed for sex chrs. All 4 types of meiotic products from male can fertilize only 1 type of egg as the remaining 3 are eliminated as polar bodies.

The unbalanced sex chrs are less dangerous than unbalanced autosomes. Monosomy X is viable (Turner syndrome), but not monosomy Y, that is lethal early in gestation. To be viable, the zygote must contain at least one X chr (Table 1).

Homogenous anomalies can be produced as the fertilisation mistakes also. These can result, particularly, in ***polyploidy*** (*P*).

Polyploids are organisms (or cells) with *n* sets of chrs repeated more than 2 times. They are most common among plants, *e.g.*, saffron crocus (*3n*), [10], cotton (*4n*) [1], Kenai birch (*5n*) [26], hexaploid kiwi (*6n*) [17] and less among the animals, *e.g.*, triploid tardigrades (*3n*) [9], tetraploid *Salmonidae* fish (*4n*) [64], octoploid (*8n*) fish *Acipenseridae* [60], duodecaploid (*12n*) frog *Xenopus ruwenzoriensis* [54], etc. As an

exclusion in prokaryotes, the large (≈ 600 μm) bacterium *Epulopiscium fishelsoni* has tens of thousands of copies of its chr [48].

P is often observed in cancerous cells [78]. In normal tissues it is found in cardiomyocytes, hepatocytes, megakaryocytes [13, 58, 75], myoblasts fusion hybrids [77], cerebellar glia [46], neurons [8, 45, 46, 50], corneal endothelium of the eye [34], oral squamous epithelium affected by lichen planus [7], thyroid follicle cells [32], endocrine cells of the pancreas [23], Arias-Stella reaction endometrial cells [73], granulosa lutein cells in ovary [63], cytotrophoblastic cells [41], seminal vesicle epithelium [49], lymphocytes in HIV patients [3], smooth muscle cells of arteries [5], mammary gland, urothelium and mesothelium [8, 36]. The highest ploidy levels were 64C in liver cells [32], 32C in cardiomyocytes [40], and 128C in megakaryocytes [56].

Table 1. Zygotes as results of different gametes fusion.

Gametes	*O*	*X*	*XX*	*XXX*	*XXXX*
O	†	*X*	*XX**	*XXX*	*XXXX*
X	*X*	*XX*♣	*XXX*	*XXXX*	*XXXXX*
Y	†	*XY*♣	*XXY*	*XXXY*	*XXXXY*
XY	*XY**	*XXY*	*XXXY*	*XXXXY*	†
YY	†	*XYY*	*XXYY*	†	†
XX	*XX**	*XXX*	*XXXX*	*XXXXX*	†
XYY	*XYY*	*XXYY*	*XXXYY*	†	†
XXY	*XXY*	*XXXY*	†	†	†
XXYY	*XXYY*	†	†	†	†

Note. ♣Normal zygotes from fusion of balanced gametes. *Normal zygotes from fusion of unbalanced gametes. †Not viable conceptuses.

As a rule, the *P* is not tolerated in higher vertebrates. Particularly, in humans it causes of 10% of spontaneous abortions [24] and is presented as ***triploidy*** (3*n*) with 69 chrs (69,XXX, 69,XXY or 69,XYY), and ***tetraploidy*** (4n) with 92 chrs (92, XXXX). Usually, 3n is due to polyspermy and occurs in ≈ 1–3% of pregnancies. 2/3 of 3*n* pregnancies are males and abort or die shortly after birth.4n or 3n are observed in ≈ 2 -3 % of fertilised eggs [33]. There has been 1 report of a complete 3n child survived to ≈ 7 months. The boy had abnormal physical and CNS development and weak immune system. He died after a *Pneumocystis carinii* infection [2].

3n results from ***digyny*** (*DG*) or ***diandry*** (*DA*) the extra *n* set is from the mother or from the father, respectively). *DA* is mainly caused by doubling of the *n* set from a single sperm or may also be the result of dispermy (egg fertilized by 2 sperms) [4].

DG is usually caused by one meiotic division failure during oogenesis resulting in a 2n oocyte or by failure to extrude the 2nd polar body from the oocyte. *DA* dominates in early miscarriages, while digyny most numerous among 3n zygotes surviving into the fetal period [11]. *DA* is 4 times more frequent than digyny [33]. An asymmetric fetus is usually observed in *DG*, with adrenal hypoplasia and a mini placenta. In *DA*, a *partial hydatidiform mole* develops [4]. These parent-of-origin-specific effects on genes expression represent the *genomic imprinting*, an epigenetic phenomenon [55]. Hydatidiform moles are usually polyploid [33].

A *complete mole* is produced by 1 sperm (90%) or 2 (10%) sperm fusion with an egg which has lost its nuclear DNA. In the 1st case, the sperm then reduplicates, resulting in 46 chr set [42]. The genotype is typically 46,XX (2n) but can also be 46,XY (2n), but not 46,YY (2n). In contrast, a *partial mole* occurs when a normal egg is fertilized by 1 or 2 sperm which then reduplicates itself, yielding the genotypes of 69,XXY (3n) or 92,XXXY (4n) [42]. The risk of choriocarcinoma is characteristic for 0,5% partial and for 15% of complete moles [20]. Complete 4n results in 1–2% of early abortions and is more rare than 3n zygotes. Some 4n cells are routinely found in prenatal diagnosing, and they are regarded as not dangerous.

Mixoploidy *(M)* (when n/2n, 2n/3n or 2n/4n cell populations compose the body) extends the survival after birth. *M* is common in human preimplantation embryos. Possibly, these fail to implant or simply a selective process favors the 2n cells and therefore *M* rare in pregnancies and infants. Thus, 2n/4n *M* in living patients has been reported in at least 29 cases since 1967 [59]

Endopolyploidy is presented as *P* of some normal tissues in 2n body, *e.g.*, in myocard [53], liver [8,18], placenta [71, 79], bone marrow [51,75].

Developmental polyploidization in placenta trophoblast giant cells can reach a DNA content of up to 8C - 64C [69, 70]. As no mitosis between S phases in their cell cycle, the replicated chromatids stay cohesin-linked, so they form polytene chrs [35].

The megakaryocytes become polyploid in an *abortive mitosis* (*endomitosis*) with no *cytokinesis*. Due to suboptimal levels of Cdk1/CycB activity and premature degradation of cyclin B [57], the clusters of duplicated chrs temporarily separate and a midzone forms, but cleavage furrow regresses, and all chrs appear in one nucleus. The resulting cell is in a G1-like state, goes through S phase, repeats endomitosis; the cycle is repeated few times to form polyploid up to 128n (modal ploidy of 16n) [75].

When liver grows, the frequency of tetraploid (4n) and octoploid (8n) hepatocytes increases [74] due to endomitosis [14] (Fig. 2). These cells often have two 2n (or 4n) nuclei as result of cytokinesis failure [30] or cell-cell fusion [22], and cells with a single 4n or 8n nucleus arise readily due to anaphase inhibition or telomeres damage [76]. Hepatocytes increase their ploidy in response to DNA damage, possibly, due to altered p53 pathway responses to DNA damage [27], while most other cells apoptosize.

The cell fusion results in polynucleated differentiated *osteoclasts* (the cells, that play a vital role in bone development/regeneration) and in myocytes [72].

Euploids (*E*) are the cell or individuals that contain any number of complete *chr* sets. *E* are more tolerated in plants, than in animals. There may be a single set (monoploidy), 2 sets (diploidy), or multiple sets, the *P*, i.e. 3n, 4n, 5n, etc.) of chrs. The types of *E* are *autopolyploidy* and *allopolyploidy* [29].

Autopolyploids (*autoPs*) are the organisms or species that appear due to multiplication of chrs sets derived from a same taxon. *E.g.*, the fish white sturgeon is a natural autopolyploid (8n) [60]. Usually, *autoP* results from the fusion of 2*n* gametes whith production of triploid ($n + 2n = 3n$) or tetraploid ($2n + 2n = 4n$) young [12].
As a rule, 3n fish are sterile (*triploid block*) and used in farming to introduce sterility in trout [43] or salmon [15]. However, in some species they produce high proportions of unreduced gametes contributing to formation of tetraploids (*triploid bridge*).

Autotriploidy plants are often produced by *apomixis* (asexual reproduction by special generative tissues without fertilization). Usually, autotriploids are seedless, *e.g.*, pears, bananas or watermelons.

Allopolyploids (*alloPs*), *heteropolyploids* or *amphipolyploids*) are *Ps* with chrs origin from diverged taxa. Like *autoPs*, the *alloPs* are primarily produced by fusion of unreduced (2*n*) gametes from diploids before hybridization or by combining reduced (2*n*) gametes from 2 autotetraploid taxa after hybridization. A frog *Xenopus laevis* is allotetraploid, a natural hybrid of 2 related species with genome duplication [25].

Radish (*Raphanus sativus*) and cabbage (*Brassica oleracea*) were crossed. The hybrids in F2 were *alloPs* (4n), the result of 2n gametes fusion. A new genus, the *Brassicoraphanus* was created [38]. *Triticale*, the hybrids of wheat and rye are *alloPs* with 4, 6 or 8 chrs sets [31].

Aneuploidy (*A*). *Aneuploids* (*As*) are cells or individuals that have 1, 2, or a few chrs less or more than normally present in *chr* sets for that species.

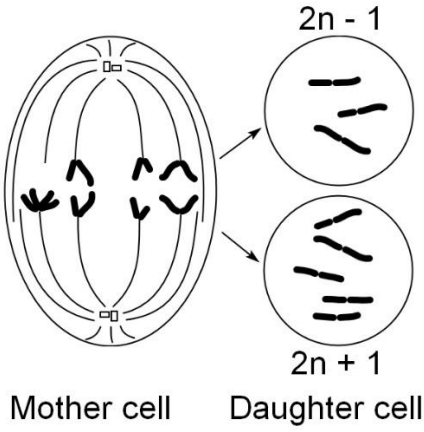


Fig. 4. Non-disjunction in anaphase of mitosis.
By D.B. Pylypiv.

The non-disjunction during cell division is a basic mechanism of *A*: both sister chromatids or 2 homologous chrs instead of equal separation between 2 daughter cells migrate to one pole. As a result, the chrs number in 1 daughter cell increases above normal and in another daughter cell it decreases below the normal quantity. Non-disjunction can take place in mitosis (Fig.4) or in the 1st, 2nd, or both of the meiotic divisions. It may happen in one or both of the parents as: 1) failure of a pair of homologous chrs to separate in meiosis I, 2) failure of sister chromatids to separate in meiosis II, 3) both events (1 and 2), and 4) failure of sister chromatids to separate during mitosis [29, 61, 66]. During meiosis in women, only 1 egg cell survives. The rest 3 cells degenerate as polar bodies. An extra chr or its loss can happen in any of 4 meiotic products (Fig.5).

The cell or an organism with 1 chr missed is *monosomic* (2n-1) and with 1 extra chr is *trisomic* (2n+1), with 2 extra chrs is tetrasomic (2n+2). Trisomy (2n+1) with 47 chrs is compatible with life and autosomal monosomy (2n-1) is not, however, one sex chr loss is compatible with life [29]. If 2 extra chrs are not homologous, the organism is double trisomic (2n+1+1). When homology pair is lost, this is a case of nullisomic (2n-2). Other *As* are possible. *A* creates genetic imbalance that often results in early lethality in gamete, zygote, embryo, fetus or early infants. The lethal effects make *A* to be rare among vertebrates. Some *A* combinations are tolerated and shown in phenotypes, particularly, in humans as syndromes. Most common extra autosomal chrs among live births are 21 (Down syndrome, 1/800 birth), 18 (Edwards syndrome, 1/6000), 13 (Patau syndrome, 1/10 000). 10% of Edwards or Patau syndrome infants survive to 1 year of age [29]. *A* is usually detected by prenatal screening [19].

About 68% of human solid tumors produce cells-*As* due to non-disjunction [21].

The pairing during meiosis in *As* occurs between 2 homologous chr at any point. In ***trisomy*** condition 2 variants behavior of chrs was observed: 1) the formation of a

bivalent, the complex of 2 homologous chrs, and a single separate homologous chr (univalent); 2) the formation of a trivalent, the complex of 3 homologous chrs [29].

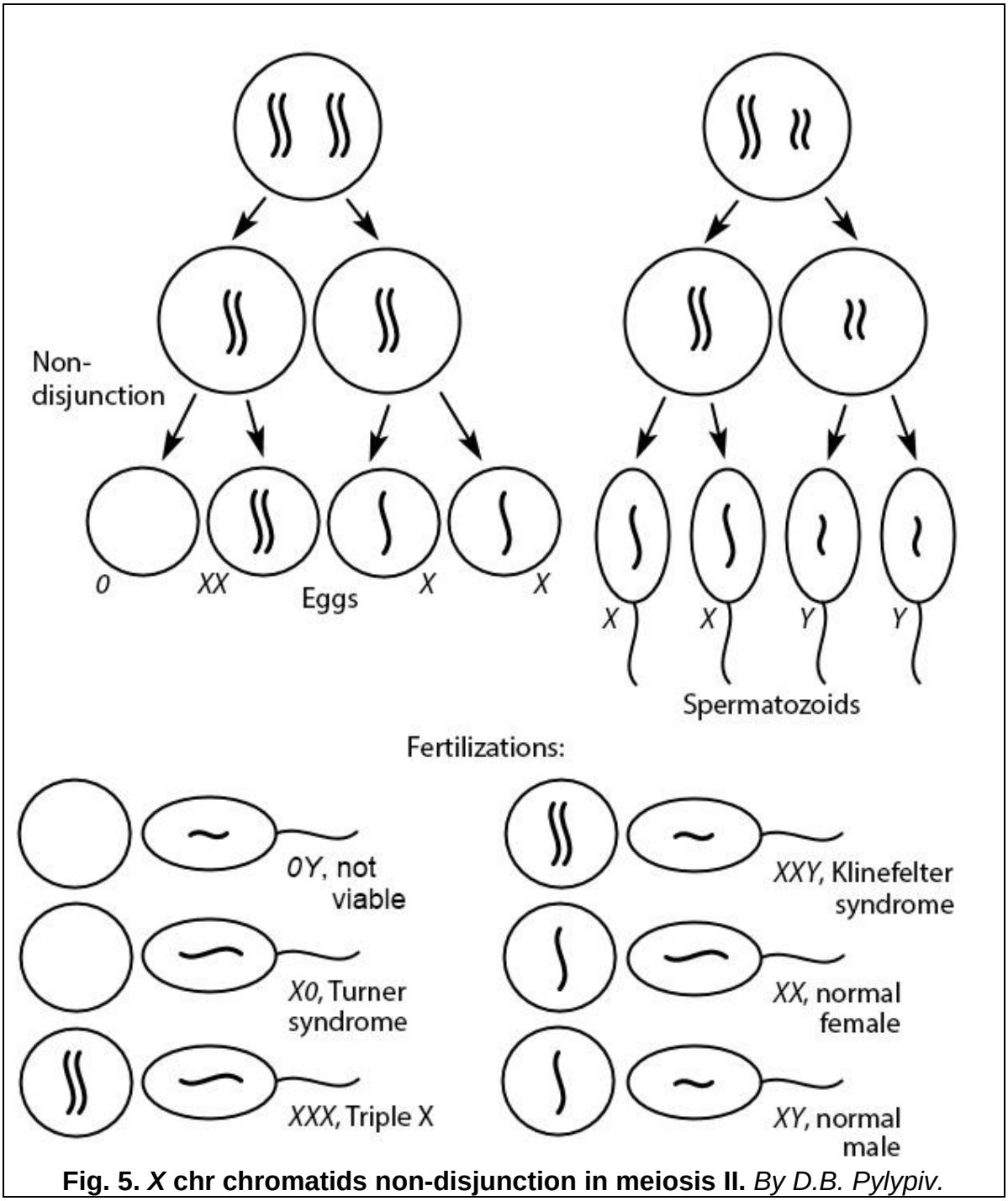


Fig. 5. *X* chr chromatids non-disjunction in meiosis II. *By D.B. Pylypiv.*

Solved problems

Problem 1. Build simple charts for most possible non-disjunctions in human male during gametes production and expected results of fertilization of normal eggs with these abnormal spermatozoids.

Solution. In meiosis during spermatogenesis several kinds of non-disjunctions can occur resulting in production of male gametes with abnormal number or absence of sex chrs, particularly. If these spermatozoa fertilize normal eggs, several of syndromes can arise, if not aborted, in offsprings (Fig. 7).

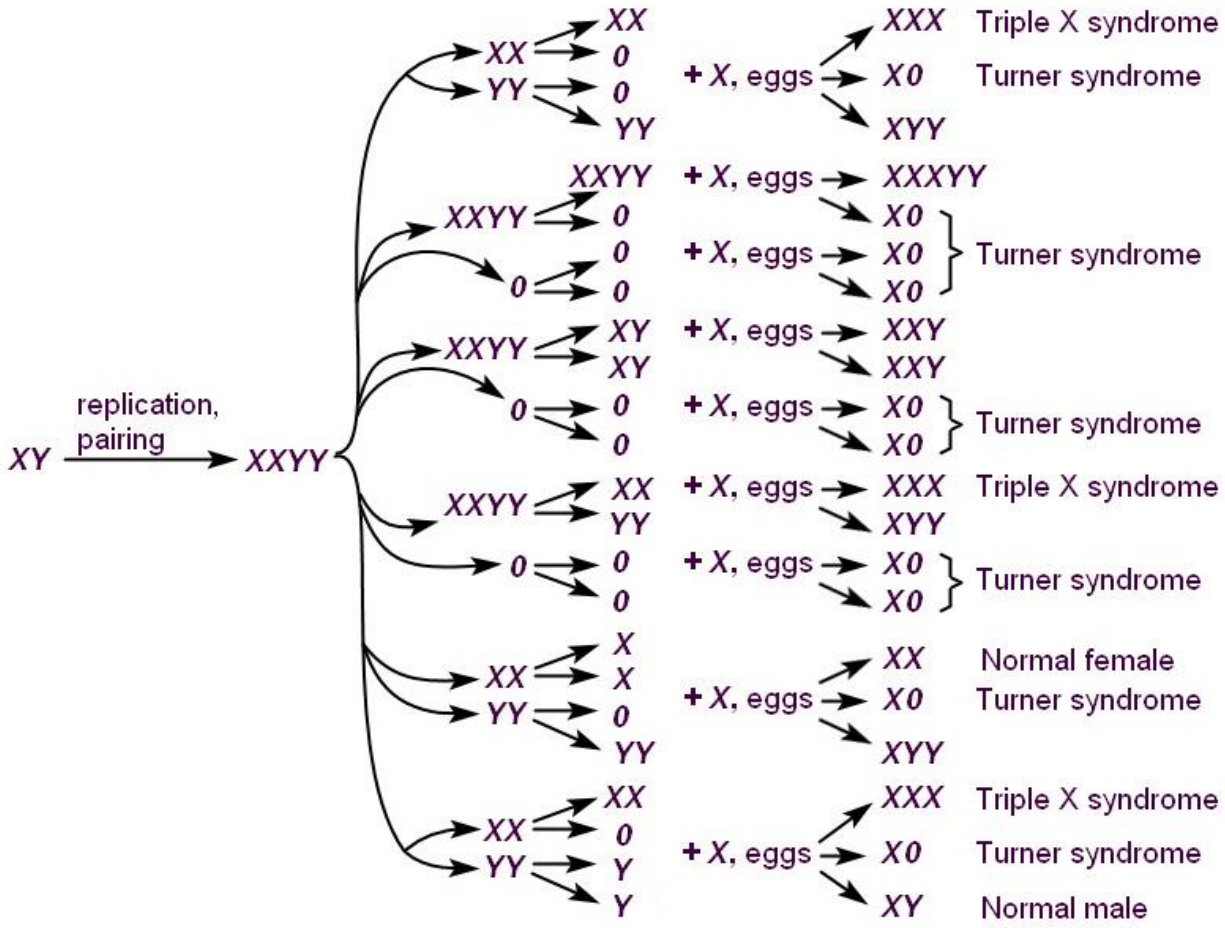


Fig. 7. Non-disjunction in male and syndromes resulted after fertilization of normal eggs with abnormal sperm. *By D.B.Pylypiv*.

Problem 2. How to recognize whether a particular tetraploid is autopolyploid or allopolyploid?

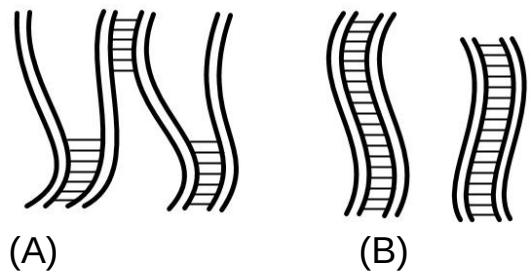


Fig. 6. Formation of tetravalents in autopolyploid (*A*) or bivalents in allotetraploids (*B*). *By D.B. Pylypiv.*

Solution: Usually, autopolyploid shows some tetravalents in diakinesis of meiosis 1 since all 4 chrs are homologous to each other and pair "two-by-two" at any place. As a rule, only bivalents are observed in an allopolyploid at this point of meiosis (Fig. 6).

