

Uvod u molekularnu biologiju i genetiku

3. Genetika



GENETIKA: znanost o nasljeđivanju

- opisuje/proučava prenošenju svojstava (eng. *traits*) i gena s generacije na generaciju
- opisuje/proučava kako se informacija (genotip) manifestira kroz formu ili funkciju (fenotip)
- opisuje/proučava koje mutacije i u kojim genima su u podlozi nasljednih bolesti
- opisuje/proučava koje su prirodne varijacije istih gena u populaciju
- opisuje/proučava načine kako stečena znanja o zakonima nasljeđivanja možemo koristiti u medicini i proizvodnji hrane

„Like begets like” (Slično rađa slično) → opažanje da potomci imaju slične osobine kao i njihovi roditelji



Međutim: postojale su i iznimke!



Stari Grci: Pitagora (6. st. p.n.e.)

- nasljedna svojstva (*nature*) prenose se s roditelja na djecu očevim sjemenom, majka pridonosi samo maternicom („inkubator”, *nurture*) → spermizam (preformacionizam)
- ako su sva svojstva od oca, kako to da majke rađaju i kćeri?

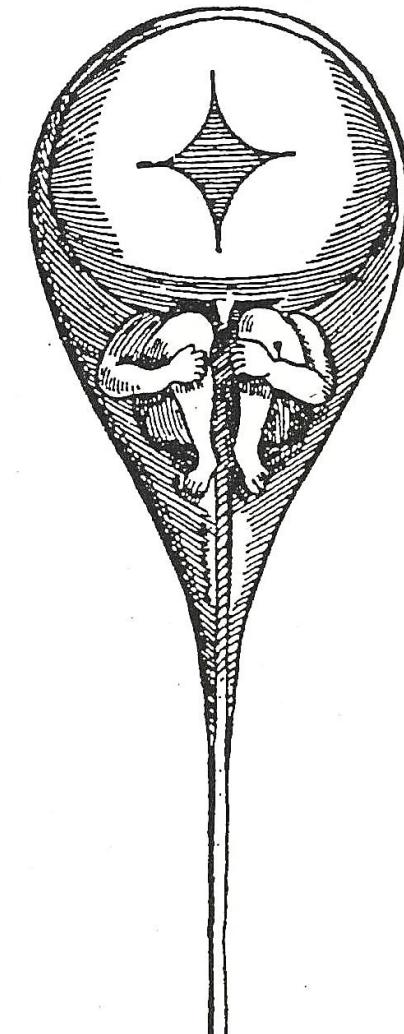
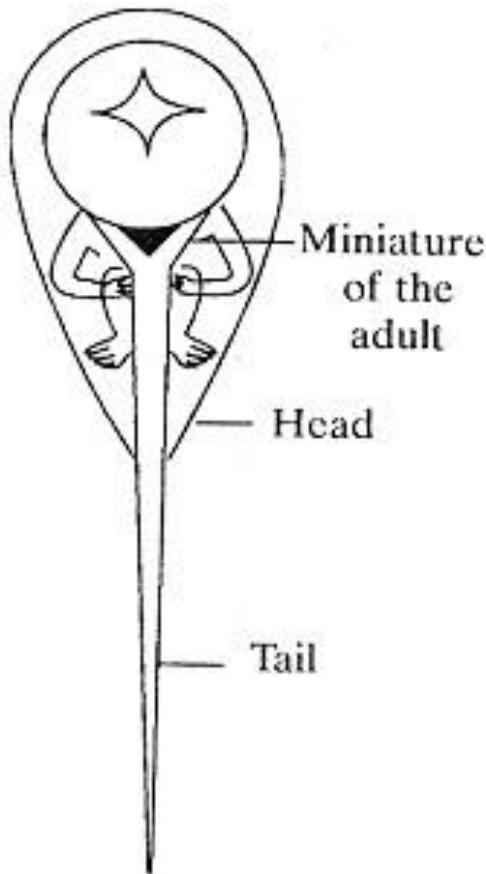
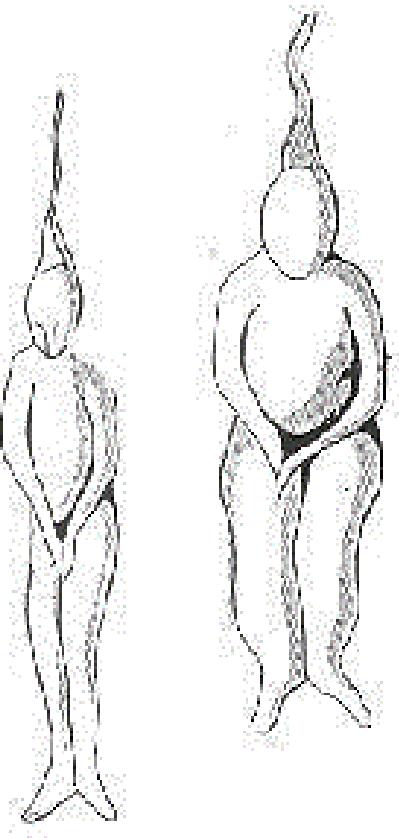
Aristotel (4. st. p.n.e.): dovodi u pitanje teoriju spermizma → djeca nasljeđuju osobine svojih majki i baka → i majka kontribuira svoja svojstva fetusu (preko menstrualne krvi)

Oblik (forma) → stvara informaciju (sjeme je nositelj nasljedne informacije oca, a menstrualna krv majke) → stvara se novi (sličan) oblik itd.

Paracelsus (homunculus) 1520.-te → „preformacija” (Pitagorina hipoteza)

Man the seed, woman the incubator

17



Homunculus

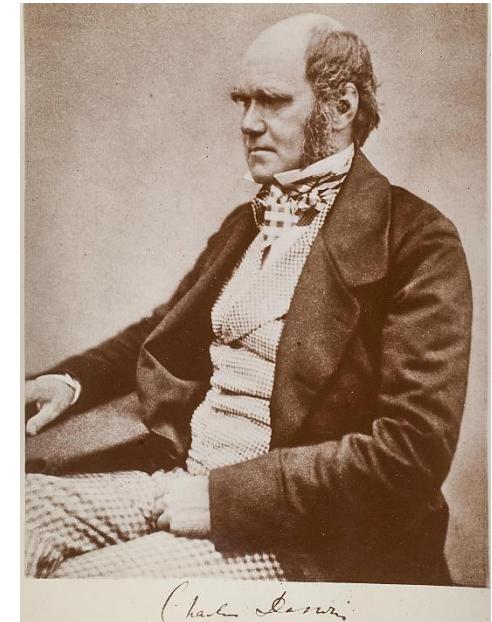
The little pre-formed person in the sperm. An imaginary representation of what a sperm might look like, if able to be seen clearly, drawn by Nicolaus Hartsoeker in *Essai de diotropique*, 1694.

Ove ideje bile su raširene do početka 19. st.

Darwin i Mendel:

- Darwin je predložio teoriju *pangeneze* (1868.): sve stanice u organizmu stvaraju sitne čestice gemule (*gemmules*) koje akumuliraju u gonadama (testisima i jajnicima) i prenose na potomstvo
- Mendel (1865.): eksperimentalno došao od zaključka da se svojstva nasljeđuju kao dvije neovisne „jedinice”(aleli) porijeklom od oca i majke...
...te da se one tijekom gametogeneze razdvajaju i pojavljuju kod potomaka ili u starim ili novim kombinacijama

U to vrijeme nisu bili opisani ni kromosomi (1879.) niti DNA (*nuklein*, 1869.) i njena funkcija (1944.) i struktura (1953.).



Osnovni genetički pojmovi u kontekstu Mendelovih križanja:

- divlji tip i mutant (mutanta) → su čiste linije

NAJUČESTALIJA
FENOTIPSKA
VARIJANTA

RIJEĐA
VARIJANTA
FENOTIPA

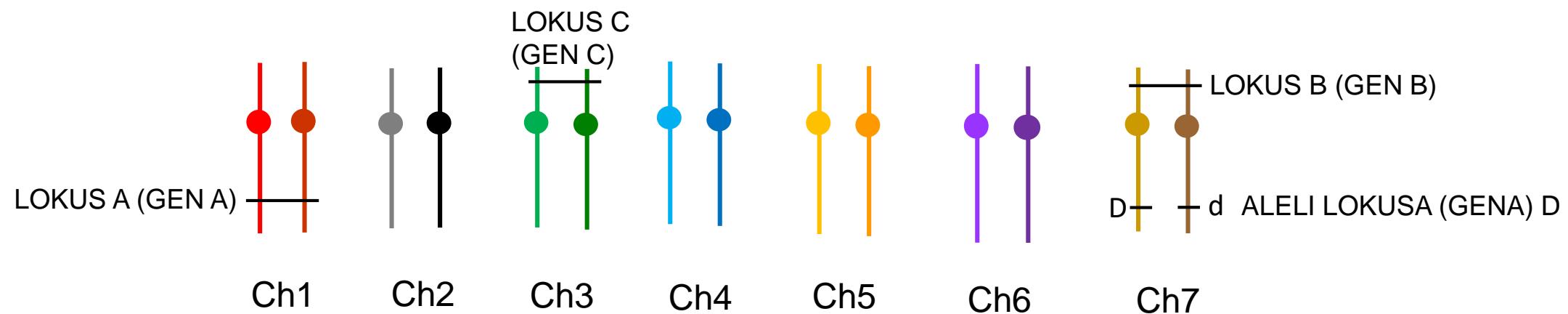
} nastaje spontanom/prirodnom mutagenezom (mutacija)

- **fenotip:** rezultat ekspresije genotipa („genski izražaj“) → vanjski izgled ili metabolički profil ili neka funkcija (fenotip je vidljiva manifestacija gentipa)
- **genotip:** DNA slijed (slijed nukleotida) ili DNA sekvenca (sekvencija) koji određuje fenotip (kemijski pohranjena informacija o budućem fenotipu)
- **fenotip:** dominantan (divlji tip) ili recesivan (mutant)
- **genotip:** homozigotan (AA ili aa) i heterozigotan/hibridan (Aa)
- **čista linija:** jedinka koja uvijek daje potomke roditeljskog fenotipa (što znači da je homozigo tj. stvara samo jedan tip gameta)

Osnovni genetički pojmovi u kontekstu Mendelovih križanja:

- **lokus/gen** je dio DNA koji sadrži uputu za neku staničnu strukturu ili funkciju
- kod diploidnih ($2n$) organizama svaki gen je prisutan u dvije kopije (dva alela) porijeklom od svakog roditelja
- tjelesne (somatske) stanice organizma koji se spolno razmnožavaju su diploidne ($2n$), a spolne stanice (gamete) su haploidne (n)

Kromosomi graška: $2n=14$ (7 homolognih parova; 7 očevih + 7 majčinih)



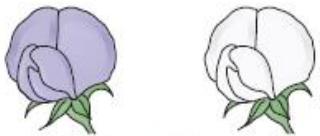
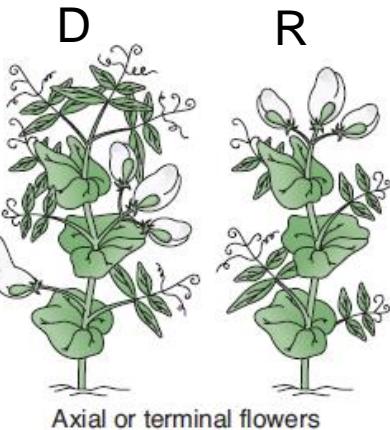
Dominantna (D) i recesivna (R) svojstva:

The seven phenotypic pairs studied by Mendel

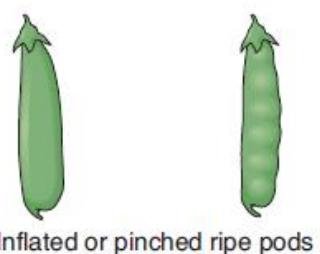
D R

Round or wrinkled ripe seeds

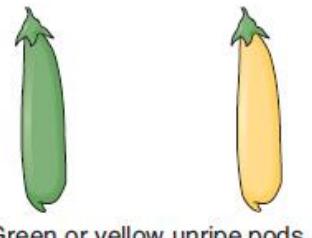
Yellow or green seeds



Purple or white petals



Inflated or pinched ripe pods



Green or yellow unripe pods

P: AA x aa (čiste linije)

F₁: svi Aa (heterozigoti)

Aa x Aa



Monohibridno križanje (1 gen)

F ₂	A	a
A	AA	Aa
a	Aa	aa

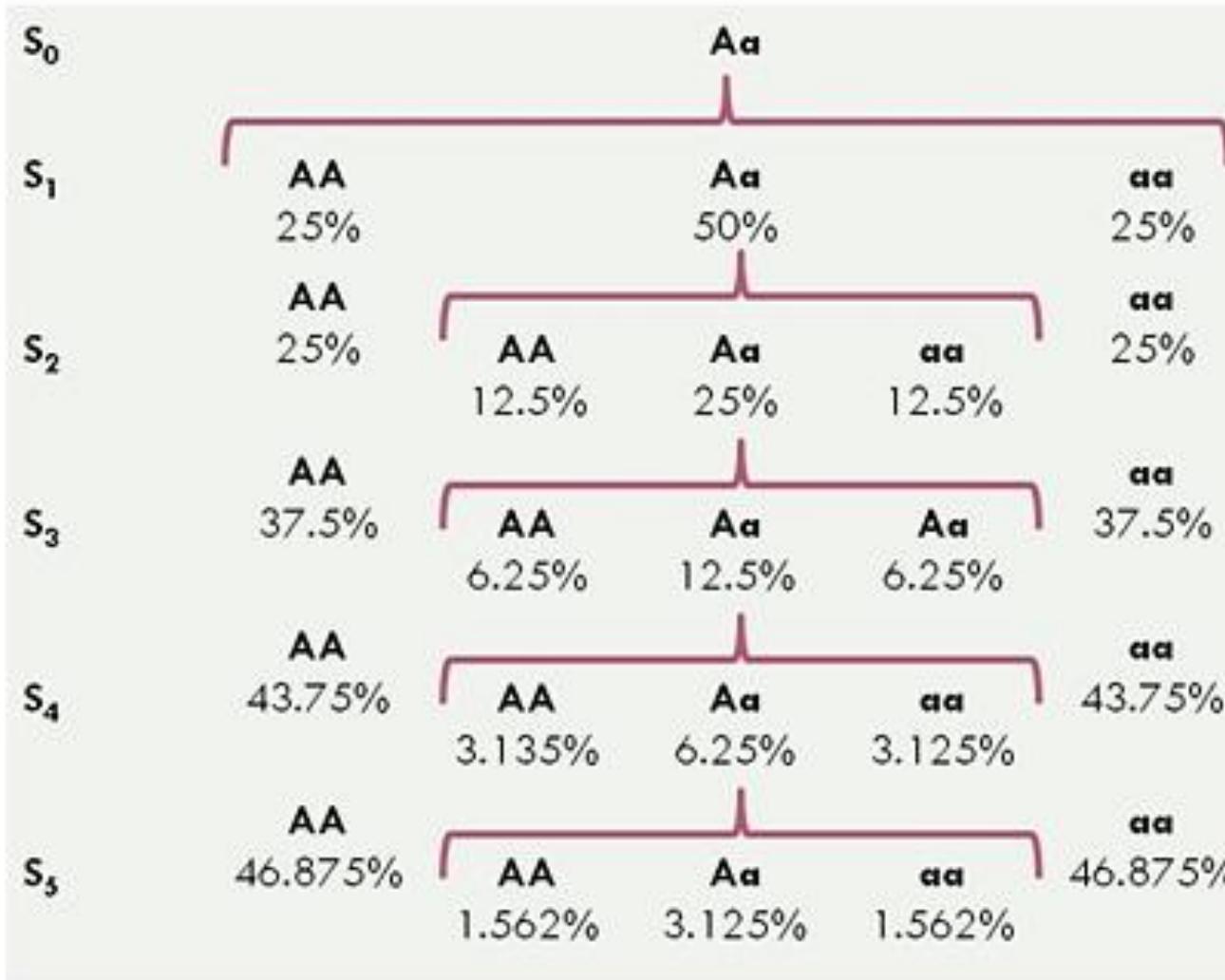
FENOTIPSKI OMJER:

3

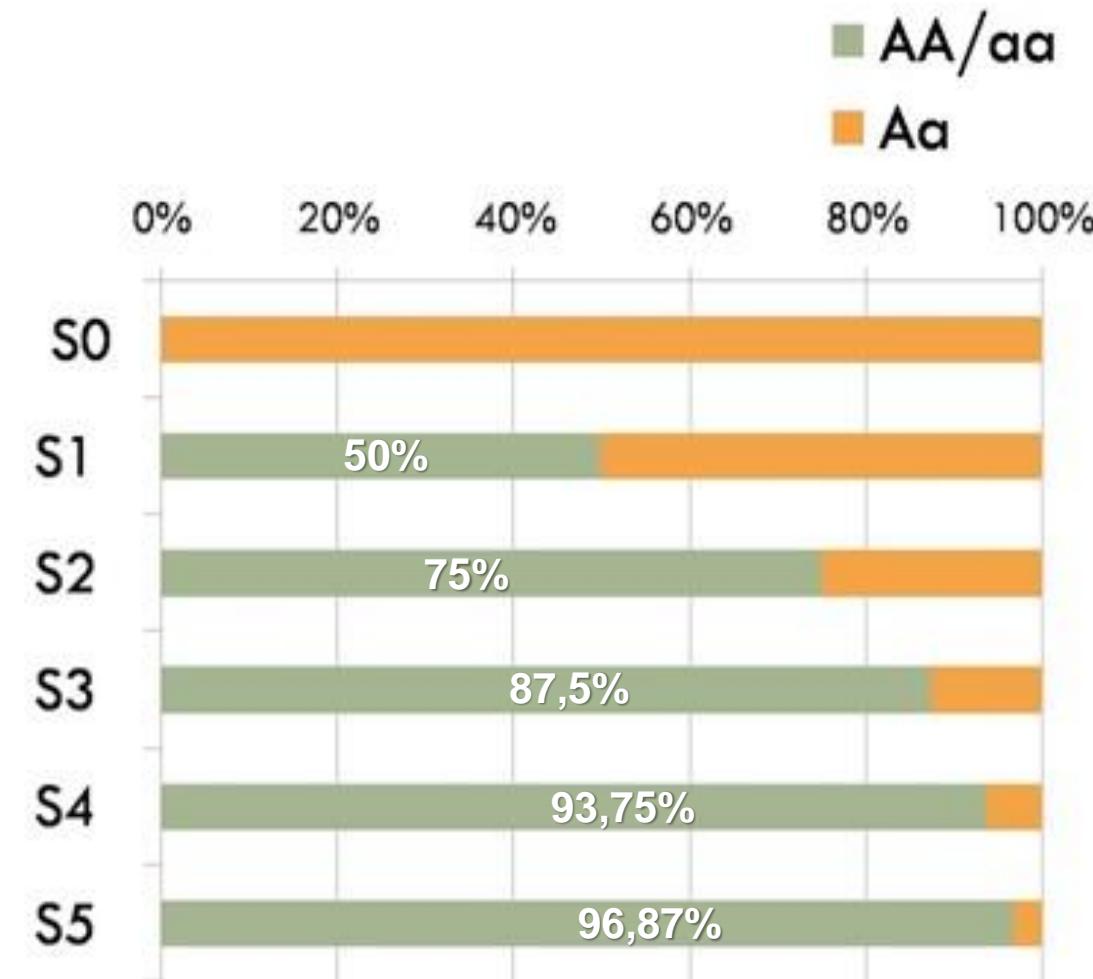
1



Čiste linije dobivaju se samooplodnjom (eng. *selfing*):



Nakon 9. kruga samooplodnje → 99,99% homozigota (za sve lokuse).



Nucleotide Nucleotide Advanced

GenBank

P.sativum mRNA for starch branching enzyme I

GenBank: X80009.1

FASTA Graphics

Go to:

LOCUS X80009 3549 bp mRNA linear PLN 18-APR-2005
 DEFINITION P.sativum mRNA for starch branching enzyme I.
 ACCESSION X80009
 VERSION X80009.1
 KEYWORDS SBEI gene; starch branching enzyme I.
 SOURCE Pisum sativum (pea)
 ORGANISM Pisum sativum
 Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
 Spermatophyta; Magnoliopsida; eudicots; Gunneridae;
 Pentapetalae; rosids; fabids; Fabales; Fabaceae; Papilionoideae; 50
 kb inversion clade; NPAAA clade; Hologlegina; IRL clade; Fabae;
 Pisum.
 REFERENCE 1
 AUTHORS Burton,R.A., Bewley,J.D., Smith,A.M., Bhattacharyya,M.K., Tatge,H.,
 Ring,S., Bull,V., Hamilton,W.D. and Martin,C.
 TITLE Starch branching enzymes belonging to distinct enzyme families are
 differentially expressed during pea embryo development
 JOURNAL Plant J. 7 (1), 3-15 (1995)

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Reference sequence information

RefSeq alternative splicing
 See 3 reference mRNA sequence splice variants
 for the LOC127125675 gene.

More about the gene LOC127125675

LOC127125675 gene
 Also Known As: KIW84_031368, SBEI

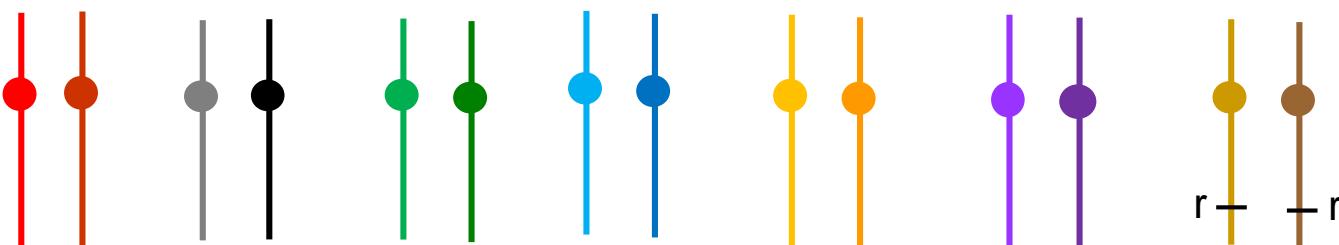
Recesivna mutacija je obično posljedica gubitka funkcije gena (eng. *loss of function*).

Relationship between modern genetic terminology and character pairs used by Mendel

Character pair used by Mendel	Alleles in modern terminology	Located in chromosome
Seed colour, yellow-green	I-i	1
Seed coat and flowers, coloured-white	A-a	1
Mature pods, smooth expanded-wrinkled indented	V-v	4
Inflorescences, from leaf axils-umbellate in top of plant	Fa-fa	4
Plant height, > 1m-around 0.5 m	Le-le	4
Unripe pods, green-yellow	Gp-gp	5
Mature seeds, smooth-wrinkled	R-r	7

<https://www.ncbi.nlm.nih.gov/nuccore/510545>

Kromosomi graška: $2n=14$



Ch1

Ch2

Ch3

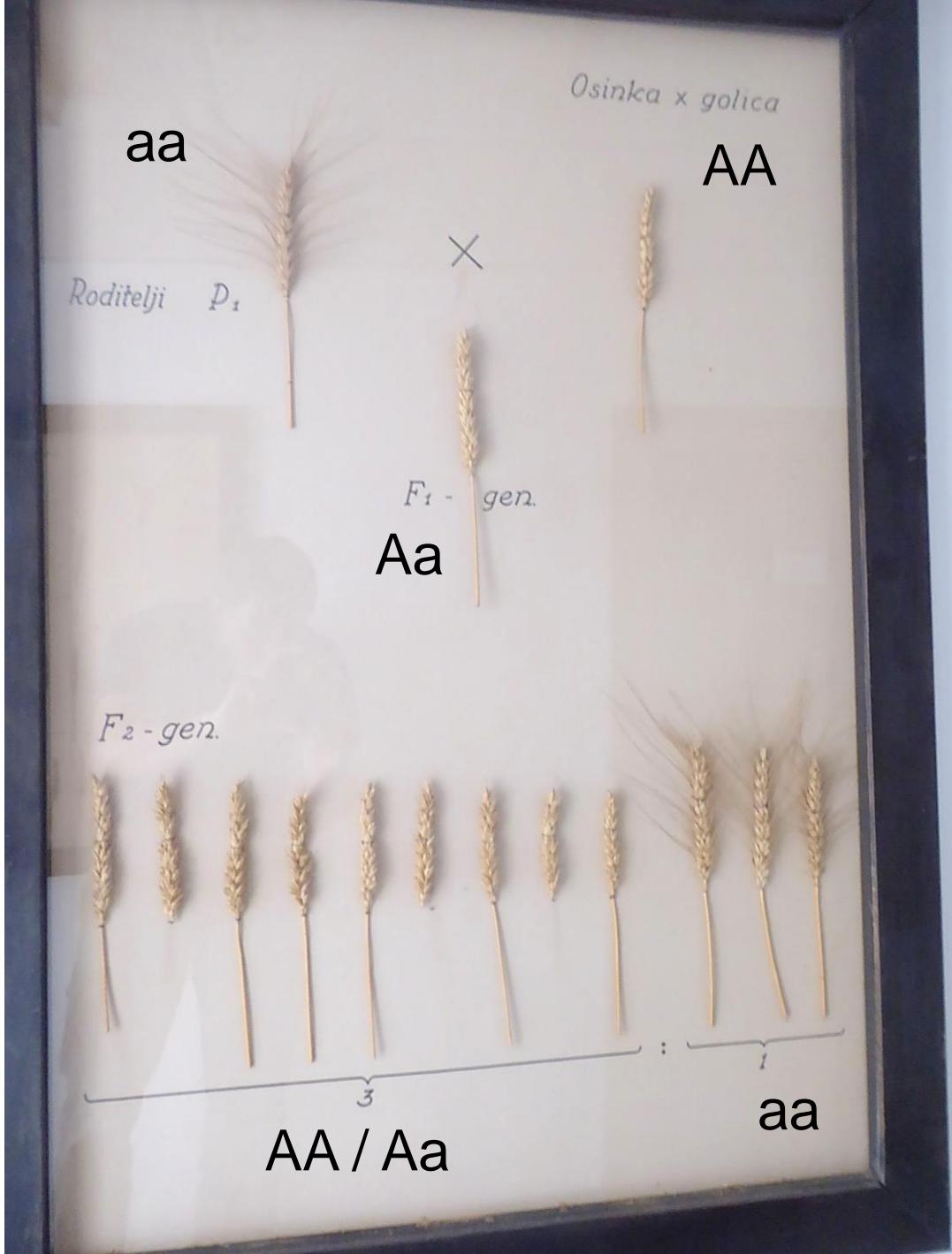
Ch4

Ch5

Ch6

Ch7

Ovo su primjeri gdje jedan gen (lokus) regulira jedno svojstvo.



Monohibridno križanje (1 gen)

	A	a
A	AA	Aa
a	Aa	aa

GENOTIPSKI OMJER 1:2:1

FENOTIPSKI OMJER:



n=1

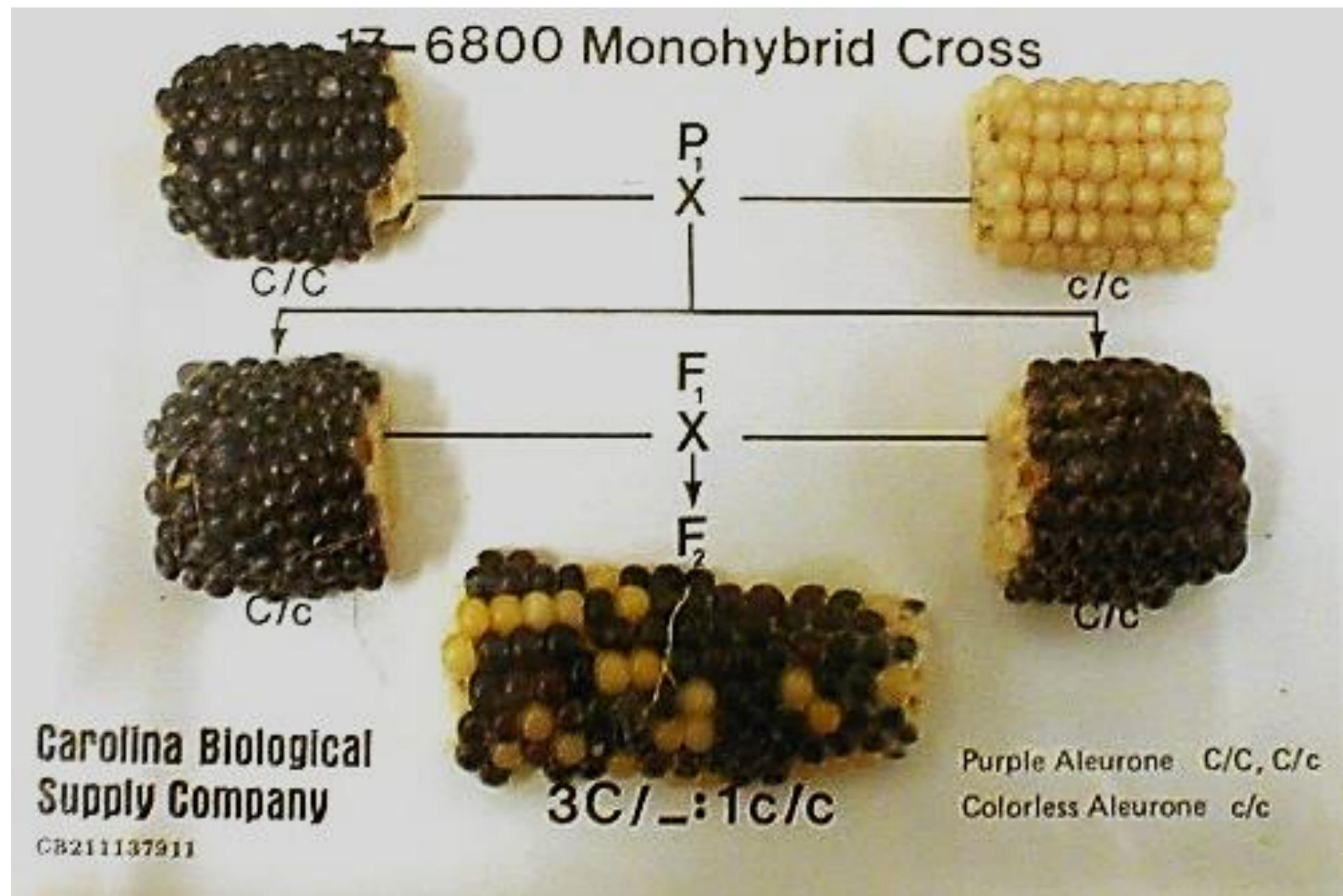
Fenotipske klase (2^n) = $2^1 = 2$

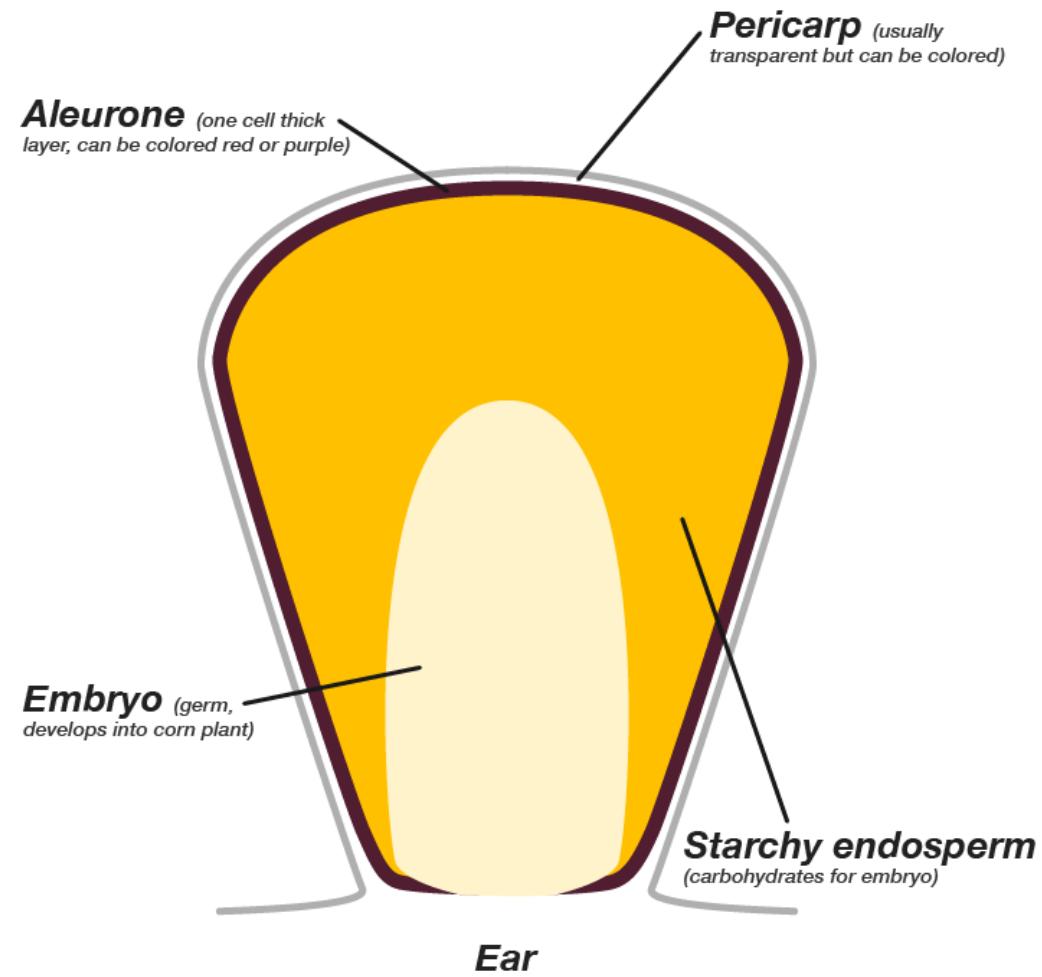
Genotipske klase (3^n) = $3^1 = 3$

Udio recessivnih homozigota $1/(2^n)^2 = 1/(2^1)^2 = 1$

- potpuna dominacija („binarni” fenotip)
 - AA i Aa imaju potpuno isti fenotip
 - 3 genotipa (AA, Aa i aa) i 2 fenotipa

Segregacija
dva alela
jednog lokusa:
kukuruz





Sirak:
varijabilnost u
dužini stabljike

- niska stabljika
- visoka stabljika

Udio rjeđeg
(recesivnog)
fenotipa $< 1/4 \rightarrow$
2 ili više gena
reguliraju visinu

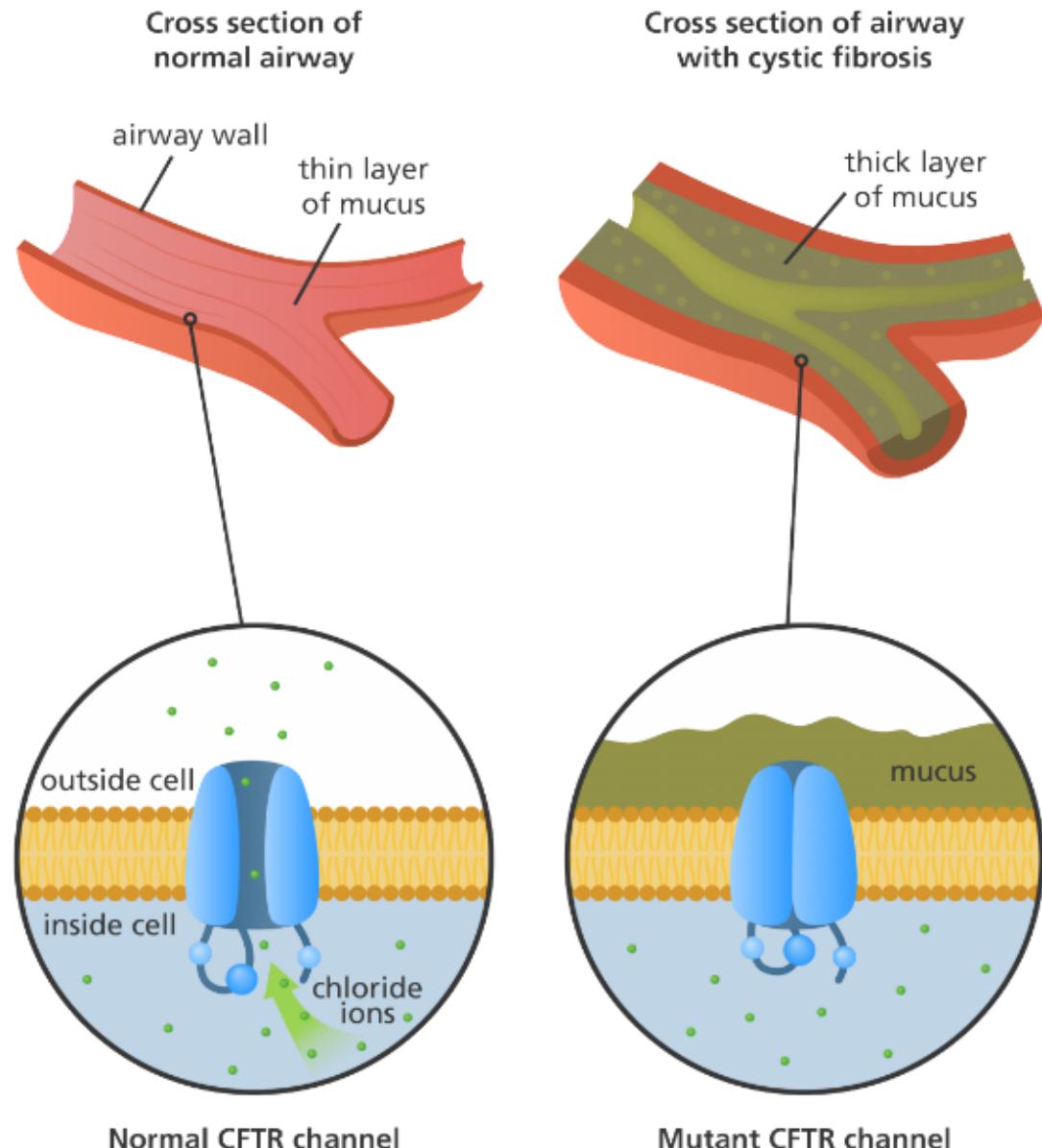
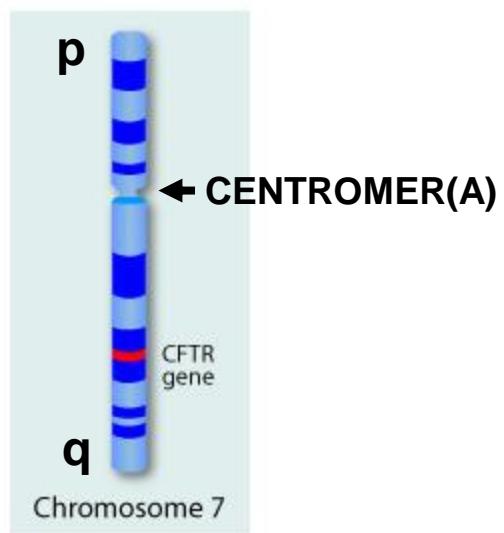
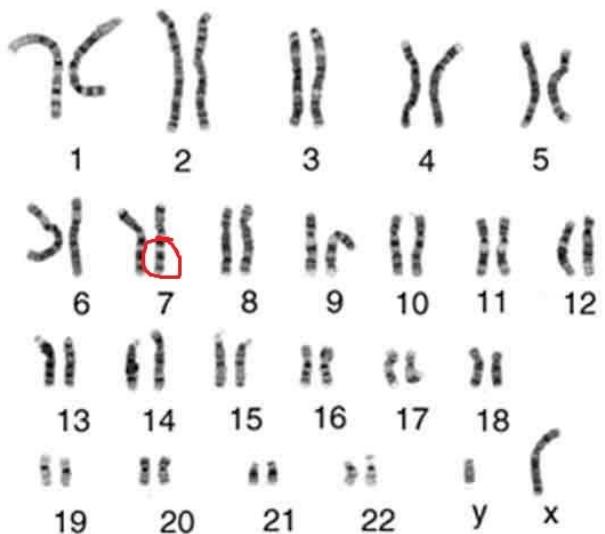
- Dw1-Dw4



Monogenske genetičke bolesti: uzrokovane mutacijom u jednom genu

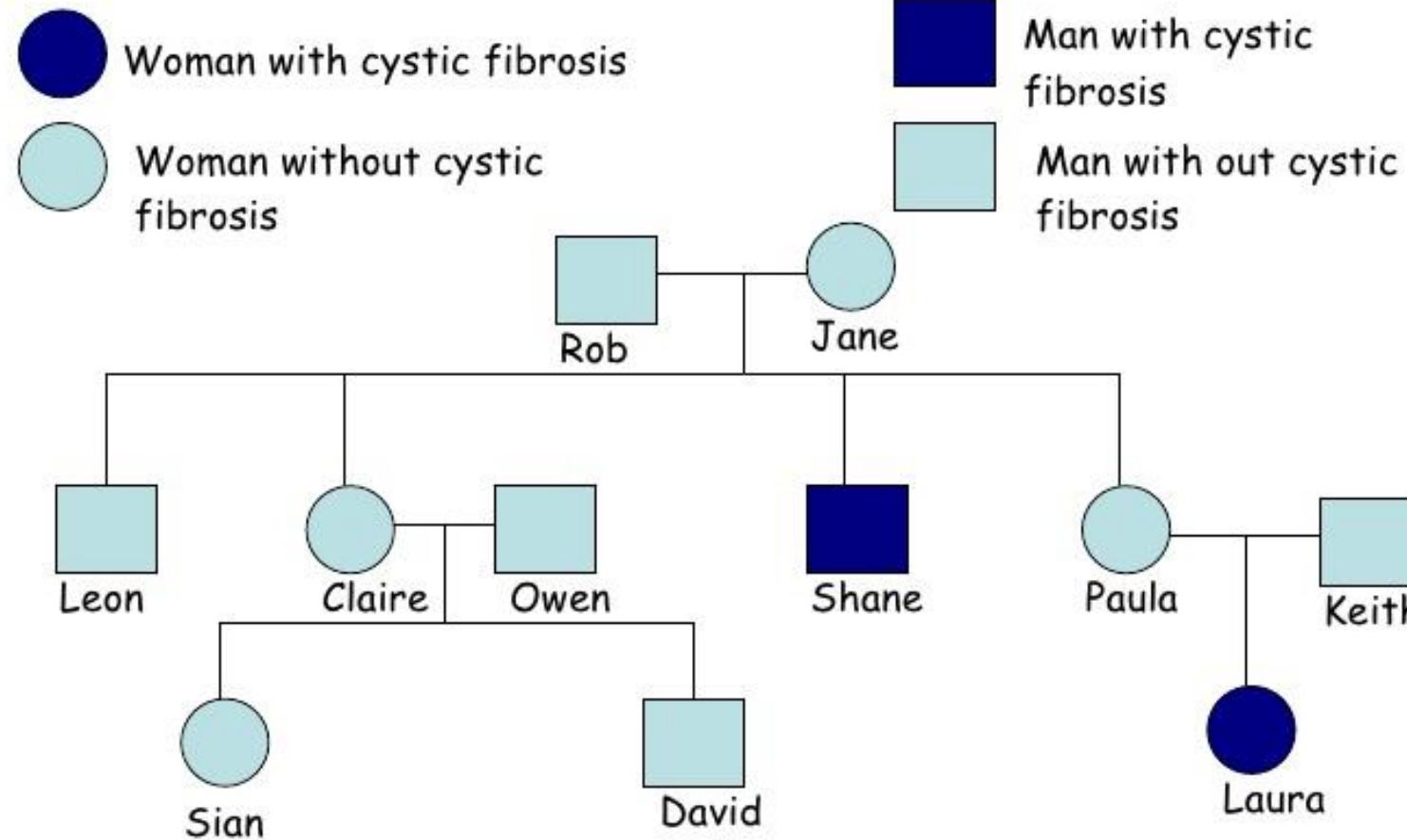


Primjer: cistična fibroza (CF)