Problem 2. Build a Punnett square for possible production of Triple X syndrome, Turner syndrome and Klinefelter syndrome due to non-disjunction in human females.

Solution. The mentioned Triple X syndrome is also called trisomy X or 47, *XXX*. It means that a female has one extra *X* chr. In Turner syndrome females have only 1 X chr. In case of Klinefelter syndrome male has one extra *X* chr. Let us build a Punnett square.

♂, sperm: normal ♀, eggs: non-disjunction	*X*	*Y*
XX	*XXX*, Triple *X* syndrome	*XXY*, Klinefelter syndrome
0	*X0*, Turner syndrome	*Y0*, non-viable

Problem 3. Some *XO* females are fertile. At least one *X* chr is required for viability of conceptus and childbirth. What sex ratio is expected among fetuses surviving to the term from the marriage of the Turner syndrome female and genetically normal man?

Solution: Parents, P: ♀ *XO* × ♂*XY*

Gametes, G: *X, O* *X, Y*

Progeny, F: *XO, XX, XY* survive, OY die at an early stage.

Thus, possible sex ratio in living births is: 2 girls/1 boy.

Problems for homework

Problem 1. The colchicine was added for overnight incubation of zygote, when it was in metaphase of 1st division after nuclei fusion in fertilization. Following treatment of zygote the chemical was washed out and zygote was incubated further. What about the results of treatment? What if instead of colchicine we used demecolcin?

Problem 2. The model in developmental and cell biology, a frog *Xenopus laevis* has 18 chromosomes in haploid set. How many chromatids are in: **a)** mitosis metaphase nucleus **b)** meiotic metaphase I nucleus **c)** meiotic metaphase II nucleus?

Problem 3. At the start of meiosis each of human chromosomes is composed of 2 chromatids. **a.** How many chromosomes in cell at the end of meiosis I? How many chromatids? **b.** How many chromosomes are there per cell at the end of meiosis II? How many autosomes and gonosomes? How many chromatids? **c.** How many chromosomes are in zygote? When will they gain the two-chromatid structure?

Problem 4. Finish the table 3 indicating aneuploids that can frequently survive to term by "++", those surviving rarely to term by "+" and those, never surviving by "-". Fill in the syndromes (if known) corresponding to chr aneuploidy.

Table 2. Monosomy and trisomy in human

Chr	Monosomy	Trisomy	Chr	Monosomy	Trisomy
1			12		
2	2q37 deletion	Trisomy 2 -	13		Patau **+**
3			14		
4	Pitt	Trisomy 4 -	15		
5			16		
6			17		
7			18		
8			19		
9			20		
10			21		Down **++**
11			22		

Problem 5. Give diagnosis. Teen girl has short stature, a webbed neck, wide-spaced nipples, a low hairline, low set ears, a small chin and jaw, and a high-arched palate. X-ray showed a horseshoe kidney. Menthally normal, however, has puberty delay. There are no Barr bodies and only one *X* chr in her cells.

Literature

1. Adams K.L., Wendel J.F. // Cur. Opin. Plant Biol.- 2005.- Vol.8, N2.- P.135–141.
2. Arvidsson C.G. //Acta Pediatr. Scand.- 1986.- Vol.75, N3.- P.507-510.
3. Auffermann W, et al. //Anal Quant Cytol. Histol.-1986.- Vol.8.-P.19-24.
4. Baker P., Monga A., Baker P. Gynaecology by Ten Teachers. London: Arnold. 2006.-ISBN 978-0-340-81662-2.
5. Barrett TB, et al. // Proc Nad Acad Sci USA.- 1983.- Vol.80.- P. 882-885.
6. Basavaiah S.B., et al.. //Cytologia.- 1990.- Vol. 55.- P.505-509.
7. Biesterfeld S., et al. // Anal Quant Cytol Histol.- 1991.- Vol.13.- P.7-10.
8. Biesterfeld S., et al. // J. Clin. Pathol. -1994 Vol 47.- P. 38-42.
9. Bertolani R., et al. // Zoologisch. Anzeiger. -2001.- Vol. 240,N 3–4 .- P. - 247–252.
10. Blattner F.R., et al. // *bioRxiv*:537688.– Feb. 1, 2019.- doi:10.1101/537688
11. Brancati F., et al. // *Eur. J. Hum. Genet.* -2003.-Vol.**11**.- 12.- P. 972–974.
12. Bretagnolle F., Thompson J.D. // New Phytol.- 1995.- Vol. 129, N78.- P.1-22.
13. Brodsky W.Y., Uryvaeva I.V. // Int Rev Cytol.- 1977.- Vol.50.- P. 275-332.
14. Celton-Morizur S., et al. //Cell Cycle.- 2010.- Vol.9.- P. 460–466.
15. Cotter D., et al. // Aquaculture. -Vol.186, N1–2.- P. 61–75.
16. Crosland M.W.J. Crozier, R.H. //Science. -1986.-Vol.231, N4743.- P. 1278.
17. Crowhurst R.N., et al. // Acta Horticulturae 297: 10.17660/ActaHortic.1992.297.5
18. Donne R., et al. // Nat. Rev. Gastroenterol. Hepatol.- 2020.- Vol. 17.- P. 391–405.
19. Driscoll D.A., et al. // The New Eng. J Med.-2009.- Vol. 360,N24.- P. 2556–2562.
20. Duffy L., et al. //J. Clin.Med. Res. -2015. - Vol.**7**, N12.- P. 961–966.
21. Duijf P.H.G., et al. // Int. J. Cancer, 2013.-Vol.132, N10.- P. 2316–2326.
22. Duncan A.W., et al. // PLoS Genet.- 2009.- 5:e1000385
23. Ehrie M.G., et al. // Diabetes.- 1974.- Vol.23.- P. 583-588.
24. Eiben B., et al. // Am. J. Hum. Genet. 1990.-Vol.47.- P. 656–663.
25. Elurbe D.M. , et al. // Genome Biol.- 2017.- Vol.18.- P. 198
26. Evans W.H. // Bot. Gaz. -1899.-Vol. 27- P. 481.
27. Fei P., et al. // Cancer Res.- 2002.-Vol.62.- P.7316–7327
28. Geer R.C., Messersmith D.J. - https://www.ncbi.nlm.nih.gov/Class/MLACourse
29. Griffiths A.J.F., Doebley J., Peichel C., Wassarman D.A. Introduction to Genetic Analysis.- W.H. Freeman, 12th ed., NY, 2020.- 816 p.
30. Guidotti J.E. et al. // J. Biol. Chem.- 2003.- Vol.278.- P.19095–19101.
31. Hammer K., et al. //Genet. Resour. Crop Evolut. -2011.- Vol. 58,N1.- P. 3-10.
32. Heide W. Die Polyploidie der Zellkerne in Leber, Schilddriuse and Samenblasenepithel des Menschen w&irend der 9. und 10. Lebensdekade und sie beeinflussende Faktoren. PhD Thesis. Univ. Dusseldorf, Germany, 1982: P.1-51.
33. Huret J.-L., et al. – http.www:atlasgeneticsoncology.org/Educ/PolyMecaEng.html.
34. Ikebe H., et al. // Exp. Eye Res.- 1984.- Vol.39.- P.497-504.
35. Ilgren E.B. , et al. // J. Embryol. Exp. Morphol.- 1981.- Vol.62.-P.183–202.
36. Isoda K. , et al. //Acta Pathol.- 1983.- Vol.33.-P.733-738.
37. Jackson R.C., et al. //Science.- 1957.- Vol.126, N3283.- P.1115-1116.
38. Karpechenko G.D. // Bull. Appl.Botany.- 1927.- Vol.17. -P. 305–408.
39. Kimball J.W.- https://bio.libretexts.org/@go/page/4845
40. Kompmann M., et al. //Arch. Pathol.- 1966; Vol.82:303-8.

41. Kropff M., et al. //Anal Quant Cytol Histol 1993.
42. Kumar V., ed. Pathologic basis of disease.- 8thed..- Elsevier.- 2010.- P. 1057–1058.
43. Lincoln R. F., Scott, A.P. //Aquaculture. -1983.- Vol.30, N1–4.- P.375–380.
44. Lukhtanov V.A. //Compar. Cytogen.- 2015.-Vol.9, N4.- P. 683-690.
45. Mann D.M.A., Yates P.O. // J. Neurol. Sci.- 1973.- Vol.18.- P. 183-196.
46. Mann D.M.A., Yates P.O. // J. Neurol. Sci. 1973.- Vol.18.- P.197-205.
47. Mehta G.D., et al. //FEBS Letters.- 2013.- Vol.587, N15.- P. 2299–312.
48. Mendell J.E., et al. //PNAS.- 2008.- Vol. 105, N 18–P. 6730–6734.
49. Müller H.A., et al. // Virchows Arch (Cell Pathol).- 1973.- Vol.12.- P.281-284.
50. Muller H.A. // Enschaften Naturwiss.- 1962.- Vol.49.- P.243.
51. Nagata Y., et al. // J Cell Biol.- 1997.- Vol.139.- P. 449–457.
52. Nussbaum R.L., McInnes R.R., Willard H.F., Hamosh A. Thompson & Thompson Genetics in Medicine, 8th ed., Elsevier Inc., Philadelphia, PA, 2016.- 1278 p.
53. Parmacek M.S., Epstein J.A. // New Eng. J. Med. 2009.-Vol.361, N1.- P. 86–88.
54. Pasquier L. Du, et al. // Front. Biosci.- 2009.- Vol. 14, N14.- P.177–191.
55. Patten M.M., et al. //Heredity. -2014.-Vol.113, N2.- P. 119–128
56. Pfitzer P., et al. // Verh. Dtsch. Ges. Pathol. 1983 -Vol.67.- P.478-82.
57. Ravid K., et al. // J. Cell Physiol. -2002.- Vol.190.- P.7–20.
58. Sandritter W., et al.// Rec. Adv. Stud. Card. Str. Met.- 1978.- Vol.12.- P.115-127.
59. Schacht J.P., et al. // Clin. Case Rep.- 2017.- Vol. 6, N1.- P.103-108.
60. Schreier D.A., et al. // J. Appl. Ichthyol.- 2011.- N27, Suppl.2.- P.24-33
61. Snustad P.D., Simmons M.J. Principles of Genetics.- John Wiley & Sons, Inc. NJ, 2006.- 896 p.
62. Sokolov I.I. Trud. Leningrad. Obshch. Estestvois., Otd. Zool.- 1954.-72, p.124
63. Stangel J.J., et al. // Am. J. Obstet. Gynecol.- 1970.-Vol.108.- P.543-9.
64. Stouder J.D., et al. Pacific Salmon and Their Ecosystems: Status and Future Options.- International Thomson Publishing, New York, 1997.- 685 p.
65. Strachan T., et al. Genetics and genomics in medicine.- Garland Science Taylor and Francis Group LLC, London, 2015.- 546 p.
66. Strachan T., Read A.P. Human Molecular Genetics.- 5th ed.- Garland Science. Boca Raton, 2018.- 784 p.
67. Swart E.C., et al. // PLOS Biology.- 2013.- Vol.11, N1.-e1001473. doi:10.1371.
68. Tjio J.H., Levan A. // Hereditas.- 1956.- Vol. 42.- P.1–6.
69. Ullah Z., et al. // Cell Div. 2009a..-Vol.4.- P.10
70. Ullah Z., et al. //*Cell Cycle* .- 2009b.-Vol.8.- P.1501–9
71. Velicky P., et al. //PLOS Gen.- 2018.-Vol.14, N10.-e1007698.doi:10.1371
72. Vignery A. // Int. J. Exp. Pathol.- 2000.- Vol.81.- P. 291–304
73. Wagner D., Richart D.M. // Arch. Pathol.- 1968.-Vol.85.- P.475-80.
74. Watanabe T., et al. //Virchows Arch. - 1978. .-Vol. *B* 27:307–16
75. Winkelmann M., et al. // Klin.Wochenschr.- 1987.- Vol. 65, N23.- P.1115–1131.
76. Wirth K.G., et al. // J. Cell. Biol.- 2006. -Vol.172:847–60
77. Yaffe D., Feldman M. // Dev. Biol.- 1965. .-Vol. 11:300–17
78. Zack T.I. et al. // Nat. Genet.- 2013.-Vol.45.- P.1134-1140.
79. Zybina T.G., Zybina E.V. //Cell Biol. Int .- 2005.- Vol.29: 1071–1083.

Practical 9. Structural variations in chromosomes and their effects

Theoretical background. Structural abnormalities or structural abberrations (*SA*) of the chromosome (chr) usually result from breakage followed by loss or rearrangement of it's parts. Mostly, the breaks occure in noncoding regions.

For an diploid (2n) organism is important to keep genetic balance, *i.e.*, to have 2 copies of each gene and at least one allele of each gene not changed or changed in only little extent providing normally functioning nucleic acids and protein. However, a fully balanced set of genes is not strictly necessary for the functioning of many differentiated tissue cells, especially if they are not divide. At the same time, relatively small imbalances can have dangerous consequences, even in somatic cells. An example is *Rb* gene, implicated in the retinoblastoma development. Normally we have two functional *Rb* genes, but one allele can be mutated or lost. The normal allele (acts now as a tumour suppressor gene) provides normal function. Loss (or mutation) of the second allele results in retinoblastoma [7, 8, 11].

SA leads to gene non-functioning, activation or inactivation at the wrong time or place or to its fusion with other gene that can result in potent oncogene production.

Many of the *SAs* are fatal for gametes or they are eliminated by natural selection in next generation. Of those that are transmitted, the most frequent are translocations, small deletions and inversions. Rearranged chrs that survive are called derivative chrs ("der") and they are numbered after the centromere (*c*) they carry. *E.g.*, a reciprocal translocation between chr 7 and chr 14 will result in a der(7) and a der(14) [11].

As a rule, loss of chr material is more dangerous than gain; the changes in sex chrs are better tolerated than in autosomes. The *SA* of sex chrs can be symptomless or manifested only in adults (*e.g.* infertility); changes in chrs have *de novo* origin [7, 8,11].

The breaks in DNA can occure in any place of the chr and they are, usually, repeared. DNA repearing system is insufficient in *chromosome instability syndromes*, *e.g.*, Falconi anemia, ataxia telangiectasia, Bloom syndrome, *xeroderma pigmentosum*. These are charaterised by frequent spontaneous chr breaks and rearrangements and/or a hypersensitivity to *clastogens*, the agents inducing disruption or breakages of chr. The genes implicated in these diseases seem to have a role in DNA repair and/or in the cell cycle regulation. If lesions in DNA left not repaired, they accumulate and one of them can, finally, activate an oncogene or inactivate a tumor suppressor gene [7, 8, 11].

Several forms of *SA* of chrs were observed.

Deletion (in short "*del*") means the chr portion loss. *Del* can be of several types.

Terminal del (with single break) occurs towards the end of a chr, *e.g.*, *p* arm of one of the chrs 5 is truncated at its end in Cri du chat syndrome. It can arise as *de novo* mutation or be the result of translocation in the parents.

Intercalary/interstitial del (with 2 breaks) take place in the interior of a chr living its ends intact, *e. g.*, Willms tumor results from intercalary *del* in chr 11 *p* arm.

Micro del is a relatively small amount of *del* (up to 5Mb) that could include a dozen genes. It is often found in children with physical abnormalities.

If *del* is homozygous (the same *del* on both homologs) this has lethal effect. Even if *del* is heterozygous (one of homology chrs involved) the organism may not survive. Only some small *del*s are compatible with life, if normal homologous chr present.

The *del* region is indicated by *deletion loop* in meiotic pairing of homology chrs (Fig.1). *Del* loop, formed by normal homolog, indicates no sequence in mutated homolog to complement with.

Fig. 1. Deletion loop.
By D.B. Pylypiv.

In 1927, H.J. Muller, the Nobel Prize in Physiology or Medicine 1946, discovered that the number of mutations in X-rays treated fruit flies increased. He found that the higher the dose of X-rays and other ionizing radiation the flies were exposed to, the greater the number of mutations that occurred [10]. The ionizing irradiations, especially, γ and X rays are the most powerful mutagens, that cause *del*s.

The chrs with *del* can never revert to a normal state. In 2n organism harmful and lethal recessive mutations are masked by the dominant alleles. When normal dominant gene lost, recessive can manifest itself. A large amount of *del* would result in immediate abortion. The demonstration of effects of mutated recessives when normal alleles deleted is called *pseudodominance.* It allows us to locate the position of the genes on the chrs and build *cytological maps,* that correspond well to the *linkage maps*.

Many of human genes were mapped using *in situ* hybridization method combined with *del* panel, produced by growing of human cell lines with different *del*s. The principle of the method is in complementary joining of labelled DNA fragment to the chr or not joining to it, if complementary fragment was deleted [8].

The *del*s are recognized by: 1) lack of reversed mutations 2) recessive lethality 3) pseudodominance 4) formation of *del* loops [8].

It is interesting, that sperm cells of human or animals are functional even if they contain some chrs with *del*s, however, pollen from 2n plant is abortive, if it contains *dels*. The ovules and pollen of polyploid plants are tolerant to *dels* [8].

Some *dels* and their mixtures are consistently found in cells of solid tumors. Their contribution to the cancer's development is not clear yet.

Del results, but not always, in the loss of important gene (*partial monosomy*). *Del* is always an unbalanced and noted as "del", followed by a bracket with the chr number, and a second bracket with the breakpoint(s) and the deleted region (*e.g.*, del(5)(q14q34)); 2 breakpoints are recorded when the deletion is clearly interstitial; only 1 breakpoint is noted when the deletion is terminal. A true terminal *del* would leave the chr without a telomere. However, this is not the case. The terminal *del* is, usually, interstitial, being capped by a telomere [11].

Constitutional del is present since birth in autosome or sex chr. Deletion in an autosome has major phenotypic consequences, *e.g.*, del(18p); del(18q); del(4p): Wolf-Hirschhorn syndrome; del(5p): 'Cri du chat' syndrome. These profoundly handicapped persons can't transmit their *dels* to any offspring. The rearrangement most often occurs *de novo* (only 10 to 15% of *del* cases come from the malsegregation of a parental rearrangement. The *del* may be accompanied by *partial trisomy* of another chr (*duplication/deficiency*). *Del* in sex chr (gonosome) causes problems in sexual differentiation and gametogenesis (except distal Yq *dels*), *e.g.*, del(Xp): Turner syndrome.

Acquired del appears *de novo* during an organism's life, e.g., the loss of a tumour suppressor gene (e.g.: del(13)(q14.00q14.09): retinoblastoma) [11].

Duplication (*dup*). In *dup* a gene, region of the chr or whole chr is duplicated, resulting in extra genetic material, which can mutate or contribute to the same or new function. Usually, changes in one copy of the duplicated region is tolerated, as necessary for organism function is provided by another copy of the region. Thus, once-identical genes undergo evolutionary changes and diverge in 2 different genes [7, 8].

The *dup* regions can be adjacent to each other, be on some distance on the same chr or even on another chr. The adjacent *dup* sections can be in tandem (AB**CDCD**EF) or reversed (AB**CDDC**EF) order. The *dup* segments may attain different loop-like configurations during pairing in meiosis in heterozygotes for adjacent *dup* (Fig. 2). Asymmetric alignment followed by unequal crossover at meiosis increases the order of *dups* in homozygous for *dup* organisms [8] (Fig. 3).

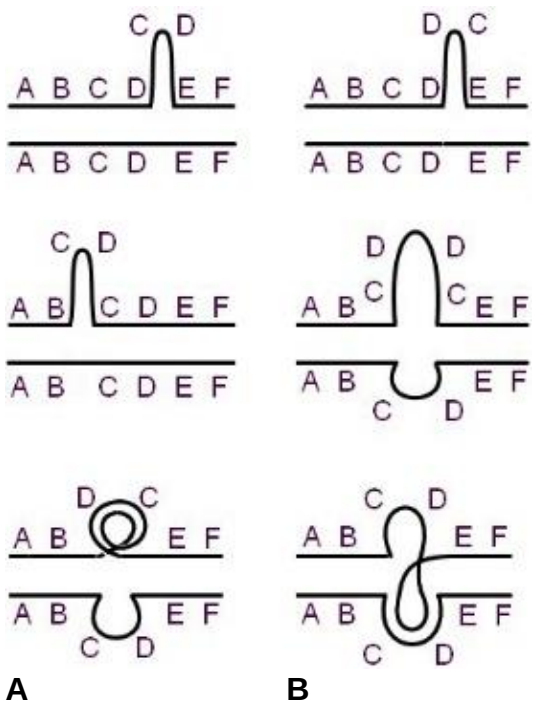


Fig. 2. Pairing structures of normal chromosome and chromosome with duplication region in tandem (A) and reversed order (B). *By D.B. Pylypiv.*

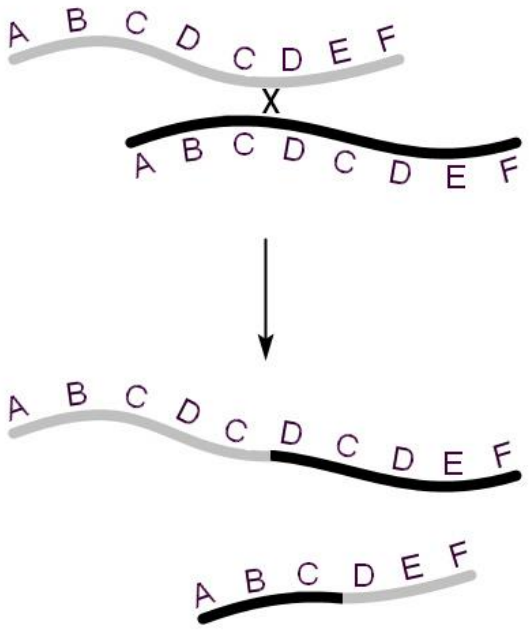


Fig. 3. Triplication in duplication homozygote due to assymetric alignement of chromosomes followed by crossingover. *By D.B. Pylypiv.*

An example is globins family, that includes, particularly, myoglobin and hemoglobin (Hb) genes. Human organisms have different Hbs composed of different subunits at different ages, *e.g.*, an adult has two α and two β subunits and fetus has two α and two γ subunits. The subunit production is coded by related gene loci (Fig. 4**)**, some of them are linked, some are not, however, all, obviously, originate from one ancestral globin gene due to *dups*.

People with certain thalassemias, the heritable blood diseases, have a Hb subunit that is part γ and part β (Kenya Hb) or one that is part δ and part β (Lepore Hb). Possibly, unequal crossover in γ-δ-β region result in hybrid gene loci production that code for hybrid subunits [21]. The *dup* and *del* occur in both aberrations (Fig.5).

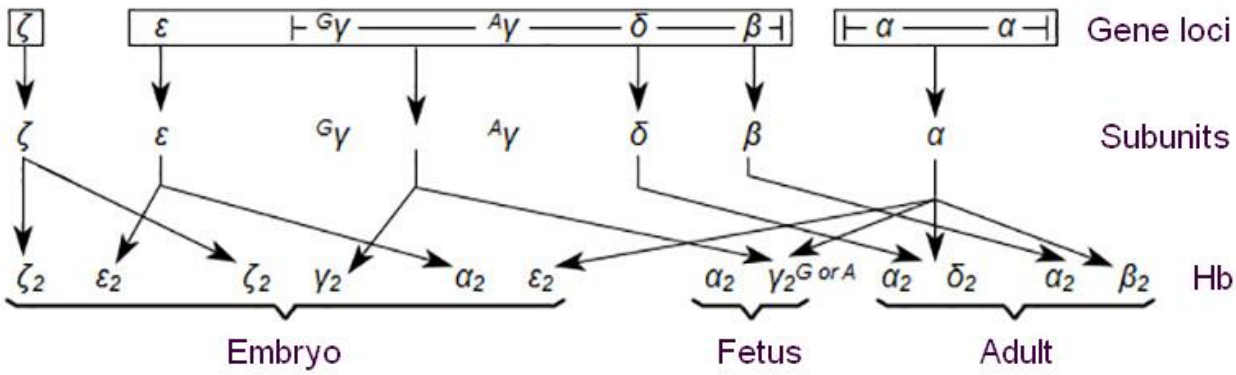


Fig. 4. Human hemoglobin genes. Each letter indicates a different gene locus and subunit. The subunits associate in particular combinations for particular period of life, providing the organism with functional hemoglobin molecules.
Adapted from Weatherall D.J. and Clegg J.B., 1979 [22]

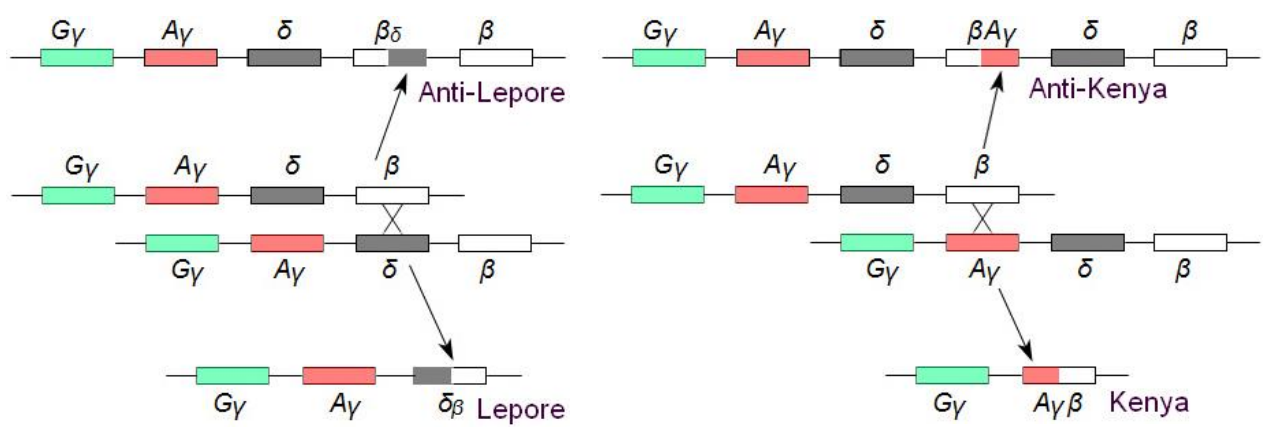


Fig. 5. Production of Lepore (A) and Kenya (B) variants of human hemoglobin subunits. *Adapted from Weatherall D.J. and Clegg J.B.,* 1979 [22]

Most *dups* in humans consist of a chr arm or part of the arm *dup* and attachment to non-homologous chrs. The abnormalities resulting from *dups* are regarded as consequences of genetic imbalance [7, 8, 11]. Usual sources of *dups* include: ***ectopic recombination***, ***replication slippage***, ***retrotransposition, aneuploidy, polyploidy.***

In ***ectopic recombination*** (*Er*) crossover occurs at non-homologous, rather than at homologous loci of the *chrs* pair. The exchange between misaligned homologs results in simultaneous reciprocal *del* in one chr and *dup* in another. This change, as a rule, is harmfull, however, in some cases can be useful for organisms [2]. *Er* is, usually, mediated by sequence similarity and direct repeats at the breakpoints. *Transposons* as the sources of repetitive DNA, that facilitate recombination, are often found at *dup* breakpoints. They also can insert *dup* fragments into unusual (*ectopic*) sites. Cells can be induced to uptake DNA *in vitro* and to insert *dup* fragment into chr.

Replication slippage *(Rs)*. In replication DNA polymerase makes a copy of the DNA strand. At some point of the process, the polymerase dissociates from the

DNA strand and replication stalls. Then this enzyme reattaches to the DNA, however, by occasion, not in the correct place. This results in copying the same region of the strand. *Rs* is elevated by presence of repeats, but only a few bases of similarity needed.

Retrotransposition, mainly by L1 (class I) of transposable elements, that can act on mRNA by reverse transcribing it to DNA and inserting randomly into the genome as *retrogenes*. As a rule, resulting sequences have no introns and often contain poly regions, that are also integrated into the genome. Many retrogenes, comparing to their parental genes, have changed regulation, which can contribute to new functions.

Aneuploidy as result of nondisjunction, usually, duplicates chrs and their genes. It has detrimental effects on viability. Some aneuploids are viable, *e.g.*, Down's.

Polyploidy or *whole genome* (*n*) *dup* is due to meiotic nondisjunction resulting in additional copies of the genome. Two rounds of entire genome *dup* occurred in ancestors of humans [4]. The entire genome *dup* keeps the same relative dosage of individual genes and is less harmful than aneuploidy. Most duplicates are lost within a short period of evolution, however, a considerable fraction of them survive [14]. Preferential retention of regulatory genes in duplicates, most notably, the group Hox genes, that specify the body plan of an embryo, has led to adaptive innovations [3, 5]. Duplicates are often unable to produce viable young in mating with non-polyploid parent species organisms due to different numbers of chrs in fusing gametes. New species evolve from mating between duplicates only. During evolution whole genomes of several fish and plants have been duplicated.

To write *dup*lication, after "dup" in bracket the chr number is placed and in second bracket the breakpoints (if they are known) and the duplicated region are indicated, e.g., dup(22)(q11.2), the case of *Chromosome 22q11.2 microduplication syndrome*; *dup(7)(q11.2) or 7q11.23 duplication syndrome*; the duplication of genetic material on a specific region on the X chr (Xq28): *MECP2 duplication syndrome* (*trisomy Xq28*).

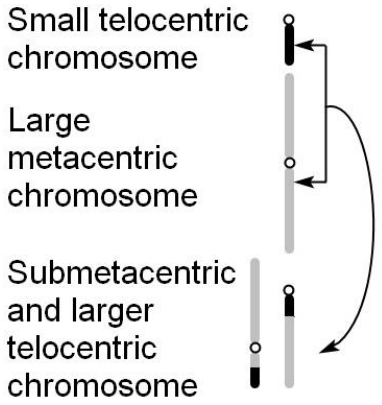


Fig. 6. Reciprocal translocation.
By D.B. Pylypiv.

Translocation (*t*). A portion of one chr is transferred to another chr. *Non-reciprocal t* is unidirectional genes transfer between chrs. The most common are *reciprocal ts*, in which segments of two different chrs exchange (Fig. 6). New linkage between genes is established, when interacting chrs are non-homologous. Gene fusion may be created when the *t* breaks and joins two otherwise-separate genes. The *t* can change the size of chr and positions of its *c* and even number of chrs. It is detected in a karyotype, a preparation of the complete set of metaphase chrs in affected cells. For example, when *t* occurs between large metacentric chr and small telocentric chr it results in submetacentric and more large telocentric chr. *T* can be *balanced* (with even exchange of genetic material with no information and functionality lost or add) or *unbalanced* (with unequal exchange resulting in extra or missing genes and functions).

The chrs pairing during meiosis in heterozygous for reciprocal *t* organism results in 'cross'- like pattern in metaphase plate. There are 2 common types of disjunction: *adjacent-1 segregations* (N1 with T2 and T1 with N2, these meiotic products are *dup*icated, deficient for different regions and often inviable) and *alternate* (T1 with T2 and N1 with N2, these meiotic products are viable) (Fig.7). These are produced in 1:1 proportion and known as *semisterility*. In plants semisterility means 50% of gametes sterile (abortive pollen). In animals gametes are viable, however translocation (duplication-deletion) products have lethal effects in zygotes. The semisterility indicates *t* heterozygotes. Exists rare possibility of homologs migration to the same pole (*adjacent-2 segregation*). New linkages of genes can result from reciprocal *ts*. Thus, reciprocal *ts* are detected genetically by semisterility and by apparent linkage of genes known to be on separate chrs [8].

Ts are important in agriculture, particularly, they decrease crop yield due to abortive pollen production. *Ts* are used for insect pests control by crossing *t* carriers with wild insects, providing 50% lethality in offspring.

The *ts* have medicinal significance also. An example is the reciprocal exchange between the small ('petite', p) arm of chr 5 and large (q) arm of chr 11. The offspring of the parent with this *t* had a *del* at 5p and *dup* in 11 q. They demonstrate '*Cry do chat*' and *11 q duplication syndrome* symptoms.

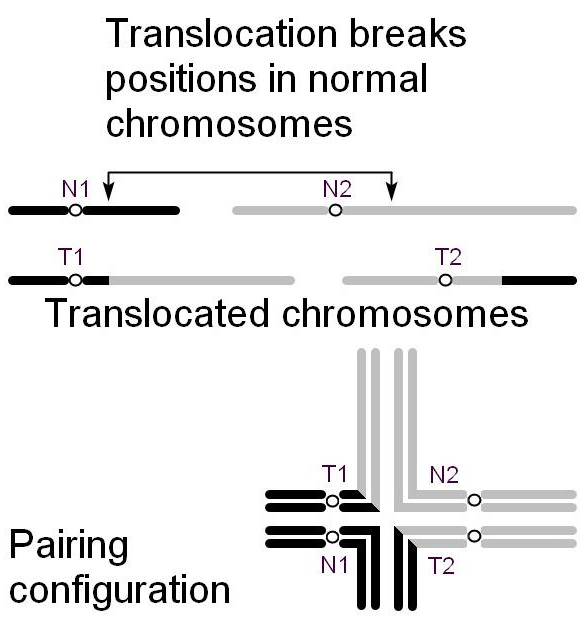


Fig. 7. Cross-like meiotic pairing in reciprocal translocation heterozygote. The metaphase plate is within the plane of the page. In T1-N2 segregants gray parts are duplicated and black translocated parts are deleted and in N1-T2 gray translocated parts are deleted and black parts are duplicated. Both often not viable. The N1-N2 and T1-T2 segregants are complete and viable. *By D.B.Pylypiv.*

In a ***Robertsonian translocation*** (*rob t*), whole arms are interchanged between non-homologous chrs. It is caused by breaks and reciprocal exchange at or near the *cs* of 2 acrocentric chrs. The results are large metacentric chr (composed of almost whole 2 chrs fused together) and 1 very small chr that may be lost with little or no effect to the organism. In case of the small chr loss, the human karyotype contains 45 chrs with no harmful effects, since genes lost on the short arms of acrocentric chrs are common to all of them and present in variable copy number (nucleolar organiser genes) [7]. Each of acrocentric chrs has potential for *rob ts*. Most common *t* in humans is between chrs 13 and 14 chrs (0.97/1000 birth) [1]. Carriers of *rob ts* have no any phenotypic abnormalities, but risk of unbalanced gametes that lead to miscarriages or abnormal offspring. *E.g.*, carriers of *rob ts* of chr 21 long arm onto the long arm of chr 14 have a higher risk of having a "*translocation Down syndrome*" child. The father has a lower (3%) risk of *t* transmission to offsprings,

than mather has (10-12%) [19]. In most cases Down's is the result of 21 chr trisomy and in 5% of cases it results from chr 21 *t* in the parent. Heterozygous for the *t* parent is healthy, however, 1/2 of the gametes produced with *adjacent-1 segregation* in this person contains the duplicated 21 chr segment causing syndrome, when fused with normal gamete in zygote.

As a rule, reciprocal *ts* are harmless and found through prenatal screening. However, carriers (≈ 1/491 live births [16]) have elevated risks of producing gametes with unbalanced chr *ts*, leading to infertility, miscarriages or children with abnormalities. The carriers need genetic counseling and testing, if they decide to have children. In gametes the *ts* occure due to errors in meiosis. With sperm or egg they are passed to zygote and, if life-compatible, to all cells of the child. In some types of infertility one parent carries a balanced *t* and conceived fetuses are not viable. *Ts* between the sex chrs can also result in syndromes, *e.g.* , XX male syndrome caused by a *t* of the SRY gene from the Y to the X chr.

Many human diseases, particularly, solid tumours, are caused by *ts*, e.g., Ewing's sarcoma, synovial sarcoma (Fig. 8).

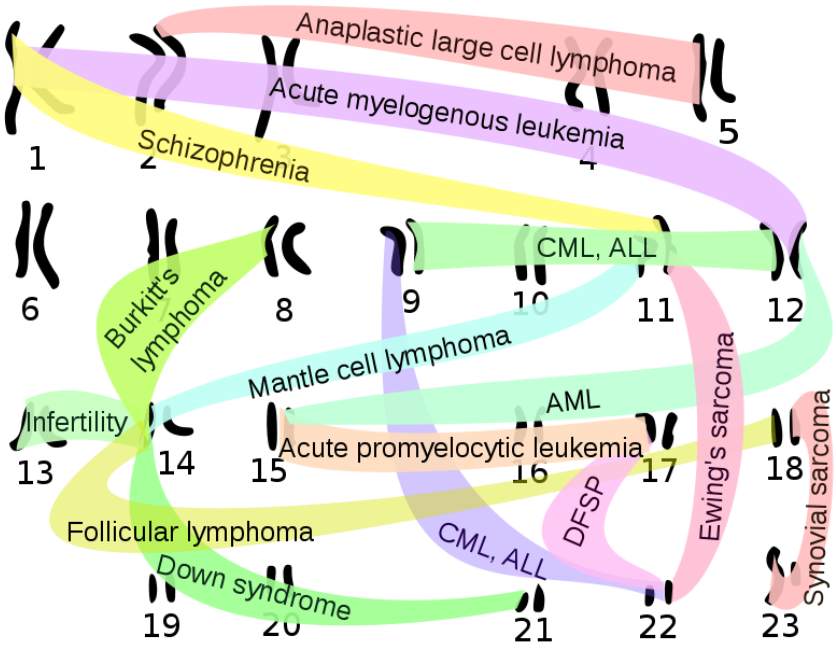


Fig. 8. Diseases resulted from translocations. ALL- acute lymphoblastic leukemia, AML- acute myeloid, CML – chronic myelogenous leukemia, DFSP- dermatofibrosarcoma protuberans. *By Häggström M.* 2014 [9]

How to write a *t* between the chrs according to the International System for Human Cytogenetic Nomenclature (ISHCN)? Let us regard next as an example. Mantle cell lymphoma is a cancer of the "B-cell" lymphocytes, the cells which help the body fight infections. It results from *t* between chrs 11 and 14 that involves large arms (q) that break at the bands 13 (chr11) and 32 (chr 14). Thus, this event is noted as: t(11; 14)(q13; q32).

Here 2 genes are fused: Cyclin D1 gene from chr 11, that gives fusion protein cell-proliferative ability, and immunoglobulin heavy locus on chr 14, that contains a gene for heavy chains of human antibodies and induces massive transcription of fusion protein. These render the changed white blood cells to form tumors in lymph nodes [13]. Soon cancer cells enter blood and lymph vessels and spread to other lymph nodes, bone marrow (blood cells 'factory'), digestive tract, liver and spleen.

Some kinds of cancer are caused by *ts* acquired during life, but not since conception. *Ts* in somatic cells result in abnormalities in the affected cell line only, as in chronic myelogenous leukemia (CML), caused by Philadelphia chr *t* (Fig.9). It is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes (neutrophils, eosinophils, basophils) and their precursors is found. This cancer was 1st among those that were linked to a genetic abnormalities [18]. The Philadelphia chr, was identified by P.Nowell and D.Hungerford in 1960 as unusually small chr 22 in patients with CML. Using the advent of chr banding techniques, J. Rowley (1973) showed that the Philadelphia chr was actually a product of a reciprocal *t* involving small segments at the ends of the q arms of chrs 9 and 22. Molecular analyses revealed that the translocation fused the *BCR* (breakpoint cluster region) gene on chr 22 with the *ABL* gene for protein kinase from chr 9. The BCR-ABL chimeric gene product is a tyrosine kinase that constitutively activates signaling pathways involved in cell growth and proliferation and causes white blood cells to become malignant. Thus, fusion of genes due to *t* resulted in potent oncogen. The investigators were able to use the sequence information to overexpress and crystallize the BCR-ABL tyrosine-kinase and to develop this enzyme inhibitors for effective CML treatment [18].

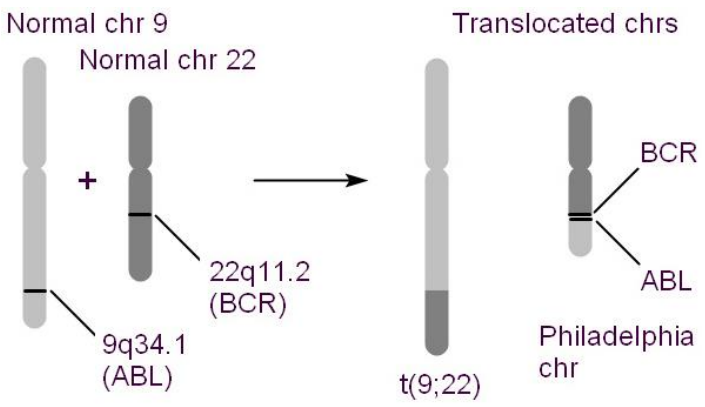


Fig. 9. Translocation t(9;22)(q34.1; q 32), a cause of chronic myelogenous leukemia. The fusion of BCR, the breakpoint cluster region, and ABL, the protein kinase gene, results in potent oncogen production, the BCR-ABL. *By D.B. Pylypiv.*

Isochromosome (*i*) is a chr with all identical arms (all are short (p) or all are long (q) arms). The *i* formation is equivalent to a simultaneous *dup* and *del* of genetic material. There is *partial trisomy* of the genes present in *i* and *partial monosomy* of the genes deleted in the lost arm [11]. An *i* can be abbreviated as "i" with the chr number and arm placed into brackets, *e.g.* , an *i* of chr 17 with two q arms can be noted as i(17q), or i(17)(q10): duplication of the q arm and loss of the p arm [11] and this is the genetic cause of the medulloblastoma, a common primary brain cancer in children [20]. The *i* is also frequent as an acquired anomaly in cancers (*e.g.* i(17q), secondary anomaly in chronic myelocytic leukaemia) [11]. The i(17q) caused monosomy of the p arm and trisomy of the q arm lead to neoplasia. Many tumor suppressor genes candidates are found lost on the p arm, allowing the tumor cell population to be maintained [15].