Cistična fibroza je monogenska (autosomna) recesivna bolest:

Family tree – Cystic fibrosis



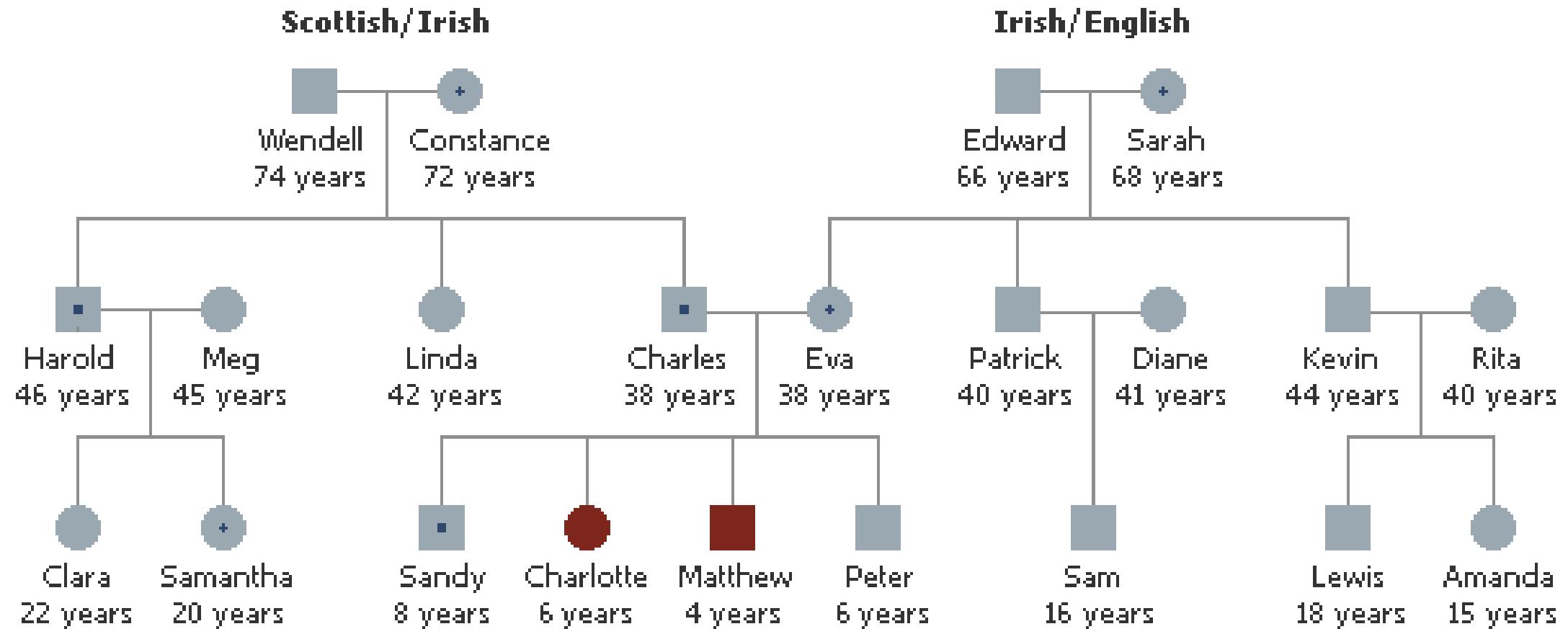
OBITELJSKO STABLO (RODOSLOVLJE/RODOSLOV)

- oboljelo dijete potomak je dvaju roditelja koji su prenositelji mutiranog alela (ne pokazu fenotip tj. nisu bolesni)

gen se nalazi na spolnom kromosomu (npr. X kromosomu)

gen se nalazi na bilo kojem od ne-spolnih kromosoma ← autosomno vs. spolno-vezano svojstvo

Nasljeđivanje cistične fibroze:



Key

Male

Female



Cystic Fibrosis



Healthy carrier of cystic fibrosis gene

Genetičko savjetovanje:

- vjerojatnost da će dijete biti bolesno
- vjerojatnost da će dijete biti prenositelj

CFRT nukleotidni slijed: divlji tip (DT)

Nucleotide Sequence (4443 nt):

EGZONI

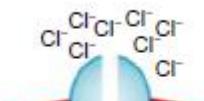
CFRT aminokiselinski slijed: DT

CFTR gene (1480 aa):

MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSDLNKEERSLA
NALRRCCFFWRMFYGFYLGEVTKAQPLLLGRIIASYDPDNKEERSIAIYLGIGLCLLFIVRTLLLHP
AIFGLHHIGMQMARIAMFSLIYKKTLKSSRVLDKISIGQLVSSLNNLNKFDEGLALAHFVVIAPLQVAL
LMGLIWELLQASAFCGLGFLVLALFQAQLGRMMMKYRDQRAGKISERLVITSEMIENIQSVKAYCWEA
MEKMIENLRQTELKTRKAAYVRYFNSSAFFSGFFVFLVLPYALIKGIILRKIFTTISFCIVLRMAV
TRQFPWAVQTWYDSDLGAINKIQDFLQKQEYKTLEYNLTTTEVVMENVTAFWEEGFGELFEKAKQNNNNRK
TSNGDDSLFFSNFSLLGTPVLDINFKIERGQLAVAGSTGAGKTSLLMIVMGELEPSEGKIKHSGRISF
CSQFSWIMP GTIKE NIIFGVSYDEYRYSVIKACQLEEDISKFAEKDNIVLGEGGITLSGGQRARISLAR
AVYKDADLYLLDSPFGYLDVLTEKEIFESCVC KLMANKTRILVSKMEHLKKADKILHEGSSYFYGTF
SELQNLQPDFSSKLMGCDSDQFSAERRNSILTETLHRFSLEGDAPVSWTETKQSFKQTGEFGEKRKNS
ILNPINSIRKFSIVQKTPLQMNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRRQSVL
NLMTHSVNQGQNIHRKTTASTRKVSLAPQANLTEDIYSRRLSQETGLEISEEINEEDLKECFDDMESI
PAVTTWNTYLRYITVHKSLIFVLIWCLVIFLAevaASLVLWLLGNTPLQDKGNSTHSRNNSYAVIITST
SSYYVFYIYVGVA DTLLAMGFFRGLPLVHTLTVSKILHHKMLHSVLPQAPMSTLNTLKAGGILNRF SKDI
AI LD DLLPTIFDFIQLLLIVIGAI AVVAVLQPYIFVATVPVIVAFIMLRAYFLQTSQQQLKQLESEGRSP
IFTHLVTSLKGWLWTLRAFGRPYFETLFHKALNLHTANWFLYLSTLRWFQM RIEMIFVIFI A VT FISIL
TTGEGEGRVGII LTLMNIMSTLQWA VNSSIDVDSL MRSVSRVFKFIDMPTEGKPKTKSTKP YKNGQLSKV
MIIENS HVKKDDIWPSSGGQMTVKDLTA KYTEGGNAILENISFSISPGQRVGLGRTGSGKSTL SAFLRL
LNTEGEI QIDGVWS DITS LQQWRKA FG VVIPQKVIFSGTFRKNLD PYEQWSDQEWKVADEVGLRSVIEQ
FPGKDFV LVDGGCVL SHGHKQLMCLARSVLSKA KILLDEPSA HLDPV TYQIIRRTL KQAFADCTVILC
EHRIEAMLECQQFLVIEENKVRQYDSIQKLLNERSLFRQAISPSDRV KLFPHRNSSKCKSKPQIA ALKEE
TEEEVQDTRL

- CFRT: *cystic fibrosis transmembrane conductance regulator*
 - kod oboljelih osoba alel divljeg tipa može biti mutiran na više različitih mesta u *CFRT* genu

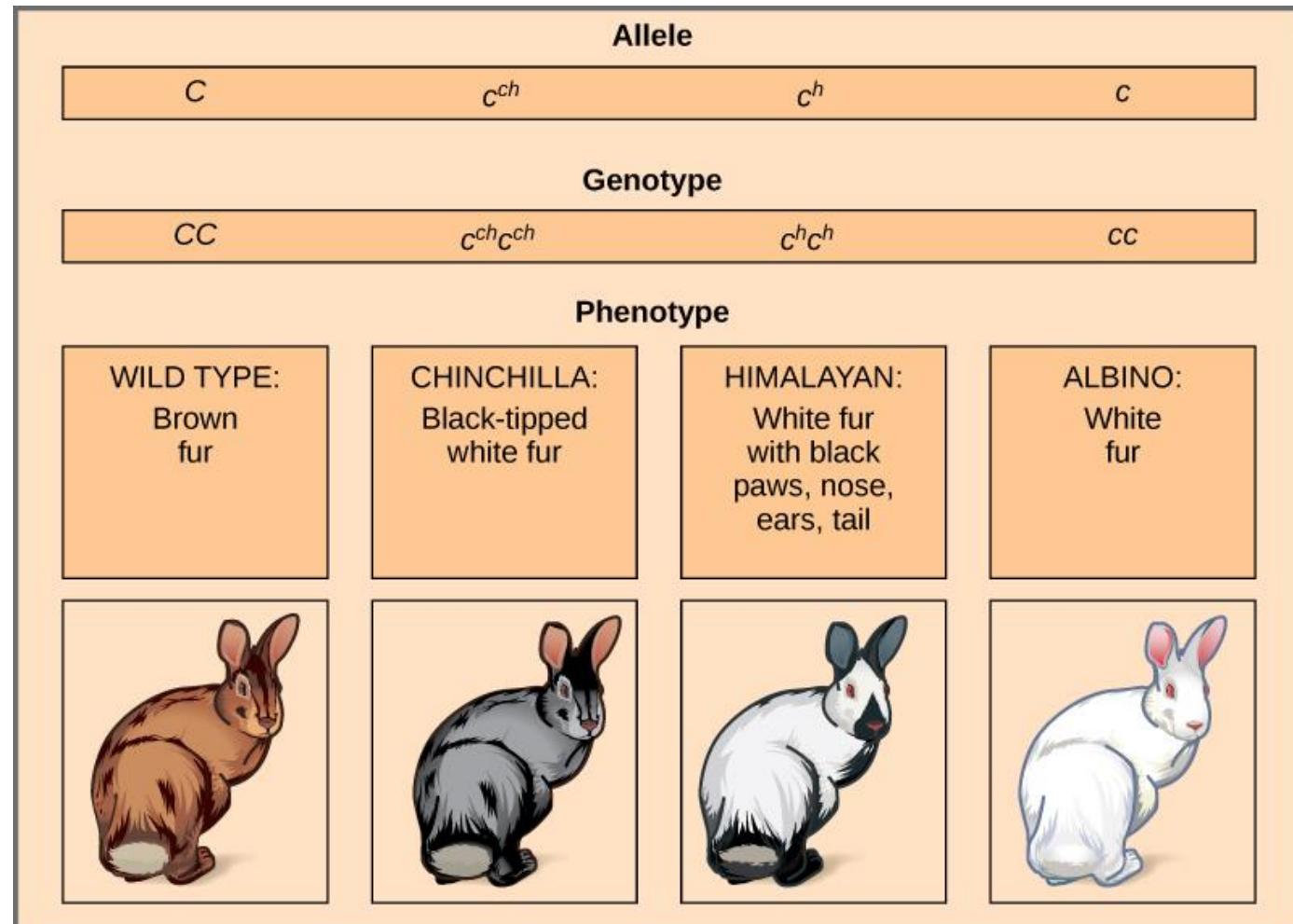
Moguće su različite mutacije u CFTR genu:

Normal	I	II	III	IV	V	VI
 <p>Mature functional CFTR</p> <p>Golgi</p> <p>Nascent CFTR</p> <p>Endoplasmic reticulum</p> <p>Full-length CFTR RNA</p> <p>Nucleus</p> <p>CFTR DNA</p>	 <p>Absent functional CFTR</p> <p>Golgi</p> <p>Absent nascent CFTR</p> <p>Endoplasmic reticulum</p> <p>Unstable truncated RNA</p> <p>Nucleus</p> <p>CFTR DNA</p>	 <p>Absent functional CFTR</p> <p>Golgi</p> <p>Protease destruction of misfolded CFTR</p> <p>Endoplasmic reticulum</p> <p>Full-length CFTR RNA</p> <p>Nucleus</p> <p>CFTR DNA</p>	 <p>Defective channel regulation</p> <p>Golgi</p> <p>Nascent CFTR</p> <p>Endoplasmic reticulum</p> <p>Full-length CFTR RNA</p> <p>Nucleus</p> <p>CFTR DNA</p>	 <p>Defective CFTR channel</p> <p>Golgi</p> <p>Nascent CFTR</p> <p>Endoplasmic reticulum</p> <p>Full-length CFTR RNA</p> <p>Nucleus</p> <p>CFTR DNA</p>	 <p>Scarce functional CFTR</p> <p>Golgi</p> <p>Scarce nascent CFTR</p> <p>Endoplasmic reticulum</p> <p>Correct RNA</p> <p>Nucleus</p> <p>CFTR DNA</p>	 <p>Decreased CFTR membrane stability</p> <p>Golgi</p> <p>Nascent CFTR</p> <p>Endoplasmic reticulum</p> <p>Full-length CFTR RNA</p> <p>Nucleus</p> <p>CFTR DNA</p>
CFTR defect	No functional CFTR protein	CFTR trafficking defect	Defective channel regulation	Decreased channel conductance	Reduced synthesis of CFTR	Decreased CFTR stability
Type of mutations	Nonsense; frameshift; canonical splice	Missense; amino acid deletion	Missense; amino acid change	Missense; amino acid change	Splicing defect; missense	Missense; amino acid change
Specific mutation examples ³¹	Gly542X Trp1282X Arg553X 621+1G → T	Phe508del Asn1303Lys Ile507del Arg560Thr	Gly551Asp Gly178Arg Gly551Ser Ser549Asn	Arg117His Arg347Pro Arg117Cys Arg334Trp	3849+10kbC → T 2789+5G → A 3120+1G → A 5T	4326delTC Gln1412X 4279insA

VAŽNO: osoba (diploid) može imati samo dva alela, ali u populaciji može biti stotine alela jednog gena (lokusa).