Both dicentric *i* (2 *cs*) and monocentric *i* (1 *c*) have been found. A U-type strand exchange between sister chromatids at the short arm (produces dicentrics) or misdivision of *c* (produces monocentrics) are likely the mechanisms for the formation of *i* (Fig.10).

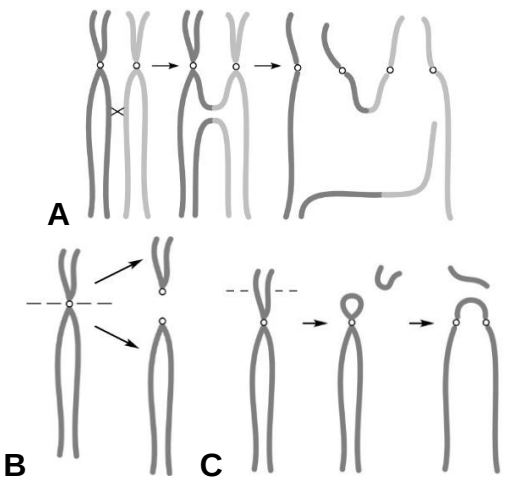


Fig. 10. Production of dicentric isochromosome in meiosis (A) and monocentric (B) or dicentric (C) in mitosis. *By D.B.Pylypiv.*

Thus, there are 2 events, that lead to *i* formation:

1) ***Unproper division of centromere.*** Under normal separation of sister chromatids in metaphase, the *c* will divide longitudinally, (parallel to the long axis of the chr) [12]. An *i* is created when the *c* is divided transversely, *i.e.*, perpendicular to the long axis of the chr (Fig. 10, B).

2) ***U-type exchange*** that can occur in sister chromatids or between homologous chromatids (Fig.10, A) in meiosis or between sister chromatids at the short arm after their truncation (Fig. 10 C). If it produced in 1st meiotic division, the duplicated material is from non-sister chromatids. In somatic cells, the most evident *i* origin is from isochromatid deletion with sisters union at the deletion place with subsequent division of *c* (Fig. 10 C) [11].

The most commonly *i* occures among X chrs (1/13 000) and acrocentric authosomal chrs 13, 14, 15, 21 and 22. Chr containing smaller arms are more likely to become *i* because the loss of genes in acrocentric arms can be tolerated. In 15% of Turner syndrome patients, the structural abnormality is *i* X, i.e, 2 copies of the q arm (i(Xq)) [6]. The *i* 21 can result in a trisomy 21, Down syndrome [7, 8].

Inversion (*inv*). A chr fragment has broken off, turned on 180° and reattached. *Invs* are of 2 types: ***paracentric***, on 1 arm without involvement of the *c* region, and ***pericentric***, that include the *c* and there is a breakpoint in 2 arms. Cytogenetic methods and genetic analysis can detect *invs*, however, small *invs* go undetected. *Invs*, usually, cause no harm in carriers as no extra DNA is add and no DNA is missed. However, there is an increased production of abnormal chrs and *unbalanced gametes* due to crossover within the *inv* segment in *inv* heterozygotes. Some time *inv* breaks a gene important for life and unaffected chr from the homology pair must supply the information for vital function. This *inv* is lethal, if made homozygous. Most of *inv* analyses described for *inv* heterozygotes [7, 8]. The *inv* most common for humans is on chr 9, the inv(9)(p12q13). It has no harmful effects in carrier, but increases miscarriage or infertility risks [17].

During meiosis one chr twists at the region of *inv* to pair with not inverted region of the homolog. The ***inversion loop*** is produced during pairing of homologs [7, 8].

Paracentric invs are rarely detected as they, usually, involve very small segments. The most frequent paracentric *invs* in cases of constitutional anomalies in human involve chrs 3, 7, and 14. Carriers are often fertile, and ≈ 50% of offspring have a normal karyotype, and ≈ 50% have the balanced (parental) rearrangement. There are only a few genetically unbalanced decendants, they are often not viable. Few cases of malformed descendants with an apparent balanced constitution were recorded.

Crossover within the *inv* loop (Fig. 11) produces an acentric fragment (lost) and a dicentric bridge linking the two *cs* of homology chrs at anaphase. The bridge either:

1) disrupts, depending to break sites, with *dup*/*del* regions in the daughter cells, or
2) prevents cell separation and doubles the amount of chrs in daughter cell, or the dicentric will be excluded from both daughter cells to micronucleus, or
3) the dicentric is included entirely into 1 daughter cell [11].

Thus, there will be, at telophase of 2nd division: 1 normal cell, 1 balanced *inv* cell, 1 cell devoid of this chromatid, and 1 cell with the dicentric. This dicentric will either: i) enter the fission-fusion cycle (leading to complex and numerous rearrangements), or ii) prevent diakinesis (leading to a tetraploidy), or iii) inactivate 1 of its 2 *cs*, which would stabilise the rearrangement.

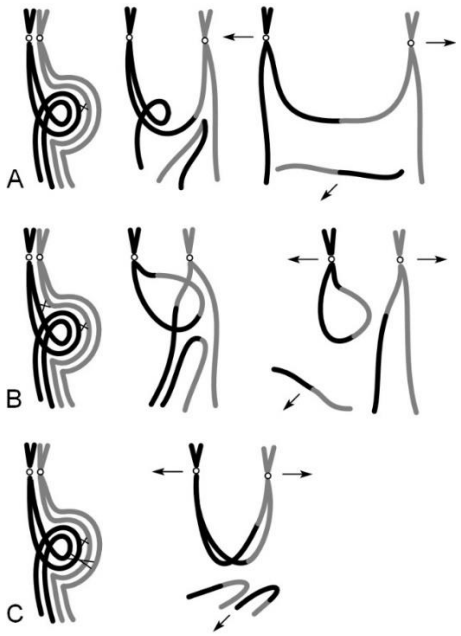


Fig. 11. Paracentric inversion and its meiotic consequences. *By D.B. Pypypiv.*

Other crossovers than pictured in (Fig. 11 A) are possible, some lead to 100% of unbalanced products (Fig. 11 B, C) [11].

Let us regard the genetic forms pictured in Fig 11, A in more details. When chrs separate in anaphase 1, the *cs* remain linked by the bridge. The acentric fragment is lost, because it can't align itself or move. The tension finally breaks the bridge, resulting in 2 chrs with terminal regions loss (Fig. 12).

The sperm or egg cells with this *dels* are not viable and even if viable, their fusions produce zygotes that are not surviving. Natural selection favours normal gametes or those with balanced *inv*. Thus, crossover, which usually produces recombinants, in this case generates lethal products. This decreases the recombinant frequency (RF) for genes within the *inv* region to zero. For genes flanking the *inv* the RF decreases in proportion to *inv* size. The *inv* heterozygotes have mechanical problems in pairing of normal chr with inverted homolog and this decreases the possibility for crossover and recombination also [8].

***Pericentric invs*.** Some pericentric *inv* are frequent, and are called chr variants, e. g., inv(Y) found in 1 to 2/1000 males or inv(9)(p11q13) found in 1/400 individuals (varies with geography). Offsprings with unbalanced forms are rare (crossover in heterochromatin is unusual). A pericentric *invs* can result in miscarriages, sterility (more often in the male), and lead to unbalanced meiotic products.

During meiosis, crossover in the *inv* loop will produce recombinant chrs (*rec*) with *dup* of one segment and *del* of another (a *duplication p - deficiency q* will be recorded as "rec dup(p)"). The *dup-del* segments are those outside of the *inv* loop (Fig. 13). If gametes carrying crossover chrs fuse with normal nucleus, the resulting zygote dies because of its genetic imbalance. If the *inv* is large, the probability of crossover in the *inv* loop will be higher, and *dup-del* segments smaller. However, the risk of the

abnormal conceptus is higher. Conversely, a small *inv* has a lower probability of crossingover in the small *inv* loop. Larger *dup-del* segments will have a stronger negative selection pressure, and the risk of a malformed offspring will be lower, than in smaller segments [11].

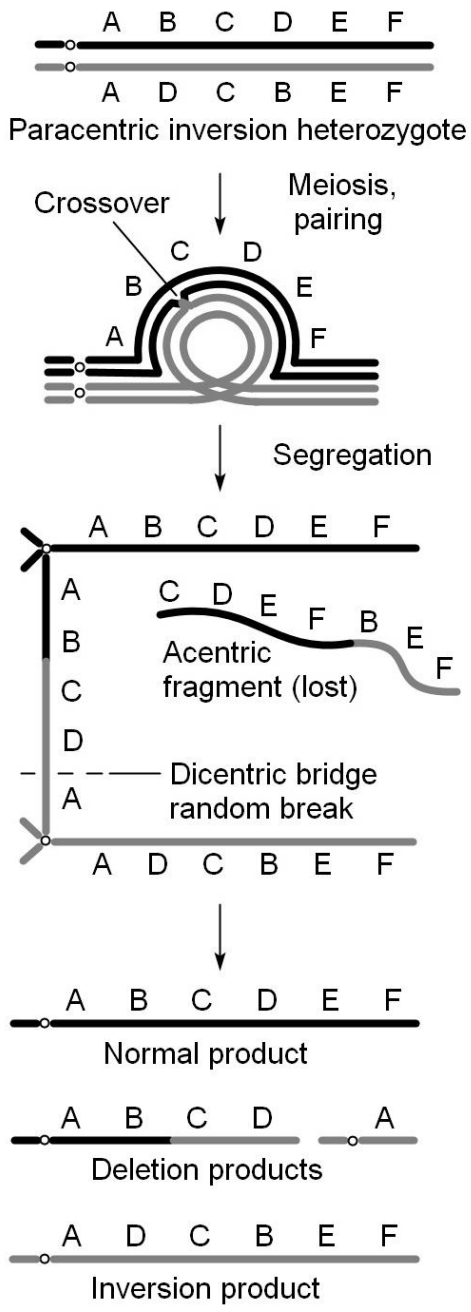


Fig. 12. Products of meiosis with a single crossover within a paracentric inversion loop.
By D.B.Pylypiv.

Next is worthwhile to note for paracentric and pericentric *invs*:

1) crossover outside the *inv* segments (out of the loop) has no harmfull consequences

2) two (or an even number) of chiasmata within the loop cancel each other

3) wherever the crossover occurs in the loop, the result will be the same: the selective recovery of non-crossover chrs in viable offspring

4) There are no *inv* loops in *inv* homozygotes. The homologous *inv* chrs pair and crossover normally, without bridges and meiotic products are viable. However, their linkage maps show inverted order of the genes [7, 8, 11].

Geneticists use *inv* for production of *dups* and *del*s in properly mapped chrs for experimental purposes. Particularly, it is possible to generate viable nontandem duplication using pericentric *inv* at the chr tip, if genes at the tip are non-essential (Fig. 14, A), or use overlapping *invs* (Fig. 14, B).

Mitotic chrs analysis is also used for *inv* studies. Here scientists are looking, particularly, for new arms ratios as results of *invs* [8].

Some meiotic *dup*/*del* products are so abnormal, that cause spontaneous abortions. To study these mutated chrs in male gametes the interaction of human sperm and zona pellucida free golden hamster eggs is used [21]. Human spermatozoid penetrates the egg *in vitro*, however, nuclei do not allowed to fuse. When mean number of spermatozoa per penetrated egg is low (1.0-3.3) after insemination *in vitro*, further incubation results in activation of spermatozoa to metaphase. Penetrated eggs are incubated overnight with colchicine to arrest chrs of sperm cells in this stage. It allows observation of male chrs in egg as separate group. High sperm/egg ratios, 4.2 and 7.5, yielded no sperm chrs [23]. This method is useful for studies of mutated chrs in sperm cells [8].

Insertion or **insertion mutation** (*ins*) is the addition of one or more nucleotide base pairs due to DNA polymerase slippage (often in microsatellite regions) or even section of the chr due to unequal crossover during meiosis. An internal segment of a

chr is deleted and transferred to a new position in other chr, or into its homologue, or in another place within the same chr. The *ins* fragment may be positioned with its original orientation or inverted. As a rule, this is a balanced rearrangement. Recorded as "ins", followed by a bracket with the number of the chr which receives the segment preceding the number of the chr which donates the segment (if different). A second bracket indicates the one breakpoint where it inserts, followed by the 2 breakpoints which define the ends of the inserted segment. For example, ins(2)(p13q31q34) and ins(5;2)(p12;q31q34): the segment q31q34 from a chr 2 is inserted respectively in p13 of this chr 2, and in p12 of a chr 5 [11].

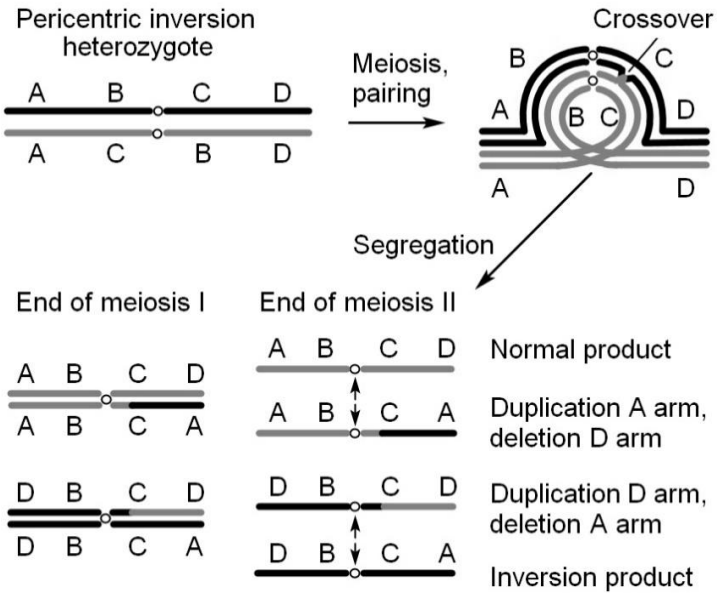


Fig. 13. Products of meiosis with a single crossover within a pericentric inversion loop. *By D.B. Pylypiv.*

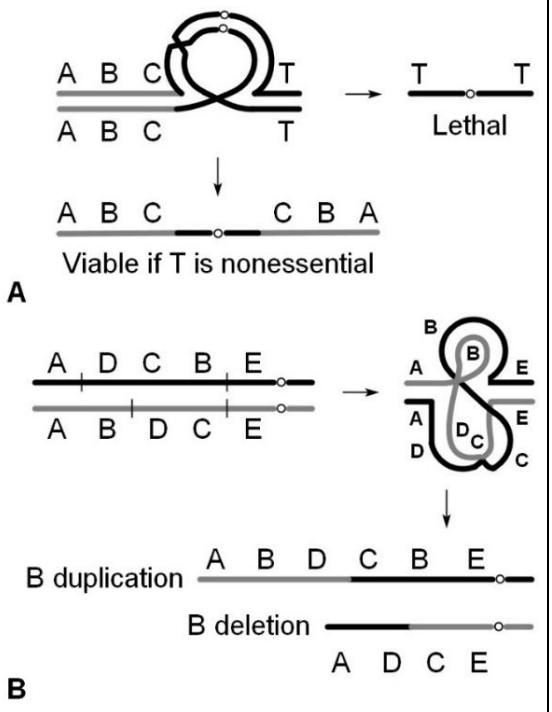


Fig. 14. Nontandem duplication by A) pericentric inversion at chromosome tip and B) by crossover between 2 overlaping inversions. *By D.B. Pylypiv.*

An insertion can be *direct* (*dir ins*) if the segment keeps its orientation in relation to the *c* as it recorded above, or an insertion can be *inverted* (*inv ins*) if the most proximal band becomes the farthest from the *c*, *e.g.*, ins(2)(p13q34q31) and ins(5;2)(p12;q34q31), the distal band number preceding the proximal one [11].

This aberration can be balanced and stable in somatic cells, and be transmitted for many cell generations. However, it is devastating at meiosis. In many cases the inserted segment will not be large enough to cause the formation of a quadrivalent. Even so, random segregation at meiosis 1 results in half of all the gametes imbalanced.

If the segment is large enough and permits occasional quadrivalent formation, then, 25% of gametes will be normal if the insertion is direct, but none if the insertion is inverted (dicentric bridge and acentric fragment to complicate the situation) [11].

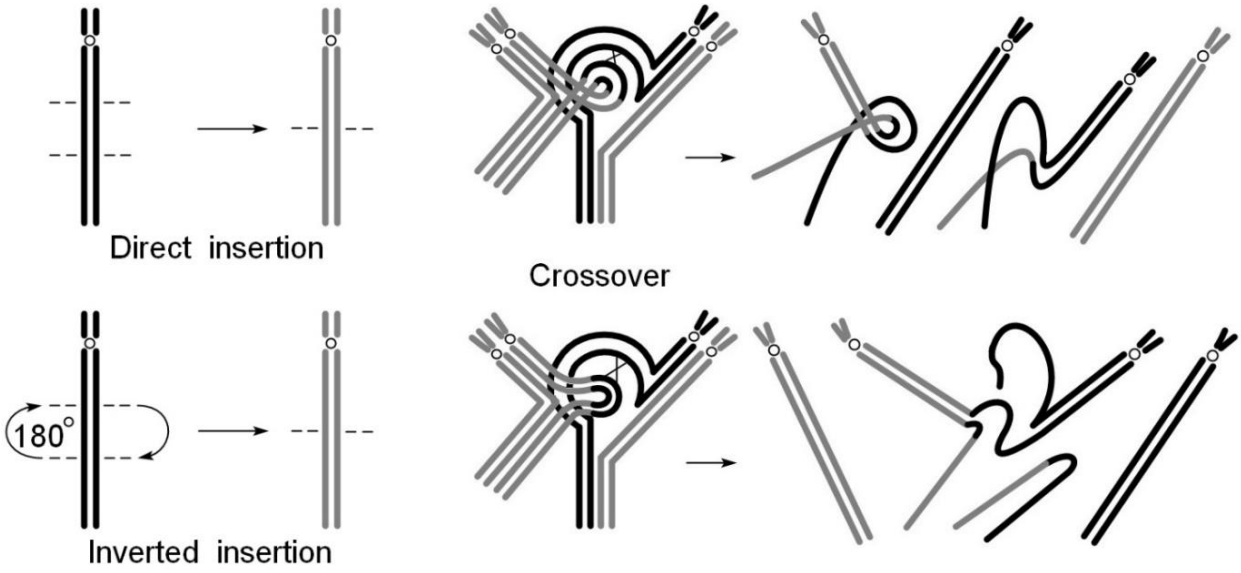


Fig. 15. Direct and inverted insertion and single crossovers results.
By D.B. Pylypiv.

Ring (*r*): a portion of a chr has broken off and formed a circle or *r*. This happens with/without loss of genetic material. *Rs* can be produced with *cs* or without them. Persisting rings are always with *c*. A centric ring involves the *del* of the tips of both arms and rejoining of the median segment to itself in a circle (Fig. 16 A, B). Although the terminal segments lost may not contain vital information, the *dups* often lead to mechanical problems at mitosis, accompanied by continuous changes in *r* size and composition [11]. If sister-chromatid exchanges follow chr replication (Fig.17), the *r* can form as dicentric *r* (two *cs*), or a pair of interlocked *rs* which will lead to bridge breakage and loss at anaphase of mitosis.

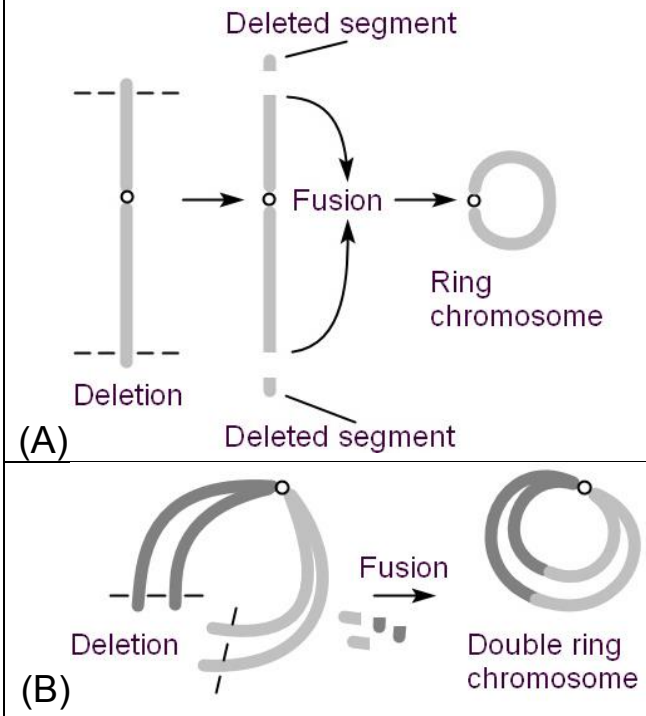


Fig. 16. Ring chromosome formation.
By D.B. Pylypiv.