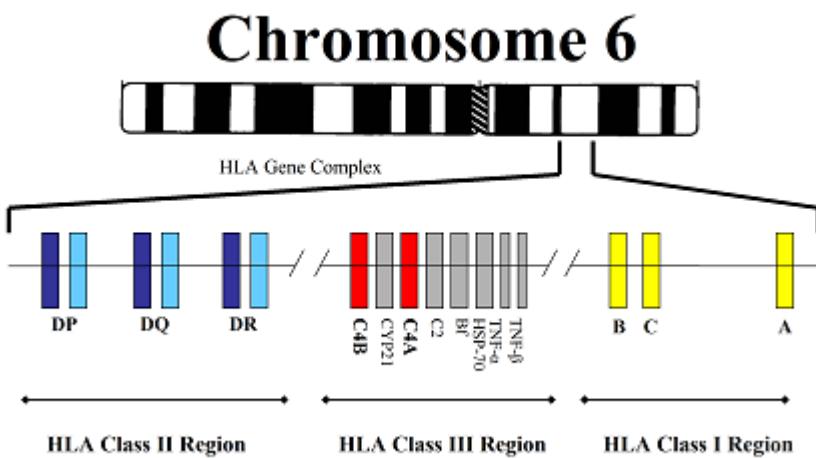
Multipli aleli: > 2 alela po lokusu (populacija)

Primjer 1: boja krvnog kunića



Dominantno-recesivni odnos alela: C > c^{ch} > c^h > c

Primjer 2: sustav tkivne snošljivosti (MHC / HLA)



MHC locus	Numbers of gene alleles
MHC class I	-
HLA-A	6,291
HLA-B	7,562
HLA-C	6,223
MHC class II beta chain	-
HLA-DPB	1,670
HLA-DQB	1,930
HLA-DPB	3,536
MHC class II alpha chain	-
HLA-DPA1	216
HLA-DPA2	5
HLA-DQA1	264
HLA-DQA2	38
HLA-DRA	29

**VELIKI BROJ
ALELA U
POPULACIJI**

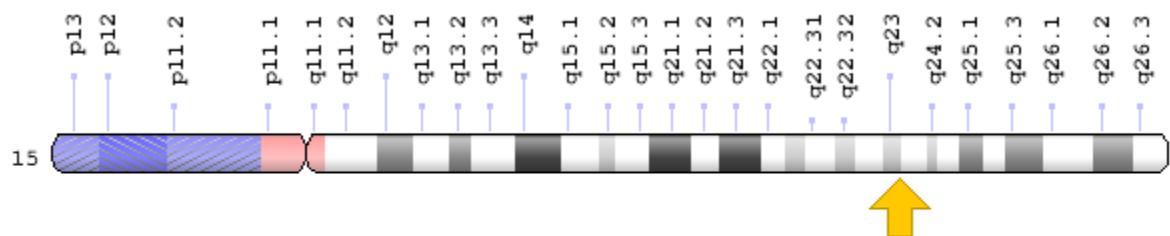
- važno kod transplantacija (potrebna je podudarnost alela donora i primatelja)

Tay-Sachs je monogenska (autosomna) recesivna bolest:

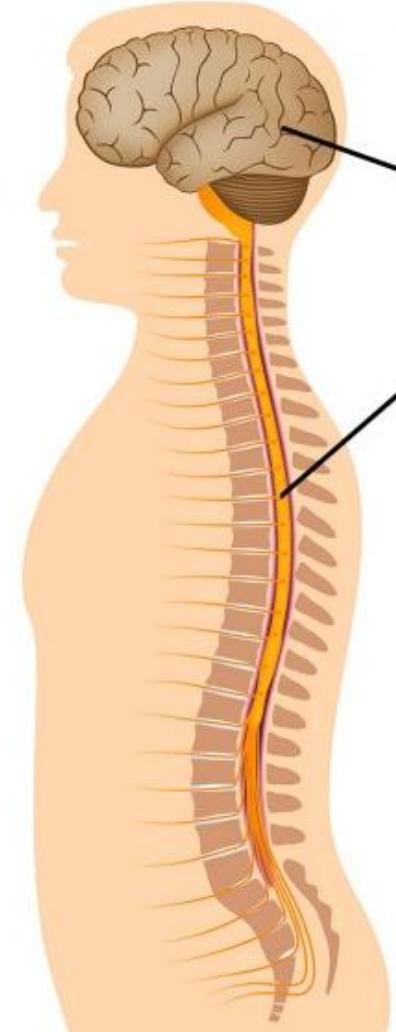
Bernard Sachs



Warren Tay

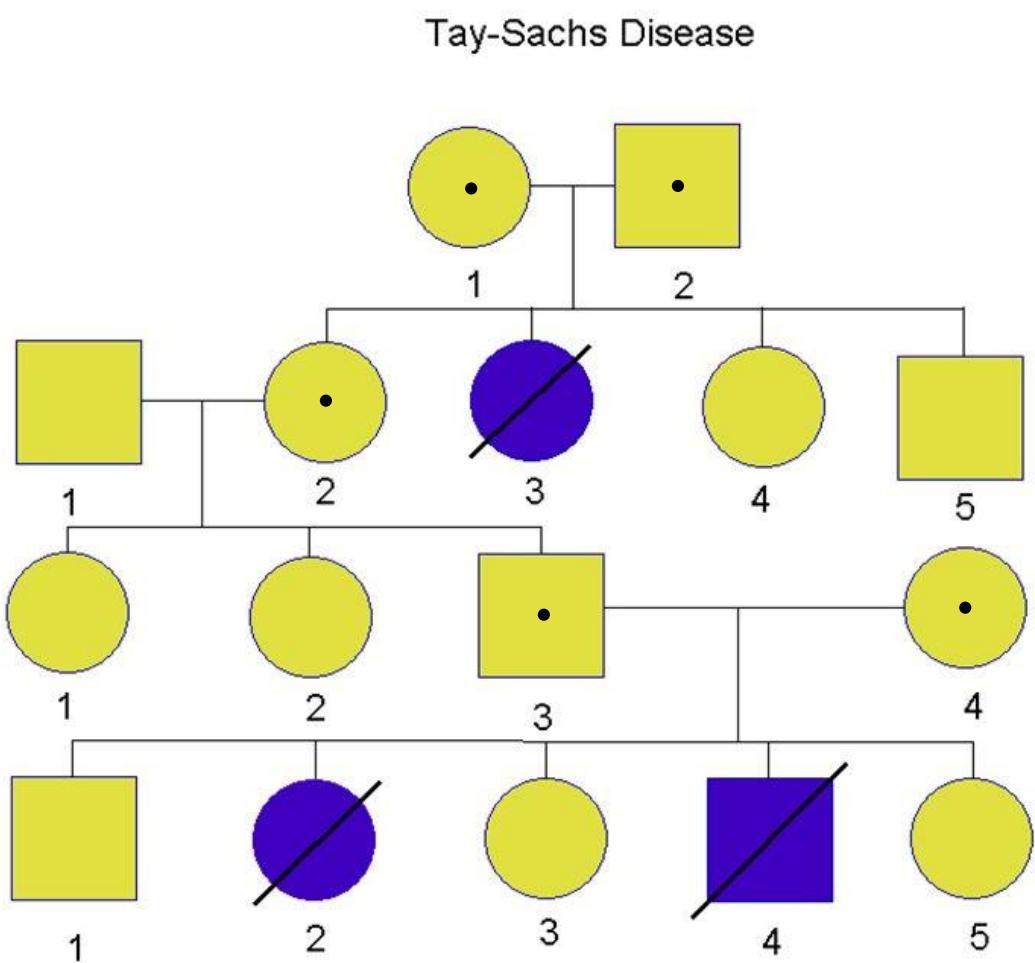


Tay-Sachs Disease



Degeneration of neurons in the brain and spinal cord

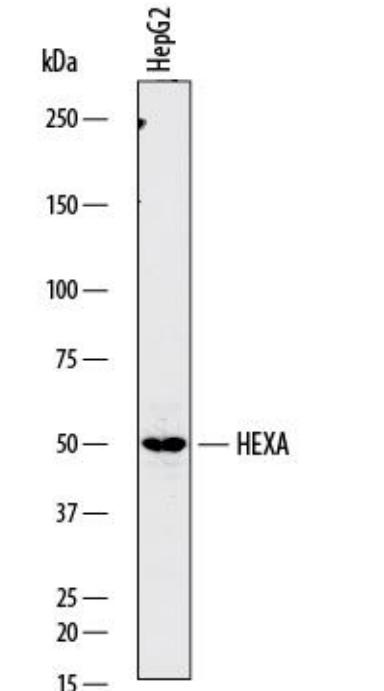
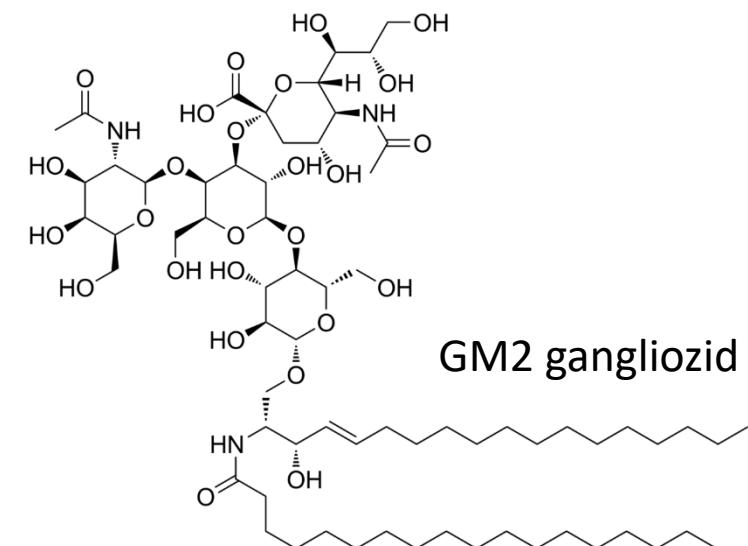
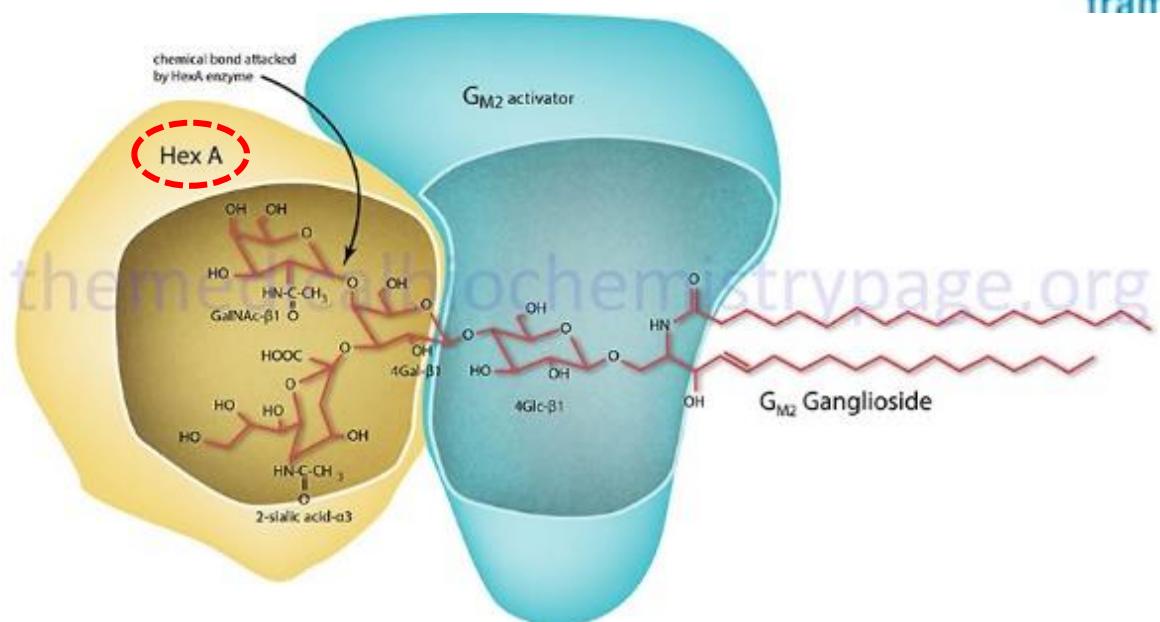
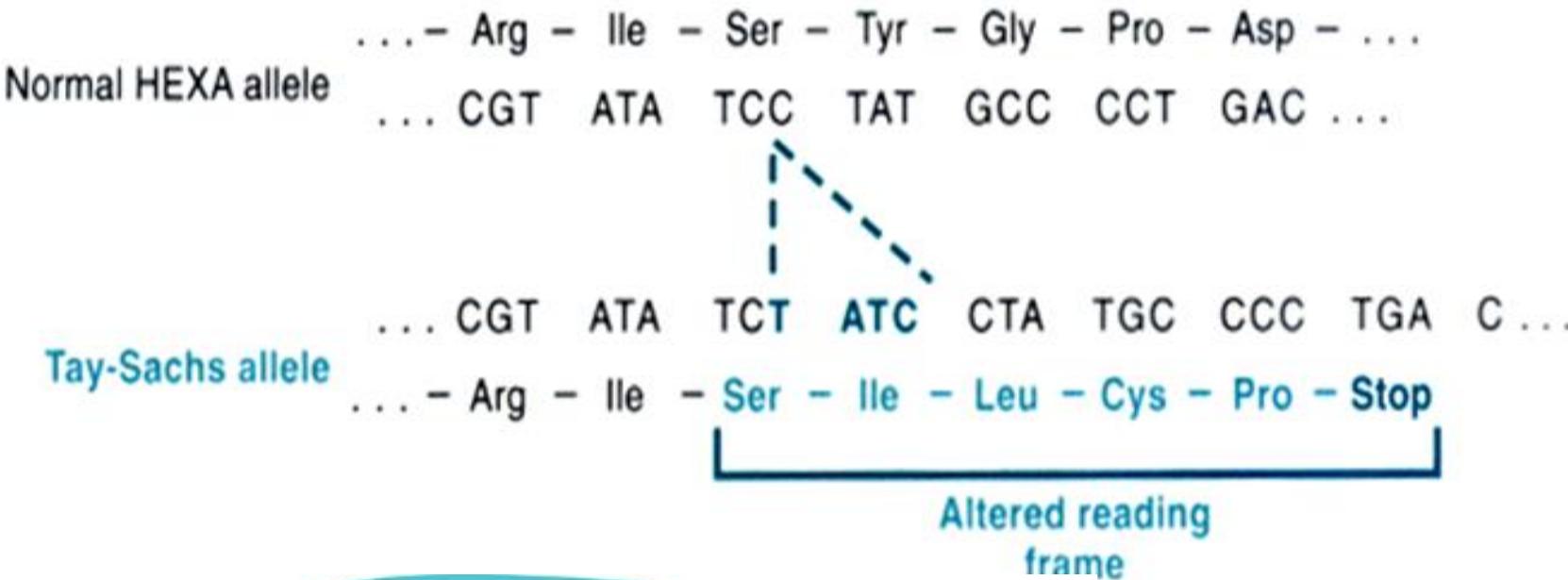
Tay-Sachs je monogenska (autosomna) recesivna bolest:



- učestalost mutiranog alela povećana je u skupini židova Aškenaza
- sklapanje brakova unutar relativno male zajednice (populacije)



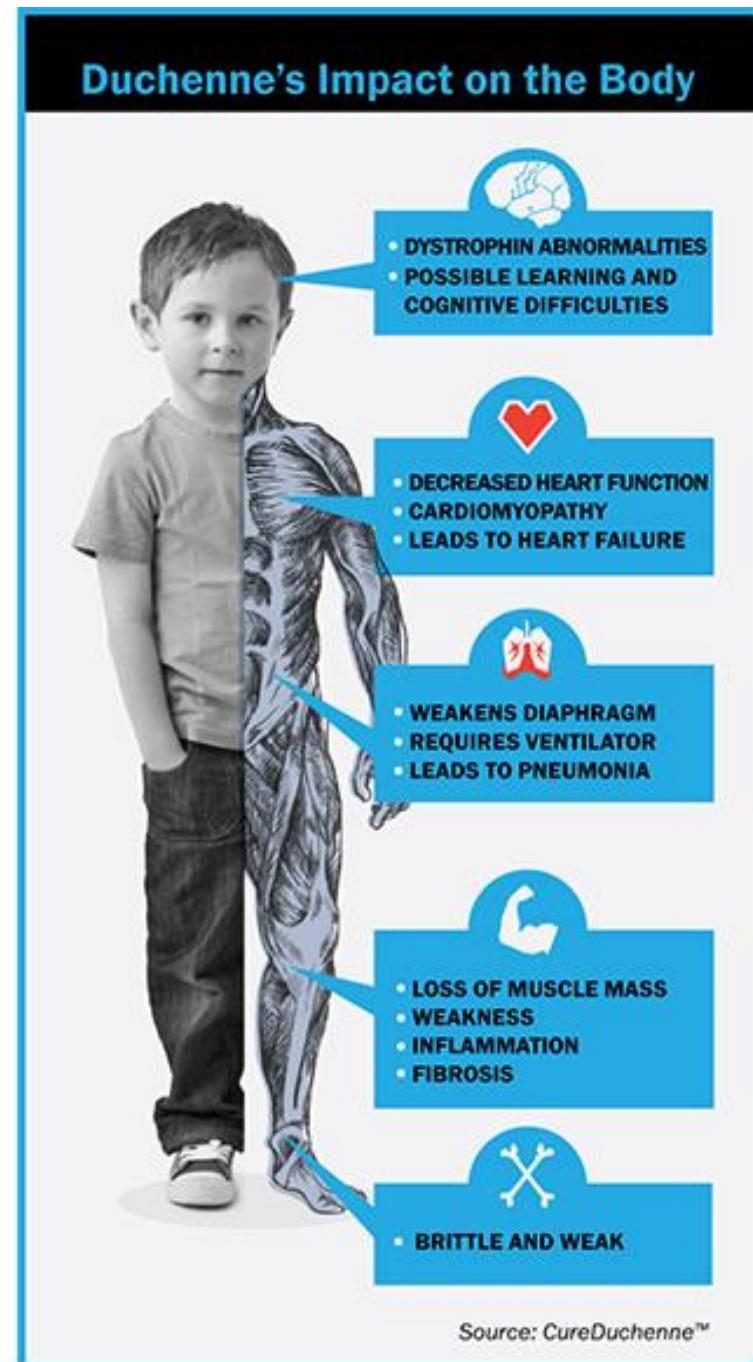
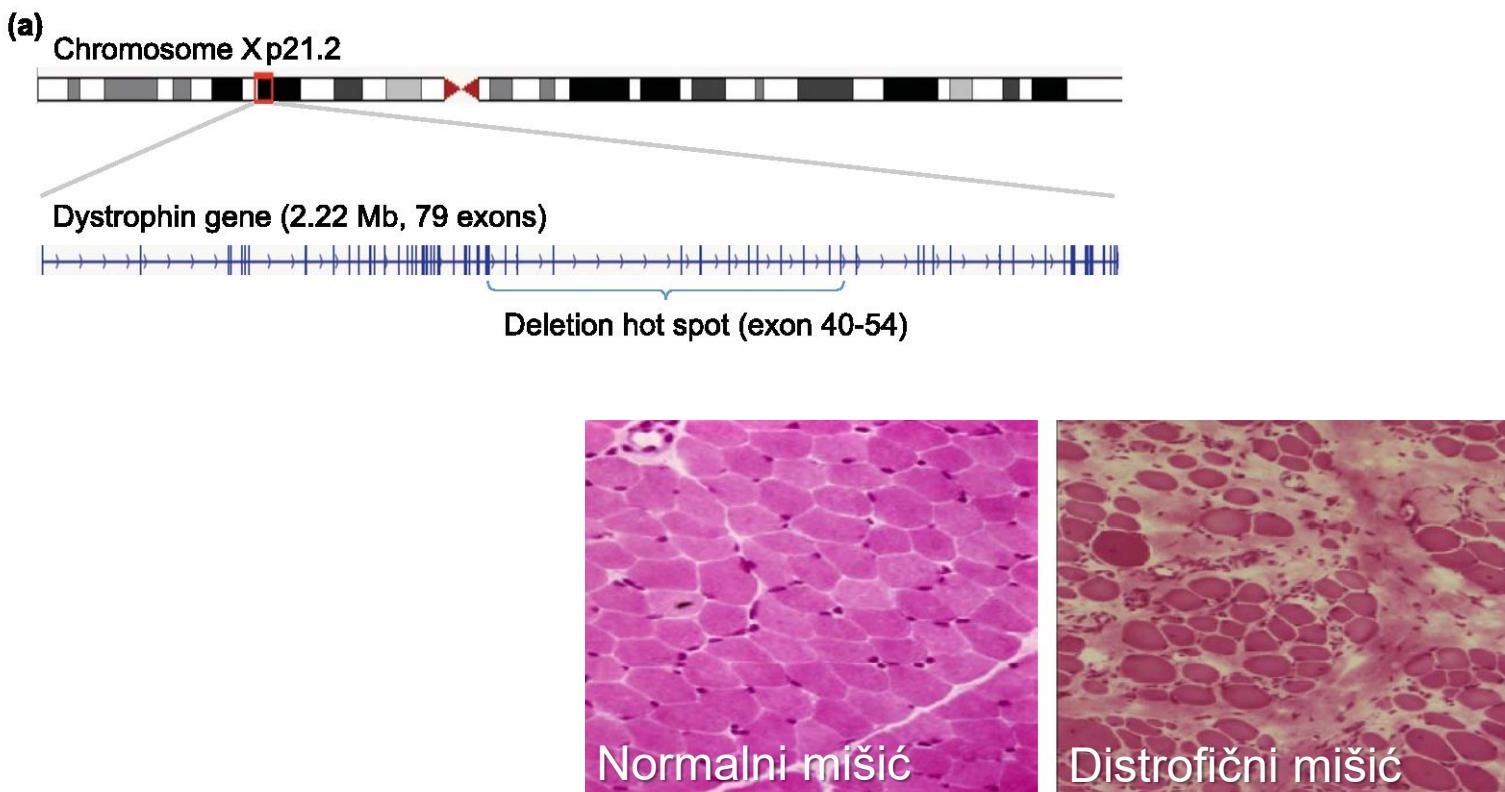
Tay-Sachs je monogenska (autosomna) recesivna bolest:



Heksozaminidaza A

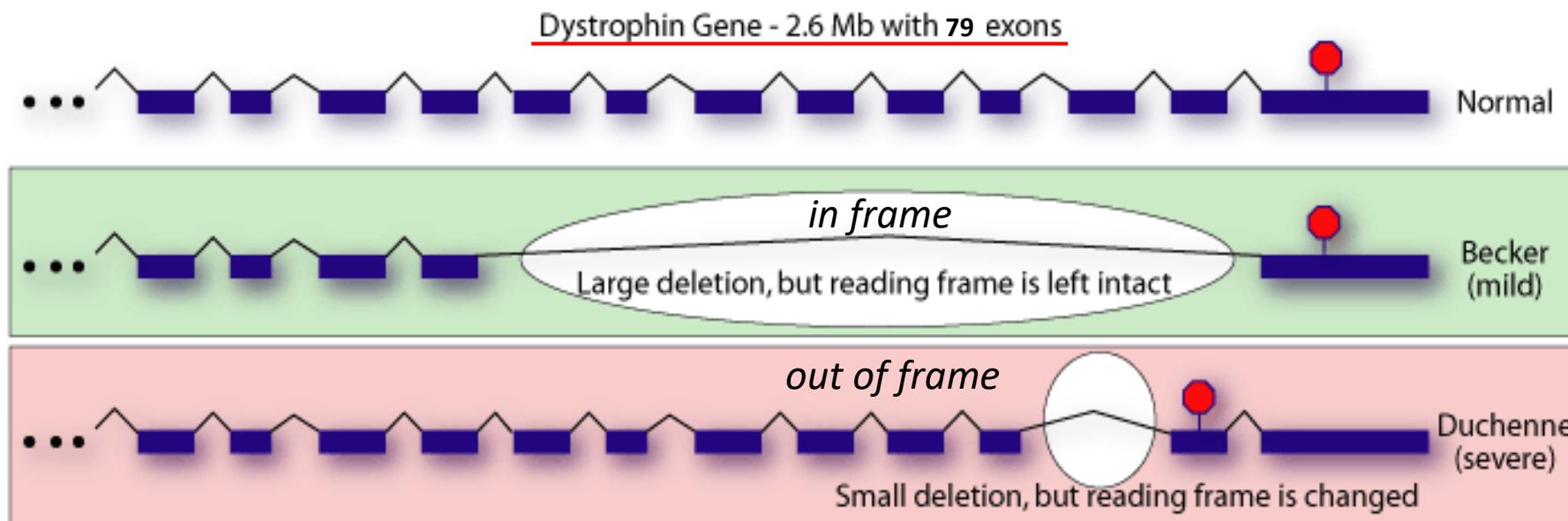
Duchenn-ova mišićna distrofija (DMD):

- odsutnost funkcionalnog distrofina, proteina koji čini oko 0.01% proteina skeletnog mišića
- atrofija mišića → gubitak mišićne mase → smrt
- X-vezana recesivna bolest, 1/3.500 muške djece



Duchenn-ova mišićna distrofija (DMD):

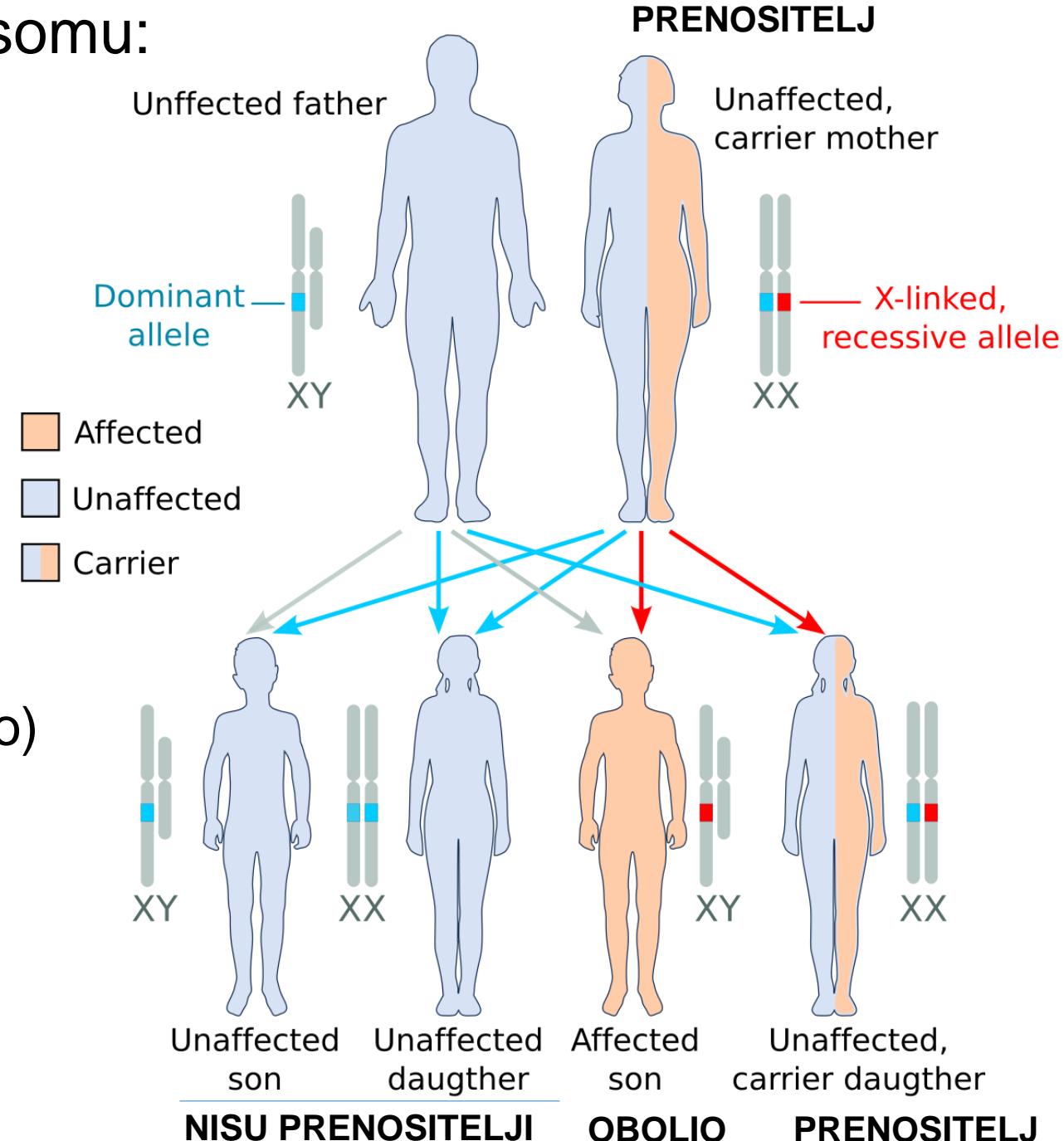
- skraćeni protein distrofin je nefunkcionalan