The ensuing fusion-fission cycle leads to variable ring sizes and additional *dups* and *del*s of genetic material. Multiple and inter-locked *rs* can also be produced. *Rs* arise most often *de novo*, and are rarely transmitted to descendants (because a *r* is unstable, cell divisions lead to impaired gametogenesis). *Rs* formation increases the frequency of mosaicisms. The effects on the phenotype are therefore variable, with signs of trisomy or of *del*. In humans, the *r* is most frequent in chr 13. The *r* formation can be written as "*r*", followed by a bracket with the

number of the chr, and a second bracket indicating the breakpoints, if they are identifiable (e.g., r(13)(p12q33). However, due to the instability of *r* composition, break-point indications may be inaccurate or represent the starting breakpoints [11].

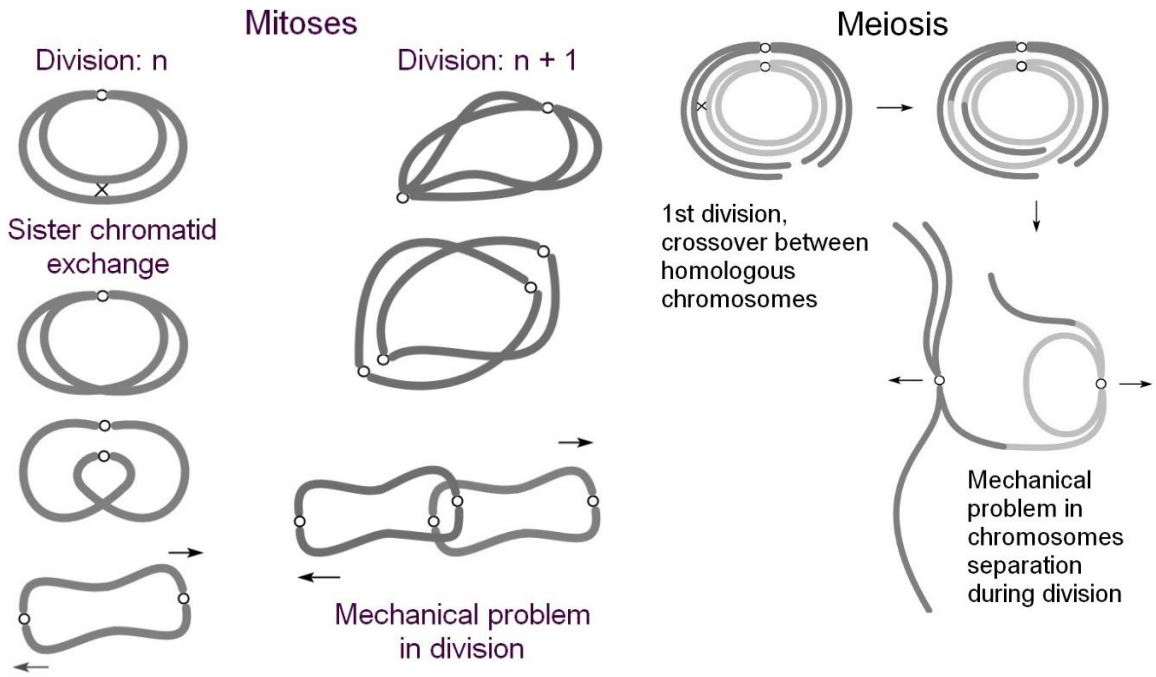


Fig 17. Rings effects on cell divisions. *By D.B. Pylypiv.*

Dicentric (*dic*) a 2 *cs* chr, can result from reciprocal *t* and several other mechanisms. It is an unbalanced abberation, leading to mechanical separation problems at anaphase (due to "bridges"). Recorded as "dic", or "psu dic" (pseudo dicentric), when one of the *cs* inactivates, precluding anaphase bridge formation. Inactivation depends from distance between the *cs.*

Persistent *dics* are frequent in *rob ts*, very rare as a constitutional anomaly, unless the short arm of an acrocentric is involved. Rare as an acquired anomaly. Dicentrics (other than *rob ts*) are highly unstable unless: 1) one of the *cs* inactivates 2) the distance between *cs* is very short so that the 2 *cs* act as one. The only proofs of the presence of 2 active *cs* are: 1) the presence of bridges at anaphase 2) the presence of non-disjunctions 3) the presence of isochrs from each of the 2 chrs, resulting from breaks in the bridge with lateral fusion (sister union fusion between chromatids).

Proof that inactivation of one *c* occurred is obvious when 2 chromatids are separated instead of being tightly attached at the *c* location (premature separation of a *c* region may also result from several other causes - reduction in paracentric heterochromatin for one, so this "proof" is not absolute) [11].

Complex rearrangements involve more than 2 chrs and/or more than 3 breakpoints. These aberrations are more frequent than it was expected. Many changes in cancer cells are of this type [7, 8, 11].

Marker is a non-recognisable, persistent chr, recorded as "mar" a subject in prenatal diagnosis. This can be either small supernumerary element in the constitutional karyotype, with/without phenotypic effects or a variable sized, often big

element in a cancer cells. As it shown by fluorescence in situ hybridization FISH (a method locating a specific DNA sequence on a chr using small fluorescently labelled complementary DNA probe), these are highly rearranged chrs, involving many participants and many breakpoints [11].

Double minute abbreviated as "dm". These appear as small, usually paired dots. Often numerous, but they are acentric and segregation is irregular and numbers vary. In the simplest case they represent interstitial *del*s, and would normally be rapidly lost from a cell population. In some mouse cell lines the supernumerary chrs and multiple *dm* are inter-changeable aberrations [11]. The *dm* may indicate (onco)gene amplification, particularly, in solid tumours.

Homogeneously staining region (hsr) is the variable sized, often important material that is multiply duplicated in (a) chr(s). *Hsr* regions can be produced in response to chronic exposure to certain toxins [11]. The *hsr* may indicate (onco)gene amplification, particularly, in solid tumours also.

Solved problems

Problem 1. The chr A) abcdefg(o)hijklmn is normal with centromere "(o)". The rest of chrs are produced due to abberations in A: B) abcdefg(o)hijkl C) dcbaefg(o)hijklmn D) abc(o)gfedhijklmn E) abcabcdefg(o)hijklmn F) abcfg(o)hijklmn G) abcdefg(o)hijklmncba H) abcdefg(o)gfedcba. Recognize them.

Solution. B) terminal *del*etion C) paracentric inversion D) pericentric inversion E) tandem *dup*lication F) interstitial *del*etion G) reversed *dup*lication H) isochromosome

Problem 2. You need to do cytogenetical analysis and chr in (A) is normal and "o" is a ***c***. Presume, that all the rest of chrs are produced from A) by only one, in each case different aberration. What kind of structural change (***dup***, ***del***, *paracentric* ***inv***, *pericentric* ***inv***) happened in each case?

A) ----------o---------- B) ----------o---- C) ----------o----------

D) -----o---------- E) ----------o----------

Solution. B) pericentric inversion C) *del*etion D) *dup*lication E) paracentric inversion

Problem 3. The translocation Down syndrome is the case, when somatic cells of the body contain 2 normal chrs 21 and large translocated fragment of chr 21 onto the 14 chr or to other chr numbers like 13, 15, or 22. In some cases, two 21 chrs can be attached to each other. A parent-carrier that has large fragment of the chr 21 translocated to chr 14 mates with normal chrs parent. What proportion of their normal children can be a carriers? What is the probability of translocation Down syndrome in this family?

Solution. The offspring genotypes depend from combination of chrs segregated into gametes durig meiosis in parents. Segregation during meiosis in father can be adjacent or alternate. The probability of children with Down syndrome in the family is 25%. These are the individuals with one normal 21 chr from mother (21m) and one normal chr 21 from father (21f) and chr 14 containing large translocated fragment of chr 21. Thus, large fragment of chr 21 is repeated 3 time. This unbalanced combination leads to Down syndrome. Miscarriages comprise 1/4 of all conceptions.

These are all with unbalanced chr14 present in triplicate. Carriers are healthy as they have balanced translocation between chr 14 and chr 21, their probability is 25% (or half of all healthy children). The probability of children without translocation between chrs 14 and 21 is 25%.

Problem 4. The behavior of chrs in laboratory animal with a heterozygous reciprocal translocation is as follows: a) alternate segregation takes place 70% of the time in the non-crossover meiocytes; and b) alternate segregation occurs 45% of the time in the meiocytes when a crossing-over takes place in one or both of the interstitial segments (the region between the **c** and the point of chr exchange in the center of the cross). If 5% of the meiocytes result in a crossing-over in at least one of the interstitial segments, what percent of the gametes produced would be the result of alternate or adjacent segregation?

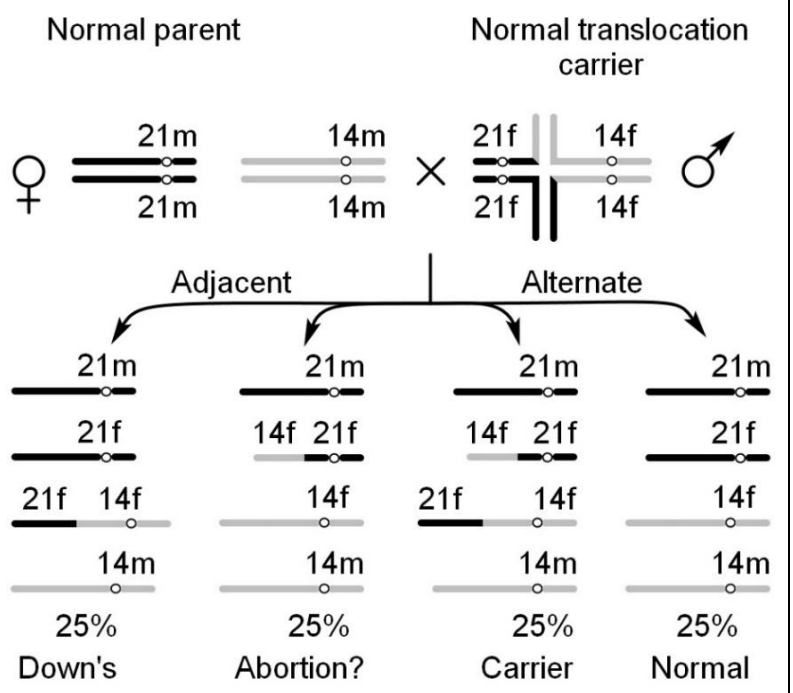


Fig. 18. Translocation Down syndrome, father is translocation carrier. *By D.B. Pylypiv.*

Solution: In crossover meiocytes: 0,45 × 0,05 = 0,0225 alternate segregation and 0,55 × 0,05 = 0,0275 adjacent segregation. In non-crossover meiocytes: 0,70 × 0,95 = 0,665 alternate segregation and 0,30 × 0,95 = 0,285 adjacent segregation. Thus: 0,665 + 0,0225 = 0,6875 = 68,75% alternate segregation and: 0,285 + 0,0275 = 0,3125 = 31,25% adjacent segregation.

Problem 5. When strain of *Neurospora crassa* with reciprocal translocation was crossed with normal strain. The following ascospore groups were detected in asci: most numerous were those in which all 8 spores are white (a) or black (b). Only few of asci contained 4 white and 4 black spores (c). Explain these results.

Solution. We observed a) 8 white spores, 0 black b) 8 black spores, 0 white c) 4 white, 4 black. During meiosis the fungus *Neurospora* produced groups of 8 ascospores, that can be oserved within each of asci. Deletions genotypes render the spores to be not viable. These can be detected by no melanine synthesis (white spores) (a). They are produced from adjacent 1 segregation meiosis. Asci containing them are numerous. Viable spores with normal chr set sythesise this pigment (black spores) (b). They are produced from alternate segregation meioses Asci containing them are numerous also. The least in number are asci (c) containing 4 white and 4 black spores, the product of crossover between the centromeres and the translocation breakpoint.

Problems for homework

Problem 1. Compouse a table, containing drowings of the structural aberrations of chrs, their complete and shortened (according to ISHCN) names and distingwishing characteristics. Provide examples of the diseases caused by these aberrations.

Problem 2. Two genes are 30 m. u. apart on the same arm of the chr. The paracentric inversion involves 1/3 of the distance between them. What recombination to expect if the organism is a) homozygous b) heterozygous for this aberration?

Problem 3. Determine the products of a single crossover within the inverted region of a homology pair chrs with the gene order *E F G H* in one chr and *e g f h* in the another, if inversion is a) paracentric; b) pericentric.

Problem 4. The genes *G, H, I, J, K,* and *L* are closely linked, but their order in chr is not known. The deletions in this region of the chr uncovered recessive alleles of the genes as follows: deletion 1 uncovers *g, h,* and *j*; deletion 2 manifests *g, j, i,* and *k*; deletion 3 opens *k* and *l.* Estimate the order of the genes. There is not enough information to order some of the genes. What can be done to complete the task?

Literature

1. Anton E., et al. // Hum. Repr.- 2004.- Vol. 19, N6.- P. 1345–1351.
2. Bush G.L., et al. // Proc. Natl. Acad. Sci. USA.- 1977.- Vol.74.- P. 3942-3946.
3. Davis J.C., Petrov D.A. // Trends Genet..- 2005.- Vol.21, N10. – P. 548–551.
4. Dehal P., Boore J.L. //PLOS Biol.- 2005.- Vol.3, N 10.- e314.doi:10.1371.
5. Freeling M., Thomas B.C. // Gen. Res.- 2006.-Vol. 16, N7.- P. 805–814.
6. Gersen S.L., Keagle M.B. (Eds.) The principles of clinical cytogenetics (3rd ed.). Springer, - New York, 2013.- 1000 p. ISBN 1441916881.
7. Goldberg M.L., Fischer J.A, Hood L., Hartwell L.H. Genetics: From Genes to Genomes.-7th ed.- New York: McGraw-Hill Education, 2021.- 1000 p.
8. Griffiths A.J.F., Doebley J., Peichel C., Wassarman D.A. Introduction to Genetic Analysis Twelfth Edition.- W.H. Freeman, 12th ed., NY, 2020.- 816 p.
9. Häggström M. // Wiki J. Med. 2014.- Vol.1, N2. DOI:10.15347/wjm/2014.008.
10. Hermann J. Muller.- https://www.nobelprize.org/prizes/medicine/1946/muller/fac.
11. Huret J-L., et al.– http.www:atlasgeneticsoncology.org/Educ/PolyMecaEng.html.
12. Kothari M.L., Mehta L.A., Roychoudhury S.S. Essentials of human genetics (5th ed.). Hyderabad, India: Universities Press, 2009.- 488 p.
13. Li J.Y., et al. //Am. J. Pathol.- 1999.- Vol. 154, N5.- P.1449–1452.
14. Lynch M., Conery J.S. // Science.-2000.- Vol. 290 N5494.- P. 1151–1155.
15. Mendrzyk F. et al. // Gen.Chromos. Cancer.- 2006.- Vol.45, N 4.- P. 401–410.
16. Milunsky A., Milunsky J.M. Genetic Disorders and the fetus: Diagnosis, Prevention and Treatment.- Wiley-Blackwell, 8th ed.- Hoboken, NJ, 2021.-1392 p.
17. Muthuvel A., et al. // Niger. Med. J.- 2016.- Vol 57, N2.- P. 142–144.
18. Nowell P.C. // The J. of Clin. Investig.-2007.- Vol. 117, N 8.- P. 2033–2035.
19. Rogers K.-https://www.britannica.com/science/Down-syndrome.
20. Roussel M.F., Hatten M.E. // Curr. Top. Devel. Biol.- 2011.- Vol.94.- P. 235–82.
21. Rudak E., et al. // Nature, London.- 1978.- Vol.274.- P.911-913.
22. Weatherall D.J., Clegg J.B. // Cell.- 1979.- Vol.16.- P. 467–479.
23. Wramsby H., Hansson A. // Arch.of Androl.-1984.- Vol.12, N1, P. 79-83.

Practical 10. Chromosome banding and karyotyping

Theoretical background. The lymphocytes, fibroblasts or other cells from donor organisms need to be cultured to have enough of them with mitotic condensed chromosomes (chrs). The incubation with microtubule-disruptive agents, *e.g.*, anticancer drugs *colchicine* or *colcemid* stop the mitosis in *metaphase.* Another of such agents, the *nocodazole* arrests the cell in *prometaphase*. Both stages are good for staining of chrs for light microscopy (LM). The staining that produces bands is called *"banding"*.

Too long incubations with colcemid result in overcondensed chrs that band poorly and moreover some cell types, e.g., from the mouse, eventually escape the colcemid arrest and proceed their the cell cycle [6].

Karyotyping is the process of a karyotype preparation from photographs of chrs, in order to study the chr complement of an individual, including the chrs number, sex chrs types and possible structural or numerical aberrations.

To study the *karyotype*, the cell's complete set of metaphase (prometaphase) chrs sorted by size, morphology, *banding patterns* and other characteristics for particular species or organism must be prepared. The bands are defined as sections of a chr which are distinguishable from their neighboring segments by intensity of staining. Each banding pattern is chr specific [21]. It allows quick sorting of all crs in homology pairs and detection of gonosomes. Bands are marked with numbers increase from centromere to telomeres. This allows clear description of structural anomalies, *e.g.*, translocations, deletions, duplications, inversions, etc. Abnormal number of chrs can be easely detected also.

Cytogeneticists rely on a regularly globally updated mapping system that allows to refer very accurately to specific chr portions. The system is standardised, and uses the schematic diagram for each of human chrs, an *idiogram* (Fig.1).

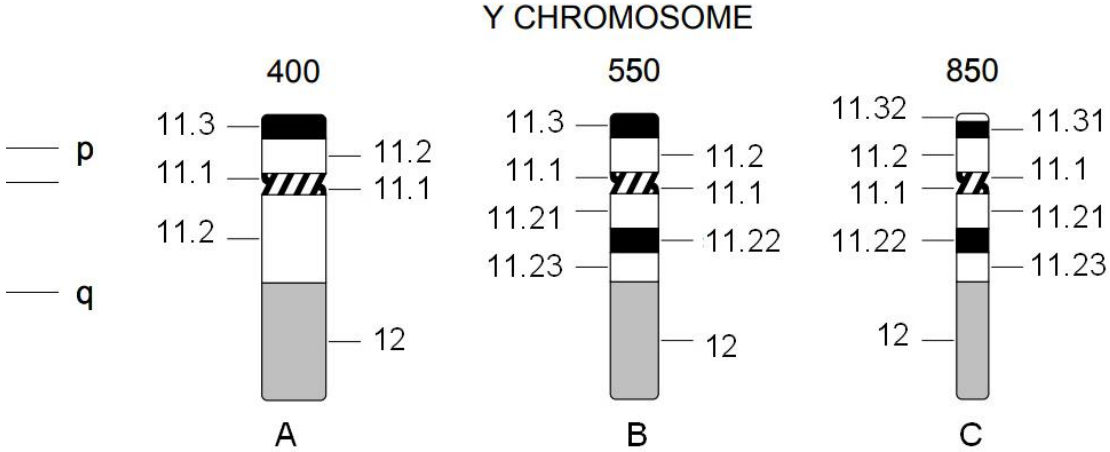


Fig. 1. *Y* chromosome idiogram at (from left to right) 400, 550 and 850 band resolution. *Modified from D. Adler* [2].

Idiograms provide a reference point for locating the positions of genes to particular numbered bands. It helps to identify abnormalities associated with a range of chr disorders. Moreover, idiograms enable us to reference sources, like the Human

Genome Project via universal vocabulary [69]. The Fig. 2 provides the idea for ideogram reeding: regions, bands and sub-bunds. The numbering system for bands and sub-bands follows the Paris Conference (1971) recommendations [28].

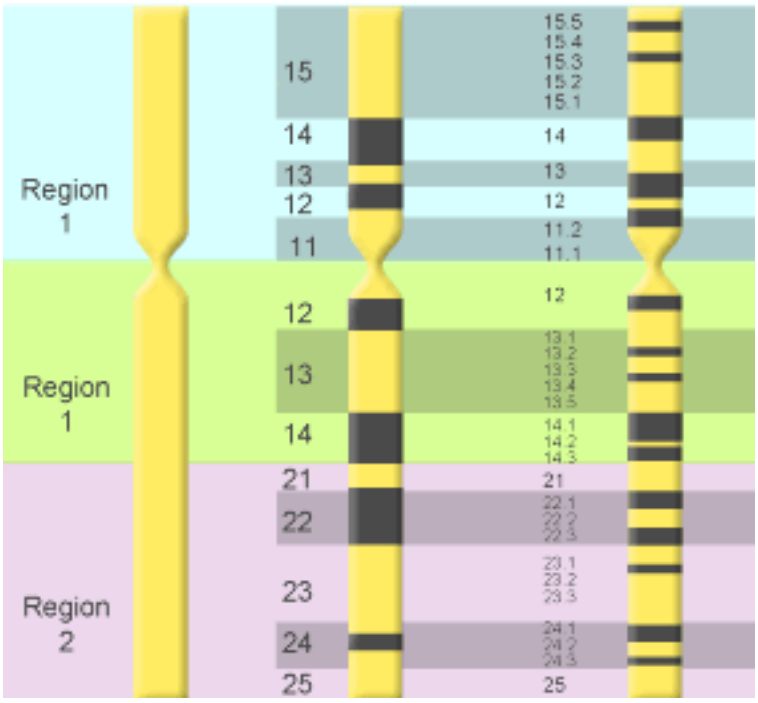


Fig. 2. Ideogram explaining genetic notation.
By Rolf V., Fowler M., Rekas M., Hurt L. [77].

Whole karyotype can be presented as an ideogram showing morphological features of the chrs ordered in a series of decreasing size. The chrs are identified by their structure (size, position of centromere, etc.) and by *banding pattern* they produce in different staining methods. Based on structure human chrs are categorised into 7 groups: A (chrs 1-3, large metacentric 1,2 or submetacentric), B (chrs 4,5 large submetacentric, all similar), C (chrs 6-12, *X* medium sized, submetacentric), D (chrs 13-15 medium-sized acrocentric plus satellites), E (chrs 16-18 short metacentric 16 or submetacentric 17,18), F (chrs 19-20 short metacentrics), G (chrs 21, 22, short acrocentrics with satellites. *Y* no satellites).

In most banding methods, *e.g.*, Giemsa (G-), quinacrine (Q-), reverse (R-) bandings, the chrs appear as rods with a series of alternate bright (negative) and dark (positive) bands. Some methods detect only specific parts of chrs, *e.g.*, *centromere regions* by C-banding; *nucleolus organizer regions* (NORs) by $AgNO_3$ staining; secondary constriction (*h region*) of human chr 9 by Giemsa 11 (Table 1).

Higher resolution views of the chr, with sub-bands of bands detection can be produced, if stain cells in prometaphase or prophase, when chrs are less condensed [28, 68] Even the narrowest band contains over million of base pairs, enough to code hundreds of genes [93].

Single stain banding techniques.

Giemsa banding (G-banding), named after German chemist- bacteriologist G.Giemsa, is traditionally used as a simple, reliable staining for chrs [11, 38, 64, 86, 89, 90].

First, the metaphase spreads are treated with proteolytic enzyme *trypsin*. Other pretreatments include chrs incubation in hot-saline citrate or in detergent, or urea solution. Then, chrs with partially digested protein are stained with Giemsa dye (a mixture of Azure B, methylene blue and eosin). It attaches mainly to the chr *heterochromatin* regions that are AT-rich (*i.e.*, contain numerous adenine and thymine base pairs), relatively gene-poor and late in replication. This results in the appearance of dark ("positive") bands of chrs in LM. The *euchromatin* regions are less

condensed, GC-rich (*i.e.*, contain a lot of guanine and cytosine base pairs) more active in replication and appear in LM as less stained bright segments ("negative bands").

Table 1. Examples of most used chromosome banding techniques

Methods	Band type	Use in studies	Reference
Trypsin-induced Giemsa	G	Hetero- or euchromatin, chr aberrations, e.g., t(9;22)(q34:q11) - Philadelphia chr	[90, 86]
Quinacrine, Hoechst 33258)	Q	Light fluorescence in dark G-bands, some centromere regions, distal q-arm of Y chr	[6, 60, 67]
Centromere stain	C	Centromere region; isochromosomes; with T-banding for dicentric rings; parental origin of foetal chrs	[12, 60, 95]
Reversed Giemsa (R-Giemsa)	R	Telomere deletion, translocation; chr size, aberrations, e.g., t(9;22)(q34:q11)	[22, 60]
Telomere	T	aberrations at the ends of chrs; human chr 22 and its translocations; dicentric rings undetectable by other procedures	[79]
Bromodeoxyuridine (BrdU) for two cell cycles	SCE	differentiation of sister chromatids, detection of their exchange and sites of reparation, mutagenesis studies	[4, 26, 27, 33, 49, 54 57 72, 74, 88, 96]
Daunomicin	D	analoques of the Q-bands, however, more stable; routine cytogenetic analysis.	[13, 56]
DAPI or Hoechst 33258	G	heterocromatin/euchromatin bands; cytogenetics of human × rodent hybrids; chr aberrations, e.g., t(9;22)(q34:q11)	[35, 94]
DAPI/ Actinomycin D	G	hetero- or euchromatin, abnormalities in chrs, e.g., t(9;22)(q34:q11)	[48]
DAPI/Distamycin A	C	p-arm of chr 15, centromere regions of chrs 16, 9, and 1, distal q-arm of Y chr.	[84].
DAPI/ Chromomycin A3 or mithramycin	G, C, R, NOR	hetero- or euchromatin, abnormalities in chrs, e.g., t(9;22)(q34:q11), the Philadelphia chr	[81, 83, 97]
Chromomycin A3/ Methyl green	R	heterocromatin, euchromatin, abnormalities in chrs	[22, 67, 81, 98]
Gimsa 11	G11	heterochromatic regions on human chrs, centromere of chr 11; h region of human chr 9, structural abberrations of chr 9;	[7, 8, 29, 31, 65, 88, 102]
Silver Nitrate Stain	NOR	Nucleolar organizer regions in all acrocentric chrs, that were active in the preceding interphase; satellite associations	[30, 39, 61, 95]
Propidium iodide + DAPI (CPD-banding)	NOR T	Detection of nucleolar organizer regions and telomeric regions	[36, 45, 47, 75]
FISH		sequences in DNA or RNA; chr regions; chr aberrations; chr territories and morphology in nuclei	[15, 24, 45, 52]
Multicolor FISH and spectral karyotyping		structural chr aberrations in diseases; specific coloring of chrs	[55, 82]

Some protein digestion from the chrs [11, 89, 91] reflects the structural difference between chr regions, responsible for the banding. The positive G-bands are the hydrophobic regions conditioning the thiazine-eosin precipitate formation [89]. They contain the condensed heterochromatin and proteins rich in disulfide (–S-S-) cross-links. These proteins preserved during the pretreatment and needed for the Giemsa complex production. Positive G-bands can be revealed by fluorochromes specific to AT-rich DNA regions also.

The negative G-bands are less hydrophobic and do not favor the formation of the thiazine-eosin precipitate [89]. They contain early replicating euchromatin with relatively loose structure and hydrophobic protein sulfur mainly as sulfhydrils (-SH). These proteins are easily removed by trypsin.

Histones are uniform in the chrs and extracted all in a same way by trypsin. The non-histones bound more tightly in the condensed chromatin regions. Thus, *non-histone proteins* are extracted differentially and are responsible for the G-banding [11].

The **Giemsa ll (G-11)** technique [7, 29, 31, 102] specifically stains the secondary constriction, or h region, of human chr 9 in bright red even in poor material.

Particularly, Gangé R. and Laberge C. [31] obtained chrs spreads from short-term whole blood cultures grown in Eagle minimum essential medium for 65 to 72 h with phytohemagglutinin. Cells were harvested into diluted calf serum in water (1:7), fixed in methanol/glacial acetic acid (3:1) for 15 to 30 min and subsequently suspended in 45% glacial acetic acid for 3-4 min before warm air drying. Then, slides were stained for 5 min with 2% Giemsa mix (Harleco, Azure Blend) in 0,1% $Na_2HPO_4 \cdot 12\ H_2O$ buffer adjusted to pH11.6 with NaOH, then, washed in running tap water 1-2 min, dried and mounted.

With this method cells and metaphase chrs appear pale blue, but two chrs C9 show a large, red-purple juxtacentromeric segment and corresponding to them two red-purple bodies in cultured interphase lymphocytes. The result of the technique depends from freshness of staining solution and pH, showing the two heterochromatic segments in pH range from 11.3 to 11.19 with optimum at pH 11.6. Sometimes the centromeres of D and G chromosome appeared red and bands were noted on chromosomes, but staining of heterochromatic regions on A1 and E16 chrs, which are known to be as large as those on C9 chrs were newer observed.

The method has been useful in study of human heterochromatin polymorphism, translocations and pericentric inversions, in differentiating of human and rodent chrs in human-rodent hybrid cells, produced mainly for gene mapping [8, 10, 29, 100]. For example, in human-chinece hamster hybrid cells human chrs and hamster centromeres stain blue (bright) but human paracentromeric heterochromatin and hamster chr arms stain magenta (dark) [88]. The variant of the method of Alhadeff et al. [3] achieves both: paracentromeric staining and species differentiation.

The G-11 technique is used in analyses of hematologic conditions, mainly the patients with leukemias. The heterochromatic regions can be located on chrs 9 in patients. In certain karyotypes it helps to determine other aberrations. Synchronised cultures of blood and bone marrow can be used for preparation of air-dried and/or flame-dried slides. Trypsin-Giemsa banding can be employed. Photographs made of both abnormal and normal cells for karyotypes allow to suspect of chr 9 abnormality

on the basis of trypsin-Giemsa banding, slides with abnormal cells can be destained with methyl alcohol : acetic acid, 3:1, washed briefly in distilled water, incubated at 60°C in distilled water for 2h, then restained using the procedure of Alhadeff et al. [3] for G-11 staining or by modified method of Stein [88]. Chrs 9 can be located in those photographs used for karyotypes.

R-banding or reverse banding (RB) was proposed by [22, 23]. The negative G-bands can be revealed by Giemsa staining or with fluorochromes, that are specific for GC-rich regions of the chrs, in a process called RB. Giemsa mix stained R ands are observed in phase contrast and acridine orange (AO) stained R bands need fluorescence microscopy.

RB is reverse to G-banding. The slides with metaphase spreads are incubated in hot phosphate buffer solution (PBS) at ~87°C, then treated with Giemsa dye. Contrary to G-banding, the RB chr patterns show darkly stained R bands, interspersed with bright G-bands. There are 3 hydrogen bonds between guanine and cytosine pair (G≡C) and 2 hydrogen bonds in pair between adenine and thymine (A=T). Thus, GC-rich dark colored R bands with euchromatin are more resistant to heat denaturation, than AT-rich regions with heterochromatin. The DNA of the chrs at AT regions has a lower melting point (Tm~ 65°C) as compared to that of the GC regions (Tm~105°C). The technique is useful in analysis of deletions or translocations involving the telomeres and allows the precise determination of chr lengths.

T-banding. T-bands are the most heat-resistant segments of R-bands produced by heating chrs spreads in a phosphate or phosphate buffer solution (PBS) and by followed staining with Giemsa mix or AO [23, 50, 79]. T-bands quality depends on the PBS concentration, temperature, and the treatment duration. Initially T-bands were regarded as telomere bands [23].

T-band regions are capable of producing chiasmata and recombine [37] . Cuny et al. [16] found 5 DNA classes with different densities in vertebrates: light isochores: L1 and L2 and heavy isochores: H1, H2 and H3. The H3, with highest saturation of GC pairs and gene density, are located within the T-bands. The chrs termini in most vertebrates contain tandem repeats $(TTAGGG)_n$ [64] Sub-telomeric region is a non-coding area with sequences becoming less and less similar to TTAGGG in direction to centromere. Telomeres and the subtelomere region compose a terminal restriction fragment (TRF) [9, 17, 18] often used in measuring the telomere length [78].

The microphotometry showed the highest density chromatin near T-bands. It is used as a marker for detection of the changes within the chrs associated with endoreduplication [17, 18]. Prolonged heating in a hot (~87^{o}C) PBS produces holes in the sub-telomeric regions in both chromatids or in the pericentromeric regions of certain chrs due to degradation of chromatin. The degraded chromatin regions correspond to these high density areas [5, 19, 20].

Q-banding **(QB)** utilizes quinacrine mustard (QM), quinacrine dihydrochloride, or related quinacrine fluorochromes (Fs) as chr stains. Structural variants of chrs are detected in fluorescence microscopy. Quinacrine intercalates into chr DNA irrespective of sequence. Under UV light Q-bands fluoresce strongly in yellow in AT-rich regions and emit light less in GC-rich regions [6] .

According to X-ray microanalysis data, quinacrine binds uniformly onto chrs. Thus, QB results from differential excitation or quenching of fluorescence. The mode of QM action on chr is not clear. Definitely, the proteins of chromatin and chrs have a key effect on the QM fluorescence of nuclei and chrs [68]. QB allows the detection of chr aberrations causing diseases, e.g., cancers. QB is useful for "population cytogenetics" also. It detected more polymorphism in human chrs by revealing the bright fluorescence indifferent chrs (3, 4, 13, 14, 21, 22, Y) found in homozygous or heterozygous state. Because of differences among populations in QB patterns frequencies, the method is used in ethnic studies. Selective values of heterochromatin of polymorphic QB variants, in the adaptation of human populations to cold and hypoxia were reported. An increase of the mean of Q-bands in females compared to males was observed. The X chr is capable of "compensate" for the large Q-band of the chr Y. Method is helpful in estimation of sex or sex chr mosaic for Y-bearing cell line by genotype and for X–Y or Y-autosome translocations screening [67].

C-banding stains the regions of chrs containing ***constitutive heterochromatin*** (CH), the highly condensed, inactive form of DNA) and can be used to identify some specific chrs also. The chrs are denatured in a saturated alkaline solution and then stained with Giemsa.

Usually, CH is presented as highly repetitive ('satellite') DNA, however in some species mainly moderately repetitive DNA sequences are present. The CH DNA is late in replication and in mammals its cytosines are often methylated. A number of proteins are either specific to or concentrated in CH and, possibly, involved in its condensed state. Traditionally, heterochromatin is regarded as genetically inert. It varies in quantity among genomes with no apparent phenotypic effects. In *Drosophila* heterochromatin is not replicated during polytenization of chrs, and in certain species it is eliminated in somatic cells and retained only in the germline. The highly repetitive DNA sequences of heterochromatin could not be translated.

CH has effects on the meiotic chiasmata number and position, and induces the inactivation of flanking genes (position-effect variegation). The CH in *Drosophila* can contain Y-chr fertility factors, factors involved in pairing and disjunction of achiasmate chrs, and certain other unconventional factors such as Responder and ABO. Possibly, the CH of other species contains unconventional factors also [89].

Nucleolar organizer regions (NORs) staining reveals the black dots of Ag-NORs above the centromeres of the metaphase acrocentric chrs of human lymphocyte culture. The method utilizes aqueous solution of silver nitrate ($AgNO_3$) to stain NORs in black, and chromosome arms in yellow. Thus, nuclei turn yellow with black nucleolus within.

Trypsin-Giemsa banding methods can be performed on the silver stained metaphases in order to identify unequivocally individual chrs [39].

Combined C-banding and NORs staining of metaphase chrs whith use of ization. Slides are first immersed in dilute HCl to remove residual cytoplasm from the chrs, then treated with saturated barium hydroxide and incubated overnight in saline sodium citrate, adjusted to pH 7.0 with HCl. After C-banding pretreatment, a two-step method of silver staining of NORs utilizing a protective colloidal developer is used. Silver staining is followed by trypsin treatment to remove extraneous silver precipitate from

the chrs which permits the C-banding by Giemsa. The method is suitable for fresh and aged mitotic chr preparations and provides staining of heterochromatin and NORs [96].

Bromodeoxyuridine (BrdU) for two cell cycles for sister-chromatid exchange (SCE). SCE could result as a consequence of DNA damage repair by homologous recombination (HR) during DNA replication and this is evidenced, particularly, in Bloom's syndrome [53], Behçet's disease [44]. The cultured human cells are exposed to the thymidine analog 5-bromo-2'-deoxyuridine (BrdU) during 2 cell cycles, resulting in the 2 sister chromatids with differential incorporation of the BrdU [54]. DNA replication is semiconservative and BrdU consumed by cells from the medium builds into the nascent elongating strand. After 2 rounds of replication, one sister has 1 strand of DNA without BrdU and 1 strand with BrdU substitution. In the other sister, both DNA strands contain BrdU.

After BrdU incorporation, the differential staining of SCEs can be done using different techniques: modified Giemsa [50], 33258 Hoechst [74], acridine orange or 4′-6′-diamidino-2-phenylindole (DAPI) [57].

Fig. 3. Sister chromatids exchange. *By D.B.Pylypiv.*

Particularly, treatment with intercalating and UV light absorbing Hoechst 33258, followed by UV light exposure, causes "bleaching" of the DNA in proportion to amount of BrdU in the double-stranded molecule, possibly, due to free-radical damage. Subsequent Giemsa staining makes this differential bleaching apparent by LM, where doubly substituted chromatids stain less than hemi-substituted. SCEs following either the 1st or 2nd round of DNA replication in the presence of BrdU lead to visible exchanges after the staining (Fig. 3). Other SCE BrdU-methods include fluorescent imaging and the use of either acridine orange (AO) in place of UV treatment followed by Giemsa staining, or an anti-BrdU antibody with either propidium iodide or DAPI counterstain for bulk DNA. Rapid photobleaching is a drawback, especially with the use of AO, and fluorescent filters are required.

***Daunomicin (D) banding*.** The anthracycline anticancer antibiotic D is used for chemotherapy of acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, Kaposi's sarcoma. As fluorescing compound D can be apllied in chrs banding also.

It binds to DNA in several ways [6]. One D molecule spans over approximately from 3 to 3.5 base pairs. The aromatic rings of D intercalate between adjacent DNA base pairs and induce local unwinding, increasing the distance between bases, and other conformational disturbances of adjacent and second-neighbor base pairs [77]. It can also induce histone eviction from chromatin [71, 72].

H acceptor and donor groups of D bind to the minor groove via H-bonds. No base preference for binding. Banding pattern appears due to differential quenching of fluorescence by base pairs D fluorescence is greatly quenched by DNA with a GC content of >32% [6]. It is almost completely quenched by DNA with AT-base content of 0-60% and only partially quenched on DNA with a high AT content (>65%). D-treated AT-rich regions fluoresce in orange-red more brightly than the GC-rich regions,

thus producing a Q–like banding. D-bands appeared to be more stable than the Q-bands and may be useful for routine clinical cytogenetic analysis.

***DAPI banding*.** DAPI (4',6-diamidino-2-phenylindole), is a fluorochrome (F) used in detection of dsDNA [59, 60]. DAPI has wide use in staining due to its high quantum yield (~ 0.92) [47], achieved only when DAPI is DNA-bound, e.g., to the chrs, and shielded from the solvent. DAPI fluoresces weakly in water. The protection of the excited DAPI by DNA prevents H+ intramolecular transfer, which produce less fluorescent species [47, 48]. Some Fs stainings, *e.g.*, by quinacrine, chromomycin A3, or distamycin A fade [59], however, DAPI fluorescence is stable.

In AT-rich DNA regions of the minor groove at a low DAPI/DNA ratio, DAPI forms H-bonds with the N atoms at position 3 of the A (adenine) and/or with the O atoms at position 2 of the T (timine) of consecutive 3 to 4 base pairs (Fig.) [40, 51]. The sequence selective DAPI binding, results in bands similar to G-bands.

At high DAPI/DNA ratio, DAPI intercalates between GC base pairs in DNA regions that do not contain at least 3 consecutive AT base pairs [101]. It binds to the major groove in these regions [47, 48]. DAPI binds to the GC-rich regions of the DNA also, thus, does not produce sharp bands as compared to other Fs, *e.g.*, quinacrine [58].

***Hoechst 33258 banding*.** Hoechst 33258 belongs to a family of bisbenzimide blue fluorescent dyes used to stain dsDNA. It binds to the minor groove of the DNA in AT-rich regions [40, 84, 85]. The binding of Hoechst 33258 with DNA and banding patterns are similar to those of DAPI at a low DAPI/DNA ratio. In addition to this differential binding of Hoechst 33258 with DNA, the fluorescence of Hoechst 33258 enhances to a greater degree in th e AT-rich regions of the DNA as compared to the GC-rich regions [14, 99]. Upon dsDNA binding, their fluorescence increases ~30-fold due to suppression of rotational relaxation and hydratation [1, 70]. This improves the resolution of the bands.

Banding with counterstain.