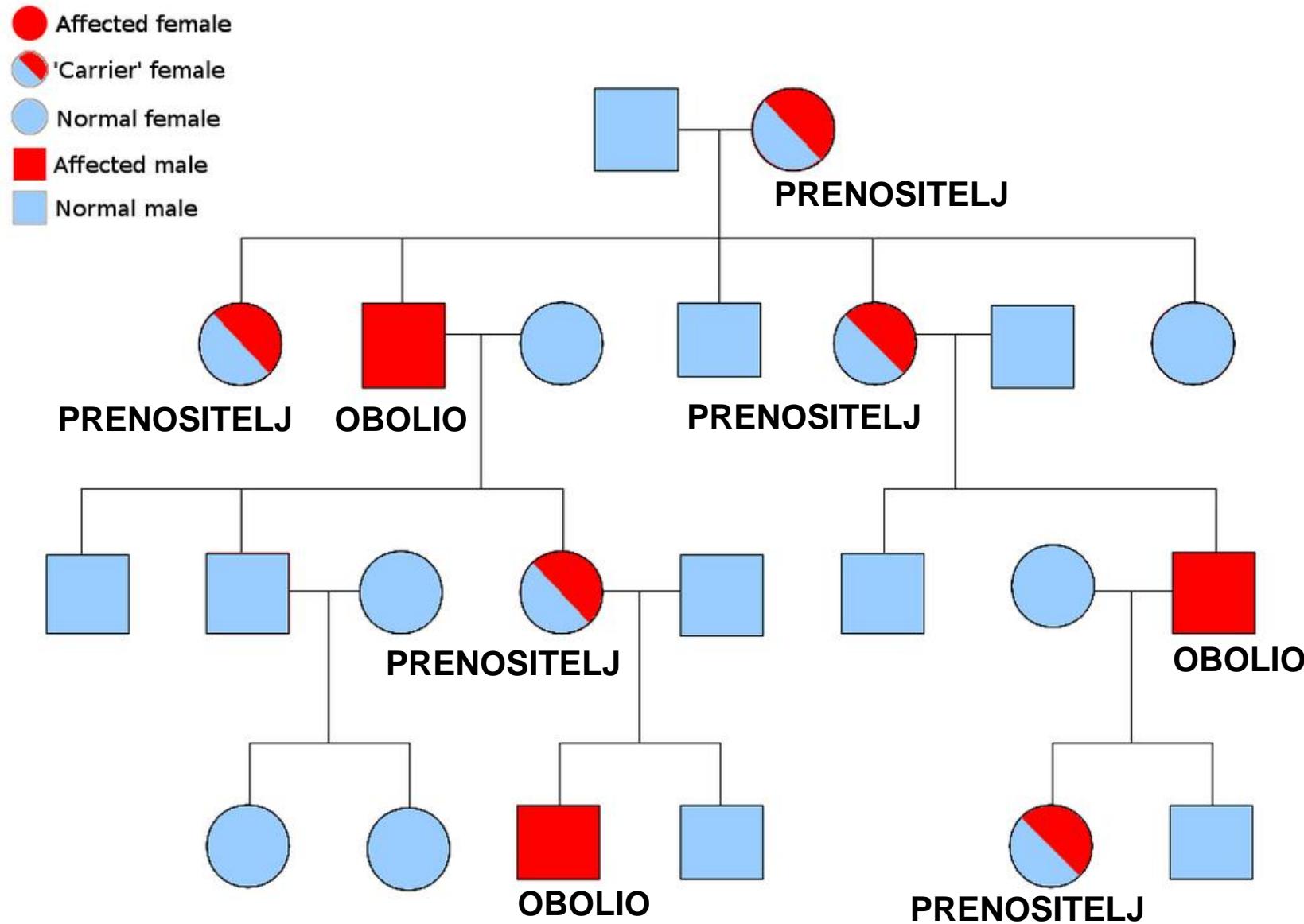
- nasljeđuje se s majke na sinove (obolijevaju) i kćeri (ne obolijevaju, osim ako i otac nije prenositelj) → spolno-vezana bolest

Spolno-vezana svojstva na X kromosomu:

- DMD (recesivno, X-vezano)
- daltonizam (recesivno, X-vezano)
- hemofilija (recesivno, X-vezano)
- *Rett-ov sindrom* (dominantno, X-vezano)

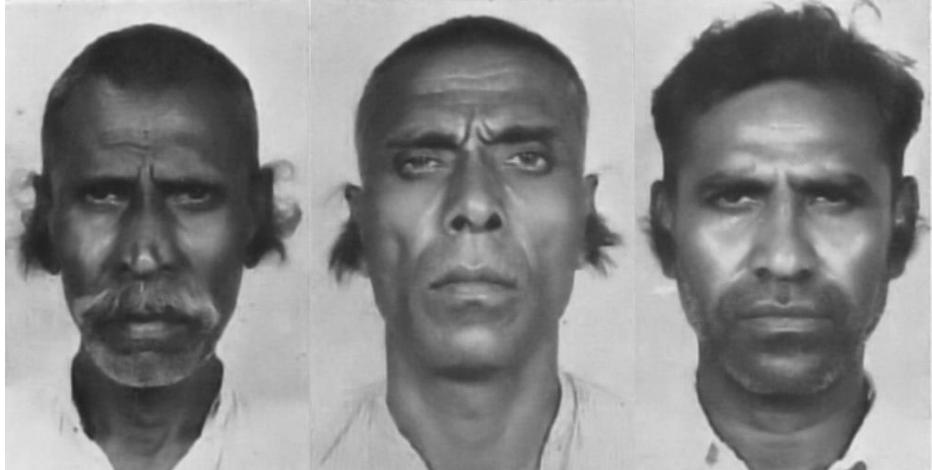


Spolno-vezana svojstva: jedan alel dovoljan je za bolest (kod muškaraca)

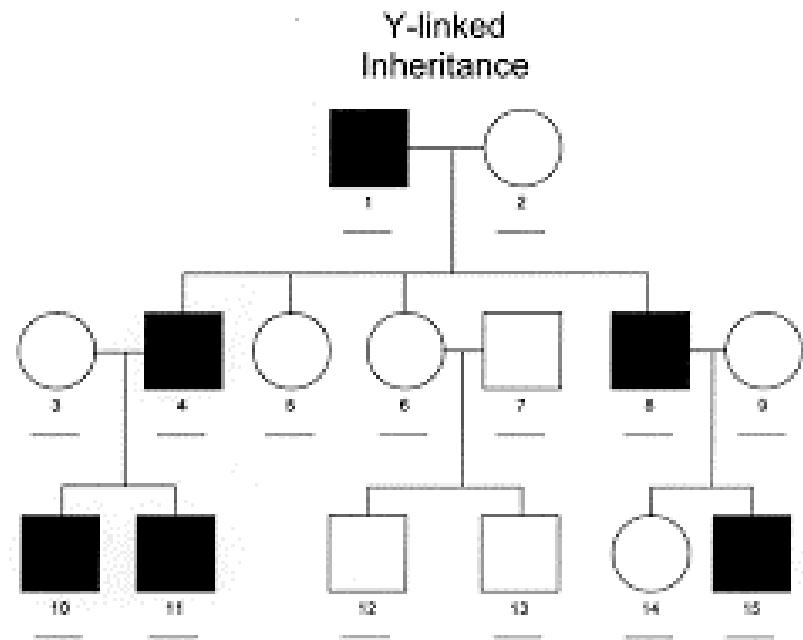


Spolno-vezana svojstva na Y kromosomu:

- izražena dlakavost ušiju (Y-vezano)
- srasli prsti na stopalu (sindaktilija)



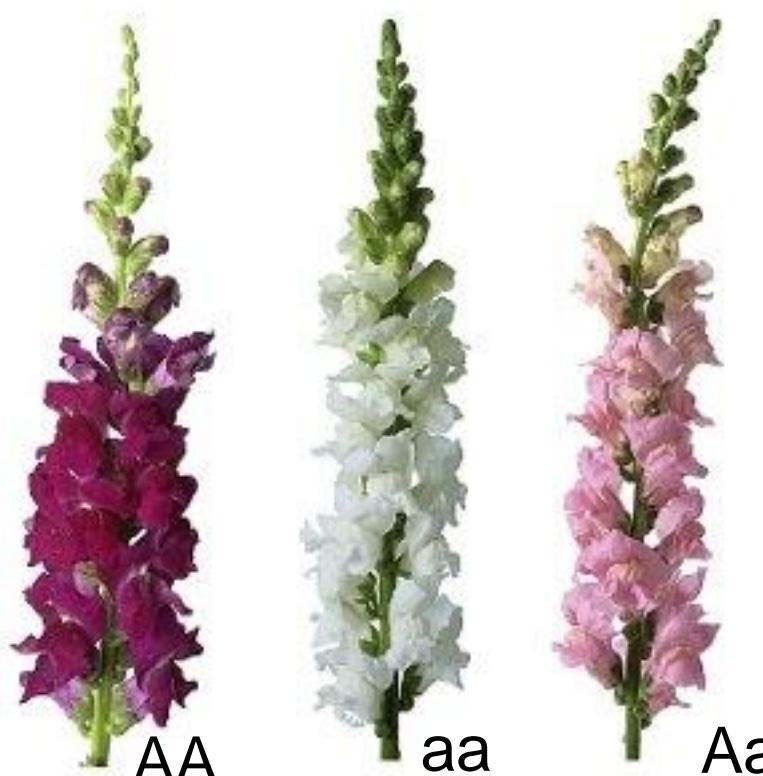
- *Ichthyosis hystrix gravior* (dermatoza)



Monohibridno križanje: nepotpuna dominacija ili parcijalna dominacija



- AA i Aa nemaju isti fenotip
- Aa ima intermedijerni fenotip (eng. *gene dosage / doza gena*)
- 3 genotipa (AA, Aa i aa) i 3 fenotipa



Monohibridno križanje (1 gen)

A	a	
A	AA	Aa
a	Aa	aa

**FENOTIPSKI OMJER
= GENOTIPSKI OMJER**

1:2:1

Monohibridno križanje: nepotpuna dominacija ili parcijalna dominacija

P:



X

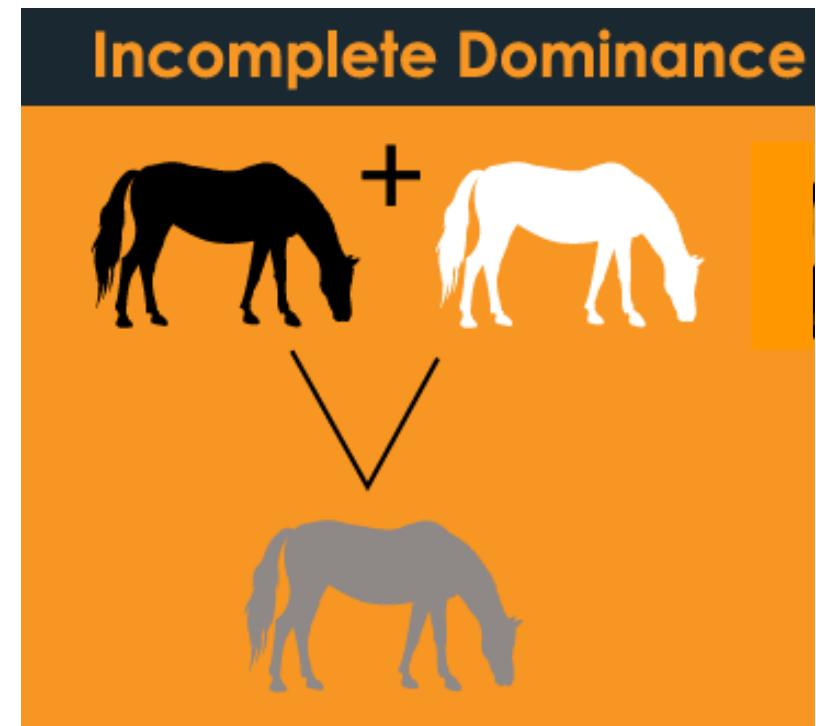


F₁:



F₂: ?

1 (smeđi) : 2 (svijetlo smeđi) : 1(bijeli)

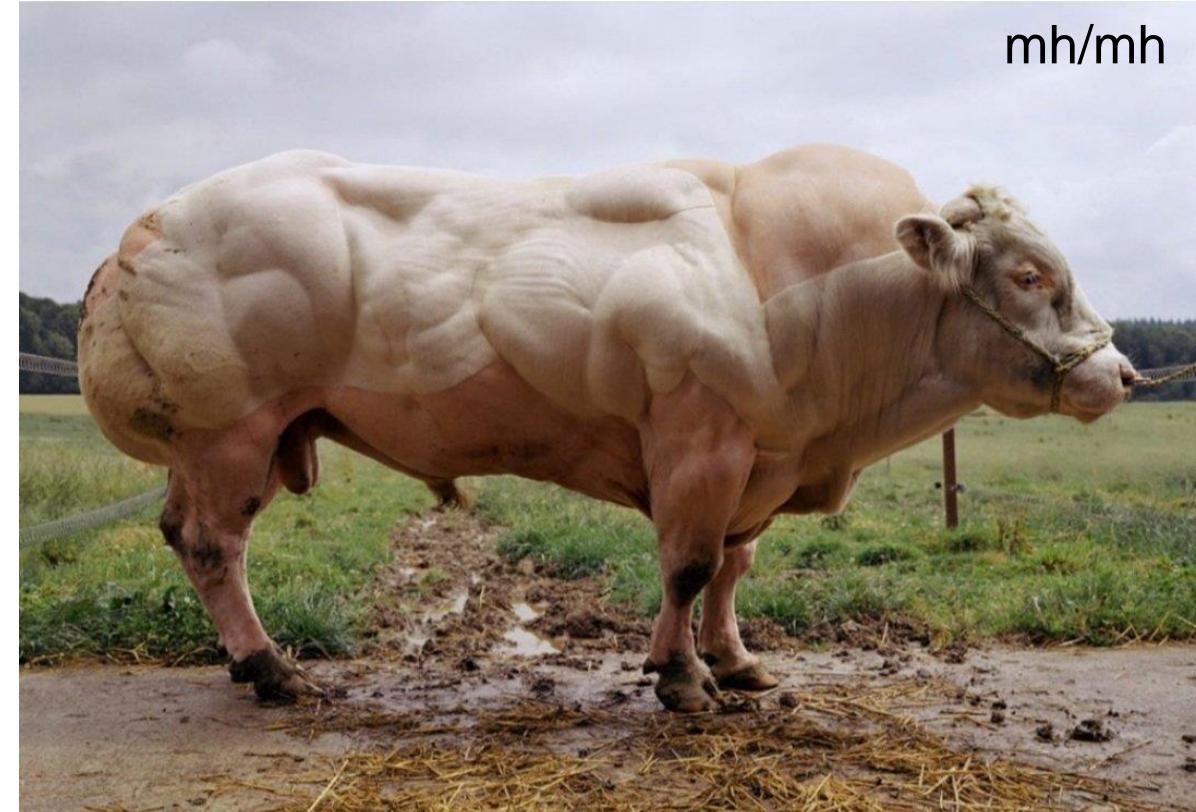


Mutacija u genu uzrokuje novu (izraženiju) funkciju: eng. *gain of function*



- miostatin (*MSTN*) je negativni regulator rasta mišića (suprimira pretjerani rast mišića kod DT)

- mutacija u miostatinu je dominantna → hipertrofija mišića (eng. *gain of function*)



A

+/+

**B**

mh/+

**C**

mh/mh



Nepotpuna dominacija *mh* alela:
intermedijerni fenotip
heterozigota (*mh/+*)