Counterstaining methods are used to induce banding with Fs that fluoresce uniformly throughout the chr and have a poor resolution. Method uses a primary stain, F and a fluorescent or a non-fluorescent counterstain. If a fluorescent counterstain is used, its emission wavelength (λ_{em}) must differ from that of the primary stain. Here 2 mechanisms form the bands:

1) *electron energy transfer* (ET) when counterstain absorbs the fluorescence of the primary stain. It occurs only in case of a spectral overlap of the λ_{em} of the primary stain and the absorbance wavelength (λ_{ab}) of the counterstain [63].

2) *direct binding competition* that displaces the primary stain by the counterstain [85].

If a fluorochrome with one type of binding specificity or quantum yield (*e.g.*,quinacrine, 33258 Hoechst, or chromomycin A3) is used together with a counterstain (*e.g.*, actinomycin D, 7-aminoactinomycin D, or methyl green) exibiting a complementary binding specificity and satisfying spectral overlap for energy transfer, contrast in fluorescence from the first dye is enhanced in certain subsets of standard chr bands.

Extensive energy transfer possibly suppresses donor fluorescence except in chromosomal regions containing clusters of at least 20 base pairs predominantly of one type, within which the donor but not the acceptor can bind and fluoresce. Quinacrine-

bright polymorphic regions are especially resistant to fluorescence quenching by counterstains with G-C binding specificity, strengthening the evidence that these latter regions have many AT base pair clusters. The ability to highlight selectively many such polymorphic regions may be useful in a number of cytogenetic problems studies [80]. Thus, binding specificity and spectral properties of the counterstains are important.
For example, the combination of the 2 fluorescent dyes DAPI and chromomycin A3 works well because their emission wavelengths are different. DAPI is specific for AT-rich DNA, while chromomycin A3 has a preference for GC-rich DNA [6].

DAPI/Actinomycin D banding. Actinomycin D (AMD) or Dactinomycin, is a chemotherapy antibiotic used to treat a number of types of cancers (Wilms tumor, testicular cancer, some ovarian cancers, rhabdomyosarcoma, Ewing's sarcoma, trophoblastic neoplasm).

The affinity of AD for GC-rich regions of DNA strands makes it useful for chr banding as counterstain for DAPI. G –type bands can be produced.

In *Allium cepa* L., telomeric heterochromatin displayed dull fluorescence after staining with DAPI and DAPI/AMD [47].

***DAPI/Distamycin A banding*.** The AT-binding oligopeptide antibiotic, distamycin A is used as a non-fluorescent counterstain for DAPI. Distamycin A-pretreated metaphases and interphase nuclei exhibit much lower fluorescence intensity than nuclei stained with DAPI only. Chr arms due to distamycin effect are pale and intercalary DAPI bands quenched, but some specific regions of constitutive heterochromatin remain bright. These are mainly the constrictions of chrs 1, 9 and 16, the short arm of chr 15, and the distal part of the Y [84].

DAPI/ chromomycin A3 (CMA) or mithramycin (MM) banding. The guanine-specific CMA or closely related MM antibiotics stain R-bands [83]. The DAPI stains more intensely C- and G-bands than the R-bands [6]. Human metaphase chrs or other species metaphase plates can be sequentially stained with CMA or MM and DAPI [82, 83, 97]. Particularly, in *V. faba*, *S. siberica*, and *O. caudatum* the NORs and/or associated heterochromatin and C-bands displayed very bright fluorescence with CMA and MM and were dim with DAPI and regions pale with CMA and MM were very bright with DAPI. Human chrs showed a small longitudinal differentiation in CMA fluorescence reversed to actinomycin D/DAPI double staining, however, of lower contrast [83].

The chrs staining with the GC-specific CMA and AT-specific DAPI, the CMApositive fluorescence of the NOR region and the telomeric bands of C-heterochromatin was observed in *A. cepa* [47].

Chromomycin A3/Methyl green (CMA/MG) banding. The GC-specific CMA acts as donor, and AT-specific MG acts as an acceptor in intermolecular energy transfer. If human chrs are stained with CMA alone, faint R-banding is seen [98].

If MG, an AT-specific dye [66] with an absorption spectrum overlapping the CMA fluorescence spectrum, is used as a counterstain, fluorescence differentiation is induced [80]. Regions in which fluorescence quenching by MG is relatively low, such as the distal part of the short arm of chr 1, are presumptively identified as enriched for GC pairs and depleted of consecutive AT pairs.

The chromomycin A3/methyl green pair produces especially striking banding, resembling reverse banding **[**22].

Combined propidium iodide (PI) and DAPI banding (CPD-banding) produces bright DAPI-positive regions of pericentromeric heterochromatin on the background of the dull fluorescing euchromatic regions and red fluorescent PI bands in the NOR region and in pericentromeric heterochromatin. PI positive bands corresponded to the DAPI-negative bands observed after DAPI-staining. Banding pattern different from that obtained by staining with PI or DAPI separately [75]

Together with DAPI, PI, a dye with low AT/GC specificity, produces uniform fluorescence in *A. cepa* chrs, whereas the NOR-adjoining regions fluoresce bright. Denaturation of chr DNA (2×SSC, 95°C for 1–3 min) followed by renaturation (2×SSC, 37°C, 12 h) alters the chr fluorescence patterns: specific PI-positive bands appear. Bright fluorescence of NOR and adjoining regions is preserved. 3-minute denaturation leads also to a bright PI-positive fluorescence of telomeric heterochromatin. The denaturation of chr DNA before staining results in changes of the DAPI fluorescence pattern and in the appearance of bright DAPI fluorescence in GC-rich NOR regions [47].

Hybridization methods.

Fluorescence in situ hybridization **(FISH)** Gall J. & Pardue M.L. (1969) described method of direct *in situ* hybridization of tritium-labeled ribosomal RNA to the amplified ribosomal genes in oocytes of the toad *Xenopus*. The hybrids were detected by autoradiography [32]. Later the indirect technique that utilizes a biotin-labeled analog of TTP incorporated to DNA probes was invented. After hybridization *in situ*, the biotin molecules in the probe bind rabbit anti-biotin antibodies. The hybridization site is indicated either fluorimetrically, by fluorescein-labeled goat anti-rabbit IgG, or cytochemically, by an anti-rabbit IgG antibody conjugated to horseradish peroxidase. If combined with Giemsa staining, the immunoperoxidase method provides a permanent record for cytogenetic analysis [52].

Many improvements of these two inseminating methods have increased the safety (now non-radioactive, mainly fluorescent probes are used), applicability and sensitivity of the *in situ* hybridization.

In many laboratories FISH substituted specific staining of chr regions.

A lot of fluorescent probes were produced, each a cloned fragment of the genome. The probes are of two types: 1) collections of probes, *e.g.*, chr specific probes; specific for all centromeres or telomeres 2) probes for unique genomic segments. FISH was used in studies of the locations of genes in the interphase nucleus.

Multicolor FISH and spectral karyotyping methods visualize all the pairs of chrs in a cell in different colors simultaneously. To overcome the problem of the limited number of spectrally distinct fluorophores in complementary probes, the DNA specific to particular chr made labeled with different fluorophores, *i.e.*, combinations of fluorophores are used in labeling of probes to generate many different colors, when probes specifically attach to chrs. Fluorophore combinations are read and analyzed in FM using up to 7 narrow-banded fluorescence filters or, in spectral karyotyping, by using an interferometer coupled with FM. In the case of an mFISH image, every combination of fluorochromes from the resulting original images

is replaced by a pseudo color in image analysis software. Thus, chrs or their fragments can be identified, allowing for chr aberrations analysis [55]. Multicolor FISH detects structural chr aberrations in diseases when other methods are not precise enough.

In spectral karyotyping, image processing software assigns a false color to each spectrally different probes combination and visualizes specifically colored chrs [82].

Methods discussed above are in use for karyotyping and identifying of chr aberrations. These techniques had a great impact on human genetics and medicine.

Experiment 1. Conventional Giemsa Stainings 1 and 2 by Moorhead et al. [64].

Principle. Giemsa mix uniformly stain chrs enabling the measurement of chr length, centromere position, arm ratio.

Before 1960th, when Giemsa became popular as mix for staining of chs [64] the acetoorcein, acetocarmine, gentian violet, hematoxylin, Feulgen, Leishman's, or Wright's dyes were used for this purpose. The Romanovsky dyes (Giemsa, Leishman's, and Wright's stain) have advantage for routine use, because the chrs spreads can be easily destained and banded by most types of banding. Contrary, orcein-stained chrs cannot be destained and banded, thus not used in routine chr staining [34].

Conventional Giemsa Staining 1.

Solutions. Giemsa stain (Gurr's, Biomedical Specialties cat. # 35086); pH 6.8 phosphate buffer (Gurr's tablets, Biomedical Specialties cat. # 33199). Working stain: 4 mL Giemsa + 96 mL pH 6.8 buffer

Equipment. LM with ocular micrometer, glas slides, cover slips, Coplin jar.

Procedure

1. Place slides with chromosome spreads in a Coplin jar.
2. Pour working stain over the slides and stain for 7 min.
3. Rinse slides in 2 changes of distilled water.
4. Dry slides in air flow and mount them with a cover slip. **Note.** If sequential banding procedures are to follow, cover slip is not used.
5. Using the highest magnification of LM and ocular micrometer measure the length of selected chr, length of its both arms and calculate the arms ratio.

Conventional Giemsa Staining 2.

Solutions. 5N HCl; distilled water; pH 6.8 phosphate buffer (Gurr's tablets, Biomedical Specialties cat. # 33199); Giemsa stain: 2 mL stock Giemsa; 4 mL pH 6.8 buffer; 92 mL distilled water

Equipment. LM, ocular micrometer; glas slides; cover slips; Coplin jars with lids.

Procedure

1. Place slides in a Coplin jar containing 5N HCl for 10 min at room temperature.
2. Rinse with tap water for 10 min.
3. Stain for in Giemsa for 10 min.
4. Rinse, dry, and coverslip.
5. Compare results of Giemsa stainings 1 and 2.

Experiment 2. G-Banding by M. Seabright [86].

Principle. Chrs are G-banded to allow better sorting in homology pairs and the detection of structural aberrations. Slides with chrs spreads are dehydrated, treated with the trypsin, then stained. Partial digestion of proteins from the chrs surface allows for sharp bands production.

Equipment. LM, ocular micrometer; glas slides; cover slips; Coplin jars with lids; 5 ml and 10 ml serological pipettes and bulbs; dehydration oven set at 95°C; slide racks; slide warming tray set at 60°C; staining rack; timer; plastic squirt bottle.

Reagents. 0.25% trypsin, in HBSS without calcium and magnesium. (Life Technologies Inc., Gibco #610-5050) Store at -5 to -20°C. Thaw at room temperature. **Note.** Do not heat (to prevent the enzyme denaturation). Dry salt phosphate buffer, pH 6.8 (Fisher Scientific #B-78); Leishman's stain powder. (Sigma #L-6254); 99,9% methanol. (J.T. Baker, VWR #JT9070-3). **Note.** Toxic. Work in fume cab. Avoid contact with skin and mucous membranes. Flamable - store in fire-proof chamber. Dispose used down the sink in fume cab with copius amounts of water. Normal saline, 0.9% sodium chloride. (Abbott Laboratories, SHMC #3619). pHydrion buffer capsules, pH 7.0 (VWR #34175-242).

Preparation of 0.025% trypsin. Mix 5 ml 0.25% trypsin and 45 ml of normal saline. -Prepare mix at the day of staining. Keep at room temperature. If color changes, do not use.

Preparation of Fisher phosphate buffer. Dissolve 1 buffer capsule (pH 6.8) in 1 liter of distilled water. Check pH with pH meter.

Preparation of stain: Swirl 500 ml of absolute methanol in flask. Add 1 g powdered stain to swirling methanol. Continue to swirl 2-3 minutes at moderate rate. Let sit for 15 min. Filter through Whatman #1 filter paper into brown bottle that contains no water (swirl bottle with methanol before use). Store away from heat and light. Shake well and filter before use. **Note.** Methanol is toxic and flameable. Work in fume cab.

Preparation of pHydrion stock buffer. Dissolve 1 pHydrion buffer capsule (pH 7.0) in 100 ml distilled water. Check pH with pH meter and record on label. Store at 2 to 8°C.

Preparation of pHydrion working solution. Mix 5ml pHydrion stock buffer and 95 ml of distilled water. Store at room temperature.

Procedure

1. Place fixed, dry slides on slide rack in 95°C oven for 20 min.
2. Cool and immerse slides in 0.025% trypsin for 10 to 120 sec.
3. Remove slides from trypsin and immediately immerse in Fisher phospate buffer to stop trypsin action.
4. Place slide cell side up on staining rack and flood with solution of 1 part Leishman's stain and 3 parts pHydrion working solution. Stain for 2 min.
5. Rinse slides thoroughly with distilled water.
6. Allow slides to drain, then place on 60ºC slide warming tray until completely dry.

Note:

1. Trypsinization time may vary depending on environmental conditions, material being banded, or trypsin stock. Always stain one slide first and check banding quality before staining additional slides. Adjust trypsinization time if necessary.
2. Be careful not to over rinse slides since over rinsing will fade stain.
3. Leishman's stain will form a precipitate when added to pHydrion buffer. Therefore, the two should not be mixed until just prior to flooding the slide.
4. Step 3 (Fisher phospate buffer) may be eliminated if desired.
5. Slides can be destained by placing them in a coplin jar containing 3:1 fixative (3 parts absolute methanol mixed with one part glacial acetic acid) for 1-2 minutes. Rinse slides with absolute methanol and allow to dry completely. Slides may then be re-trypsinized and/or restained.
6. Wright's stain may be used in place of Leishman's stain.
7. Examine the slides in LM. Using computer softvare, gather chrs from your photographs into homology pairs and groups to build the caryogram or, alternatively, number the cromosomes and interpret the caryogam with estimation of sex in the Fig. 4. Use Fig. 5 as an example how to number the chrs.

Fig. 4 G-banding: caryogram using Giemsa staining.
Courtesy: National Human Genome Research Institute -
http://www.genome.gov/glossary/resources/karyotype.pdf. [40]

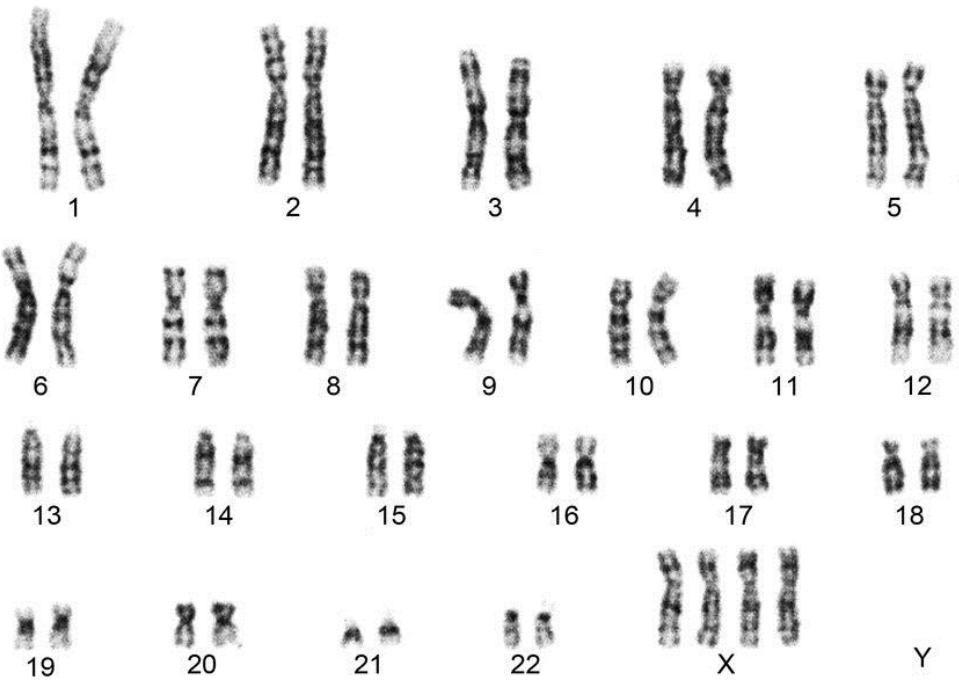


Fig. 5. G-banding karyotype of the rare chromosomal disorder tetrasomy X (48, XXXX). *By Akiyo Hineno, Tomoki Kosho, Hiroyuki Kato, Yoshiki* Sekijimahttps://www.researchgate.net/figure/G-banded-chromosomal-analysis-showing-48-XXXX_fig3_335626731, CC BY 4.0 [43]

Experiment 3. C-Banding by A. Sumner [91].

Principle Specific staining of the centromeric regions and other regions of constitutive heterochromatin, *e.g.*, the secondary contrictions of human chrs 1, 9, 16, and the distal segment of the Y chr long arm.

Equipment. Timer; circulating waterbath set at 65°C; 4 glass Coplin Jars with lids; 9.0 cm circles of Whatman #1 filter paper; squirt bottle filled with distilled water; slide warmer. Light microscope with camera.

Reagents. Xylene substitute (Shandon #99900506); absolute methanol. (J.T. Baker, VWR #JT9070-3) **Note.** Both are toxic - avoid contact with skin, mucouse membranes and inhalation. Store in approved fire-proof cabinet. Discard in organic waste container.

Glacial acetic acid. (J.T. Baker, VWR #JT9507-3); Concentrated hydrochloric acid. (Fisher #A144-212) **Note.** Both are toxic - avoid contact with skin, mucouse membranes and inhalation.

Sodium chloride crystal (#07581-1NY); sodium citrate crystal (#0754-1NY); barium hydroxide crystal (Products #3772-1NY), all from Mallinckrodt, American Scientific.

Fixative 3:1. Prepare fresh mix of absolute methanol and glacial acetic acid (3:1, v/v). **Note.** Toxic. Discard extra fixative down fum hood sink with copius amounts of water.

0.2 N HCl: add 4.15 ml concentrated hydrochloric acid to 200 ml of distilled water, mix and add water to 250ml. Stable 1 year.

2XSSC: dissolve 17.53 g Na chloride crystal and 8.82g Na citrate crystal in 1 liter of distilled water. May be refrigerated in thightly capped bottle indefinitely.

2.5% $Ba(OH)_2$: dissolve 2.5g of $Ba(OH)_2$ crystal in 100 ml of distilled water. This is a supersaturated solution. Store in thightly capped bottle at room temperature. Stable indefinitely.

Procedure

1. G-band slides to facilitate identification of chrs. Photograph 2-3 metaphase spreads for documentation and for comparison after C-banding.
2. Remove oil from G-banded slide thoroughly with at least 2 rinses of fresh Xylene substitute.
3. Destain slide by dipping in 3:1 fix; wipe bottom of slide, place on 40-60°C slide warming tray until beads of solution are formed: then blot gently with bibulous paper. These 4 steps should be repeated until beads are clear.
4. Place dry, destained slide in 0.2 N HCL for 1 hour. After 30 min turn on pre-set waterbath and start to filter $Ba(OH)_2$ through #1 Whatman filter paper into Coplin jar.
5. Rinse slide (treated in 0.2 N HCl) in Coplin jar filled with distilled water.
6. Place rinsed slide in freshly filtered $BA(OH)_2$ solution for 2 minutes.
7. Rinse with distilled water in squirt bottle (some force is required to remove $Ba(OH)_2$ crystals).
8. Place rinsed slide in Coplin jar (in waterbath) filled with 2XSSC at approximately 62.5°C for 1 hour.

Fig. 6. C-banding of a human female karyotype showing constitutive heterochromatin
By Rcann3 - Own work, CC BY-SA 4.0, [42]

9. Remove slide slowly and rinse gently in Coplin jar filled with freshly distilled water.
10. After drying, the slide should be stained as follows: For peripheral blood specimen, stain 90 sec with 1:5 Wright's stain (prepared, as stain in G-banding procedure above).
11. Observe the slides in microscope. Find staining of the centromeric regions and other regions of constitutive heterochromatin similar to pictured in Fig 6.

Experiment 4. Giemsa Reverse Banding by B. Dutrillaux and J.Lejeune [22].

Principle. Reverse banding using heat and Giemsa (RHG) involves the incubation of slides in hot phosphate buffer with subsequent Giemsa staining. The resulting chr pattern shows darkly stained GC-rich heat resistant R bands and pale AT-rich G bands denaturated by heat. R-banding is used for analyzing aberrations that involve the telomeres of chrs.

Solutions. 2% Giemsa in distilled water; tap water; phosphate buffer.

Preparation of phosphate buffer. Mix 89.9 mL of distilled water with 10.0 mL of Earle's balanced salt solution IEBSS 10, (GIBCO) and 0.1 mL of 7.5% sodium bicarbonate (GIBCO). Heat the buffer to 88-89°C in water bath.

Procedure

1. Incubate slides in hot EBSS for 10 to 15 min. (Fresh slides require 1 to 2 hours. One-day-old slides require 25 min. One-week-old slides require 7 min. As a rule, older slides require less time.)
2. Cool quickly in tap water. Do not dry.
3. Stain in 2% Giemsa for 10 to 20 min.
4. Rinse in xylene, than in tap water.
5. Air dry. Observe R- bands reverse to G-bands.

Experiment 5. T-banding by B. Dutrillaux [23].

Principle. T-bands represent the most heat-resistant segments of R-bands produced by heating chrs spreads in a phosphate or phosphate buffer solution (PBS) and by followed staining with Giemsa mix or acridine orange (AO) [23,34]. T-bands are more strictly telomeric [34].

Equipment. Fluorescence microscope, conventional LM, coverslips and slides.

T Banding by Thermal Denaturation: Method 1

1. Heat the mixture of 94 mL of distilled water and 3 mL of phosphate buffer (pH 6.7) to 87°C in a Coplin jar in termostate.
2. Spike with 3 ml of a 3% Giemsa solution.
3. Add slides to jar; incubate for 5 to 30 min.
4. Rinse in distilled water several times, dry at room temperature, and examine in conventional light microscope. Make the photos.

Fluorescent Staining

5. Destain, rehydrate through a series of alcohols, rinse in distilled water.
6. Stain in AO (5 mg/100 mL) for 20 min.
7. Rinse in phosphate buffer, mount, and examine with a fluorescence microscope (excitation light vawe length: λ = 450-490 nm; suppression: λ = 515 nm).

Note. Standard peripheral blood culture and metaphase spread preparation methods are appropriate. Slides with chrs should be aged for a few days or week prior to staining. With Giemsa (steps 1-4), the chrs are pale, unbanded, and difficult to see. With AO staining for various lengths of time, the chrs appear as follows: 45 min: green at telomeres, otherwise orange; 15 to 20 min: green at telomeres, orange areas are less intense; 30 min or more: orange color is gone, intercalating R bands appear.

T Banding by Thermal Denaturation: Method 2

1. Bring a Coplin jar containing Earle's BSS, PBS, or phosphate buffer to 87°C. The pH must be adjusted to 5.1.
2. Stain with Giemsa or AO as in Method 1, steps 2 to 7.

Experiment 6. DAPI/Distamycin A staining by D.Schweizer et [84]

Principle Developed for labeling a specific subset of C bands, the DAPI/Distamycin A staining is useful in identifying pericentromeric breakpoints in chr rearrangements and in identifying chrs that are too small for standard banding. Also, DAPI/DA is the method of choice for Yqh chr material in suspected Y autosome translocations.

Equipment. Fluorescence microscope, coverslips and slides, magnetic stirrer, pipettes, laboratory electronic scales.

Stains

Preparation of distamycin A: Dissolve 2 mg of distamycin A-HCl (Sigma) in 10 mL of McIlvaine's buffer, pH 7.0. Add a magnetic stirrer and place the foil-covered tube in a beaker of crushed ice. Stir for 15 to 30 min. Dispense in 1 mL aliquots and store at -20°C for up to 6 months.

Preparation of DAPI stock solution: Dissolve 2 mg DAPI-2 HCl (Sigma) in 10 mL of distilled water. Dispense in 1 mL aliquots, and store at -20 degrees C for up to six months.

Preparation of DAPI working solution: Add 0.1 mL of stock solution to 100 mL of McIlvaine's buffer, pH 7.0. Store at 4°C for up to six months.

Preparation of buffer: McIlvaine's buffer, pH 7.0.

Solution A: 0.1 mol/L citric acid (19.2g: q.s. to 1 liter with distilled water)

Solution B: 0.2 mol/L Na_2HPO_4 (28.4g: q.s. to 1 liter with distilled water)

The following formula can be used to prepare this buffer at various pHs:

x mL A + (100 - x) mL B = 100 mL total volume

For pH 4.1, x = 60. For pH 5.5, x = 43.1. For pH 7.0, x = 18.2

Procedure

1. Flood a slide with distamycin A solution. Coverslip and incubate the slide, in the dark, at room temperature for 5 to 15 min.
2. Remove the coverslip and rinse briefly with pH 7.0 McIlvaine's buffer.
3. Flood with DAPI working solution. Place a coverslip and incubate the slide, in the dark, at room temperature for 5 to 15 minutes.
4. Remove the coverslip and rinse the slide briefly with pH 7.0 buffer.
5. Observe with fluorescence (excitation: 340-380 nm; suppression: 430 nm).
6. Make the photographs in fluorescence microscope.

Note: *Wear gloves.* Distamycin A tends to degrade in aqueous solution unless it is frozen. DAPI stock solution can be frozen or refrigerated for several weeks without deterioration. The stained preparations tend to fade soon after made, so photograph immediately. To stabilize the fluorescence, store slides covered by slips at 4°C for a day or so [34].

Experiment 7. Silver Nucleolus Organizer Regions Staining by Howell W.M., Black D.A. [39]

Principle For differential staining of nucleolus organizer regions method utilises a protective colloidal developer in combination with aqueous silver nitrate. Spreads are treated with silver nitrate solution which binds to the Nucleolus Organizing Regions (NORs), *i.e.*, the secondary constrictions (stalks) of all acrocentric chrs. A protective colloidal developer is used to control the reduction of silver.

Equipment. Conventional light microscope, coverslips and slides, slide wormer.

Reagents. Silver Nitrate (Sigma #S-6506); gelatin powder (e.g., Sigma #G1890).

Solutions.

Preparation of a colloidal developer solution. Dissolve 2 g of gelatin powder, USP into mix of 100 mL of deionized water and 1 mL of pure formic acid by 10 min stirring. This solution is stable for 2 weeks. Keep in amber-glass bottles.

Preparation of an aqueous solution of silver nitrate. Dissolve 4 g $AgNO_3$ in 8 mL of deionized water. This solution is stable. Keep in amber-glass bottles.

Procedure

1. Pipett 2 drops of colloidal developer and 4 drops of the aqueous $AgNO_3$ onto the surface of a microscope slide with metaphase spread.
2. Mix the solutions and cover with coverslip.
3. Place the slide onto the surface of the slide wormer set at 70°C.
4. Heat within 30 sec. The silver-staining mixture will turn yellow, and within 2 min, it will become golden-brown.
5. Remove the slide and rinse off the coverslip and the stain mixture by running deionized water.
6. Blott dry the slide and examine immediately.
7. Observe the nucleolar organizer regions stained black and chr arms stained yellow. Nuclei are yellow with black nucleoli, even staining of metaphases across the slide.
8. Only a single chr carries an active NOR in female, but all three NORs are revealed in male lizard *Lepidodactylus lugubris*. (Fig. 7). Can you find the difference in NORs between male and female in human?

Note. The slide should be freshly prepared and clean. Old slides contaminated with bacteria, dust and cellular debris will give background precipitate, as these too will stain with silver. Slides that have been previously G-banded are not suitable for NOR staining. If 50% silver-nitrate solution allowed to get on skin, it can cause a chemical burn which turns black and can not be washed off. Clothing is also prone to silver-nitrate staining.

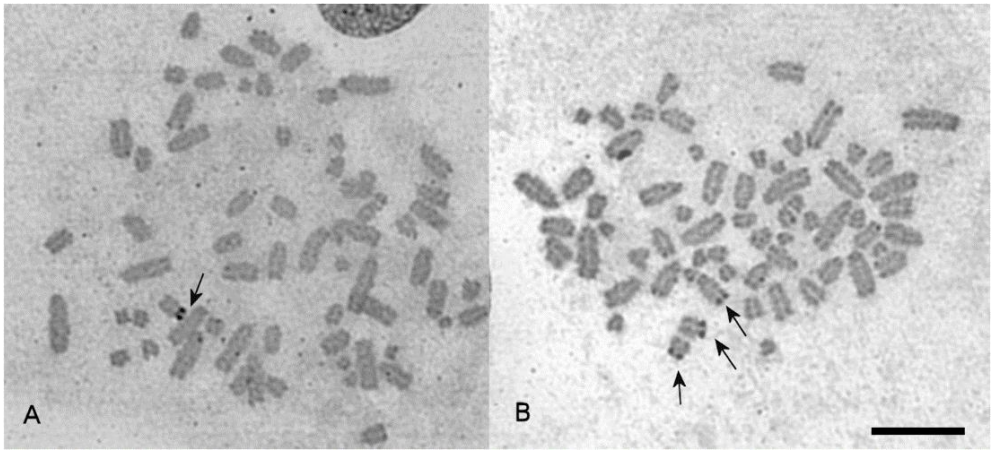


Fig 7. AgNOR staining of *Lepidodactylus lugubris* chromosomes.
(A) female B and (B) male D. There is a polymorphism in NOR activity revealed by Ag-staining: only a single chromosome carries an active NOR in female B, but all three NORs are revealed in male D. Scale bar, 10 μm. Trifonov VA, Paoletti A, Caputo Barucchi V, Kalinina T, O'Brien PCM, et al., 2015 [93].

Literature

1. Adhikary A. et al. // Nucleic Acids Res.- 2003.- Vol. 31.-P.2178–2186.
2. Adler D..-http://www.pathology.washington.edu/research/cytopages/idiograms/human/
3. Alhadeff B. et al. // Cytogenet Cell Genet 1977.- Vol 19.- P.236-239
4. Alves P., Jonasson J. //J. Cell Sci. 1978.- Vol. 32.- P. 185-195.
5. Andraszek K., et al. //Roczniki Naukowe Polskiego Towarz. Zootechn.-2010, t. 6 N3.- s. 9-16.
6. Bickmore W.A.// Encyclopedia of life sciences.- Nature Publishing Group.- 2001.- www.els.net
7. Bobrow M. et al. // Nature New Biol 1972.- Vol. 238.- P. 122-124
8. Bobrow M. et al. // Nature 1974.- Vol. 251.- P.77-79.
9. Bolzan A.D., Bianchi M.S. //*Mutation Research*.- 2006 –Vol. 612.-P. 189-241.
10. Burgerhout W. // Humangenetik 1975.- Vol. 29.- P.: 229-231
11. Burkholder G.D., Weaver M.G. //Experimental Cell Research, 1977.- Vol. 110(2.- P. 251-262
12. Chamla Y., Ruffie M. //Human Genetics.- 1976.-Vol. 34.- P.: 213-216.
13. Johnston F.P., et al., //Chromosoma, 1978.- Vol. 68, N2.- P. 115-129.
14. Comings, D.E.//Chromosoma, 1975.- Vol. 52(3.- P. 229-243.
15. Croft J.A., et al. // The J. Cell Biol., 1999.- Vol. 145, N6.- P. 1119-1131.
16. Cuny G. et al. //Eur. J. Bioch.- 1981.-Vol. 115.- P. 227-233.
17. Drets M.E. // *Genet. Molec.Biol.*- 2000. – Vol.23, N4.- P.1087-1093.
18. Drets M.E. // Cytogenet. Genome Res.- 2004 −Vol. 104.- P. 137-141.
19. Drets M.E., Mendizabal M. //*Genet. Mol. Bio.*.- 1998. –Vol.21, N2.- P.219-225.
20. Drets M.E., Mendizabal M. // *Mutation.*- 1998. –Vol 404.- P. 13-16.
21. Drets M.E., Shaw M.W. // *Proc. Natl. Acad. Sci.* 1971.-Vol.68.- P. 2073–2077.
22. Dutrillaux B., Lejeune J. // C R Acad Hebd Seances Acad Sci.- 1971.- Vol. D 272.- P. 2638-2640
23. Dutrillaux B., // Chromosoma.- 1973.- Vol.41.- P. 395-402.
24. Egozcue J. // Hum. Reprod. Update.- 1997, Vol. 3, N5.- P. 441–452.
25. Evans H.J. In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds. Handbook of mutagenicity test procedures. Amsterdam: Elsevier, 1984.- P.405–427.
26. Fenech M. //Mutat Res .- 2000. – Vol.455.- P. 81–95
27. Floser G., Haarer D. // Chem. Phys. Let., 1988.- Vol.147, N2-3.- P. 288-292.
28. Franke U., Oliver N. // Hum. Genet. 1978.- Vol.45.-P.137-165,
29. Friend K.K et al.// Somat. Cell Genet. -1976.- Vol.2, N2.- P. 183-188.
30. Funaki K. et al. // Chromosoma (Berl.).- 1975.-Vol.49.- P. 357-370.
31. Gagné R., Laberge C. et al. // Exp Cell Res.- 1972.-Vol. 73 .- P. 239-242
32. Gall J.G., Pardue M.L. //Proc. Natl. Acad. Sci. USA.-1969.- Vol.63, N2.- P. 378-383.
33. Goldfarb T., Lichten M. //*PLOS Biol*. 2010.- Vol.8, N10: e1000520.
34. Gustashaw KM. In: *The ACT Cytogenetics Laboratory Manual, Second Edition*, ed. by M.J. Barch. The Association of Cytogenetic Technologists, Raven Press, Ltd., New York, 1991.

35. Han F. et al. // Biochemistry.- 2005.- Vol. 44.- P.9785–9794.
36. Hanson, R.E. et al., //*Genome*.-1995.- Vol.38.- P. 646–651.
37. Holmquist G.P. Chromosome bands, their chromatin flavors, and their functional features// Amer. J. Hum. Genet..- 1992.- Vol.51.- P. 17-37.
38. Horobin R.W.//Biotechnic Histochem.- 2011.- Vol.. 86,N1.- P. 36-51.
39. Howell W.M., Black D.A. //Experientia.-1980.-Vol.36.-P.1014
40. Hughes D., H. Mehmet Cell Proliferation and Apostosis.- Oxford, UK: BIOS Scientific Publishers Limited.- 2005
41. https://commons.wikimedia.org/w/index.php?curid=583512
42. https://commons.wikimedia.org/w/index.php?curid=44908090
43. https://commons.wikimedia.org/w/index.php?curid=102387167
44. Ikbal M. et al.// *J Eur Acad Dermatol Venereol*.- 2006.- Vol. 20, N2.- P. 149–152.
45. Ji Y. et al. //*Genome*.- 1997.- Vol.40.- P. 34–40.
46. Kapuscinski J. // Biotech. Histochem.- 1995.- Vol. 70, N5.- P. 220–233.
47. Kim E.S., et al. // Rus. J. Genet.- 2002.- Vol.38, N4.- P. 392–398. Translat. Genetika.-2002.-Vol. 38, N4.- P. 489–496.
48. Kim S.K. et al. //J. Amer. Chem. Soc., 1993.- Vol.115,N9.- P. 3441-3447.
49. Korenberg J., Freedlender E. // *Chromosoma* 1974.- Vol.48.- P. 355–360.
50. Kumar S. et al. // Intech Open.- Feb. 17. 2021 https://www.intechopen.com/chapters/75292
51. Lacowicz J., Principles of Fluorescence Spectroscopy 2006, New York, USA:Springer Science and Business Media, LLC.
52. Langer-Safer P.R. et al. //Proc Natl Acad Sci USA.-1982.- Vol.79,N14.- P. 4381-4385.
53. Langlois R.G., Bigbee W.L., Jensen RH, German J. //*Proc Natl Acad Sci USA*.-1989.- Vol. 86, N2.- P. 670-674.
54. Latt S.A. // *Proc. Nat. Acad. Sci. USA*-1973.- Vol.70.- P. 3395–3399.
55. Liehr T. et al. // Histol. Histopathol. 2004.- Vol.19,N1.- P. 229–237.
56. Lin C.C., Van de Sande J.H. // Science.- 1975.- Vol.3, N190(4209).-P. 61-63.
57. Lin M.S., Alfi O.S. //*Chromosoma* .-1976.- Vol.57.- P. 219–225.
58. Lin M.S., D.E. // Chromosoma.-1977.- Vol. 60,N1.- P. 15-25.
59. Liu J.Y., et al. //Clin.Genet., 2006.- Vol. 69,N1.- P. 65-71.
60. Martin P.K., Rowley J.D. // Stain Technol.- 1983.- Vol.58, N1.- P. 7-12.
61. Matsui S., Sasaki M. // Nature.- 1973.- Vol.246.- P. 148-150.
62. Meyne J. et al. //Proc Natl Acad Sci USA.- 1989.- Vol. 86N18.- P. 7049-7053.
63. Montalti M. et al., *Handbook of Photochemistry*. 3rd ed2006: Taylor and Francis Group.
64. Moorhead P. // Exp Cell Res.- 1960.- Vol. 20.- P. 613-616.
65. Morse H.G. et al. // Hum Genet .-1982.- Vol.61.- P.141-144
66. Muller W. Gautier F. //Eur. J. Biochem. 1975.- Vol. 54.- P., 385-394.
67. Nabil A., Sarra F. Q-banding// Ref. Mod. Life Sci.- 2017.- Ref. Mod. Life Sci. – P. 1-3.
68. Nussbaum R., McInnes R.,Willard H.F. Thompson & Thompson Genetics in Medicine.- 8th ed.- Canada: Elsevier Inc., 2016.- 1278 p.
69. O'Connor C. // *Nature Education* .- 2008.- Vol. 1,N1.- P. 107

70. Pal S.K. et al. // Proc. Natl. Acad. Sci. USA .- 2003.- Vol. 100.- P.8113–8118.
71. Pang B. et al. // Nat. Chem. Biol.- 2015.-Vol. 11, N7.- P. 472–480.
72. Pang B. et al. // *Nat. Commun*.- 2013.-Vol.4.- P.1908.
73. Perry P.E., Thompson E.J. In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds. Handbook of mutagenicity test procedures. Amsterdam: Elsevier, 1984. – P. 495–529.
74. Perry P., Wolff S. // *Nature*.- 1974.- Vol. 251.- P. 156–158
75. Peterson D.G.et al. // *Genetics*.- 1999, Vol. 152.- P. 427–493
76. Quigley G. J. et al. // *PNAS*.- 1980.- Vol. 77 N12.- P. 7204–7208.
77. Rolf V. et al. // 2012.- https://commons.wikimedia.org/ wiki/File:Ideogram _explaining_genetic_notation.png
78. Rufer N, et al. // *Nature Biotechnol*.- 1998.-Vol.16, N8.- P.743–747.
79. Saccone S. et al. // Proc Natl Acad Sci USA.- Vol. 89.- P. 4913-4917.
80. Sahar E., Latt S.A. //Proc. Natl. Acad. Sci. USA- 1978.- Vol. 75, No. 11.- P. 5650-5654.
81. Santos N., Souza M. J. //Caryologia.- 1998.- Vol. 51, N 3-4.- P. 265-278,
82. Schröck E., du Manoir S. //1996.- Vol. 273, N5274.- P. 494–497.
83. Schweizer D. //Chromosoma.-1976.-Vol. 58, N4.-307-324
84. Schweizer D. et al. //Exper. Cell Res.-1978.- Vol.111, N2 -P.327-332.
85. Schweizer D. // Human Genetics, 1981.- Vol.. 57,N1.- P. 1-14.
86. Seabright M. // Lancet, 1971.- Vol.. 2,N7731.- P. 971-972.
87. Speit G., Haupter S. // Hum Genet.-1985.- Vol. 70.- P.126–129.
88. Stein C.K. // *Somat. Cell Molec. Genet.- 1998, Vol. 24, N3*.- P. *191-195.*
89. Sumner A.T. In: Brenner S., Miller J.H., Ed (s): Encyclopedia of Genetics, Academic Press, 2001. P. 926-927.
90. Sumner A.T. //Cancer Genetics and Cytogenetics, 1982.- Vol. 6,N1.- P. 59-87.
91. Sumner A. // Exp. Cell Res.- 1972.-Vol. 75.- P.304-306.
92. The Biology project. Human Biology. Karyotyping activity.- Univ. of Arizona.- http://www.biology.arizona.edu/human_bio/activities/karyotyping/karyotype.html
93. Trifonov VA, et al //PLOS ONE. – 2015.- Vol.10(7): e0132380.
94. Tucker J.D. // Cytogenet Genome Res.- 1988.- Vol. 48.- P.103-106.
95. Tuck-Muller C.M. et al.// Stain Technol.-1984.- Vol.59, N5.- P. 265-268.
96. Tumini E, Aguilera A. // Methods Mol Biol.- 2021.- Vol. 2153.- P. 383-393.
97. Vaio et al. // Ann. Bot.- 2005.- Vol. 96.- P. 191–200.
98. Van de Sande J., et al. // Science .- 1977.-Vol.195.- P. 400-402.
99. Weisblum B., Haenssler E. // Chromosoma, 1974.- Vol.46,N3.- P. 255-260.
100. Westerveld A. //Proc Natl Acad Sci USA .- 1976.-Vol.73.- P. 895-899
101. Wilson W.D. et al. // Biochem.- 1990.- Vol. 29,N36.- P. 8452-8461.
102. Wyandt H.E. et al.// Exp. Cell Res.- 1976.- Vol.102.- P. 85-94.