Figure 1. Comparison of Whippets with Each of the Three Potential Genotypes

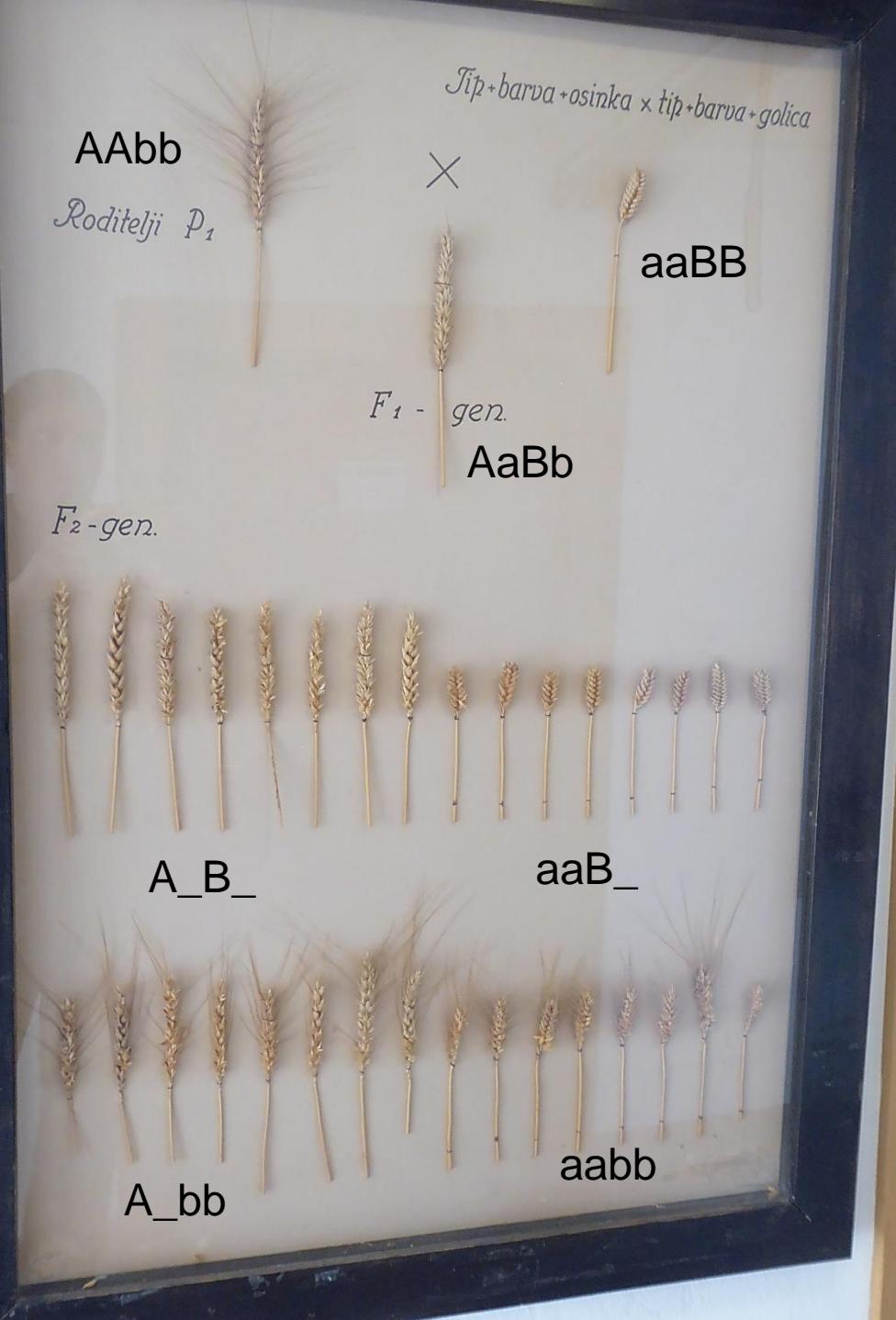
(A) Dogs have two copies of the wild-type allele (+/+).

(B) Dogs are heterozygous with one wild-type allele and one mutant cys → stop allele (mh/+).

(C) Dogs are homozygous for the mutant allele with two copies of the cys → stop mutation (mh/mh).

All photos represent unique individuals except for the top and middle panels in the righthand column.

doi:10.1371/journal.pgen.0030079.g001



Nasljeđivanje dvaju svojstava: (2 gena djeluju na 2 svojstva)

P: AAbb x aaBB

F_1 : AaBb x AaBb

Dihibridno križanje (2 gena)

	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

FENOTIPSKI OMJER:



- A- veliki klas (D)
- a- mali klas (R)
- B- klas bez osja (D)
- b- klas s osjem (R)

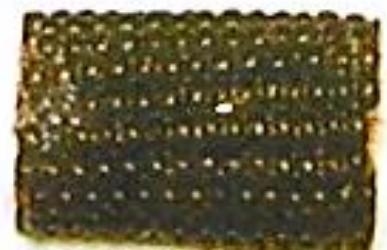
$n=2$

$$\text{Fenotipske klase } (2^n) = 2^2 = 4$$

$$\text{Genotipske klase } (3^n) = 3^2 = 9$$

$$\text{Udio dvostrukih recessivnih homozigota } 1/(2^n)^2 = 1/(2^2)^2 = 1/16$$

tamno, glatko zrno



R/R Su/Su

žuto, naborano zrno

Cultivar: Golden Bantam R-78-21
Coloration: Yellow
Starch: Endosperm: Starch: Glucose
Sweet: Endosperm:

Yellow Endosperm: Starch: Glucose
Sweet: Endosperm:

P₁
X

rr su/su

tamno, glatko zrno



R/r Su/su

F₁
X



R/r Su/su

segragacija (4 klase)

F₂



9R/_ Su/_ : 3R/_ su/su : 3r/r Su_ : 1r/r su/su

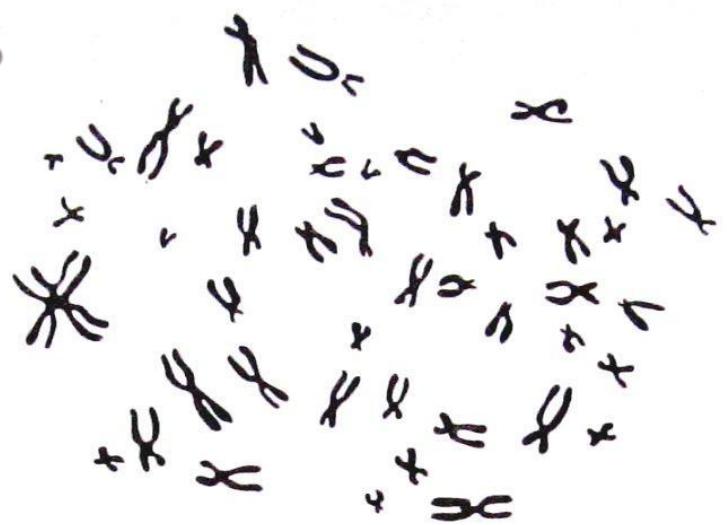
Dihibridno križanje kod kukuruza:

F₂:

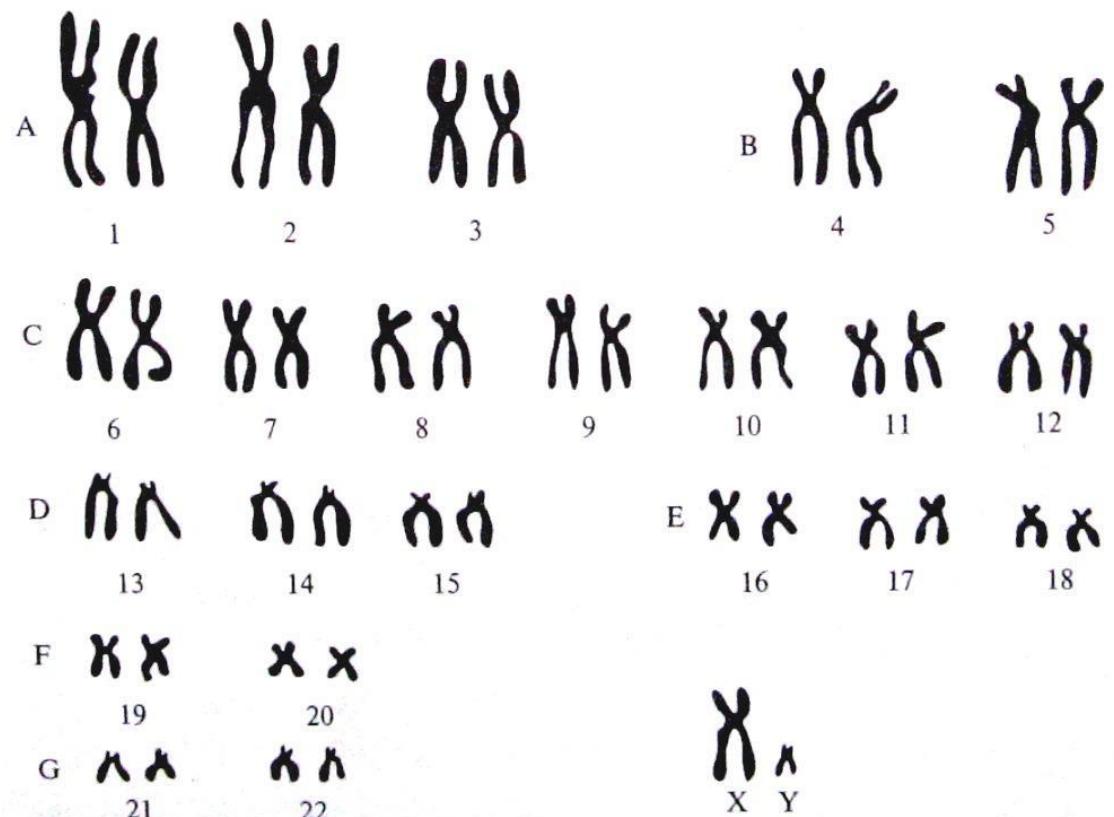


- segragacija: razdvajanje (cijepanje) roditeljskih svojstava (osobina)
- nove ne-roditeljske kombinacije (**rekombinante**): tamno/naborano i žuto/gratko zrno

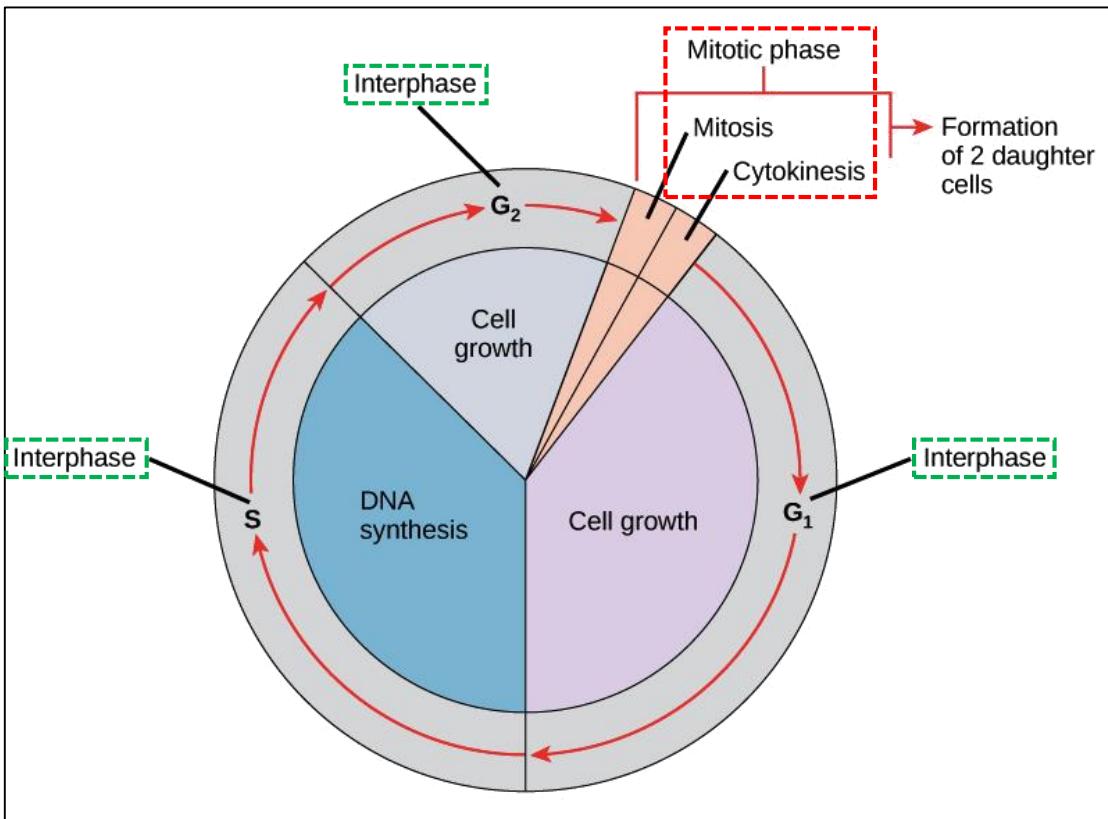
KARIOTIP



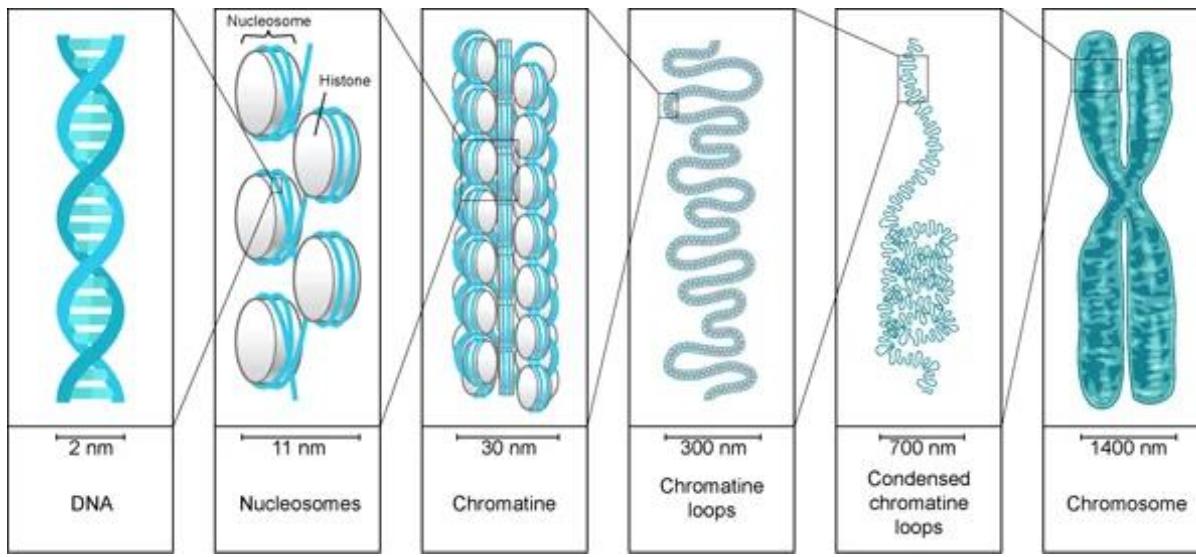
KARIOGRAM



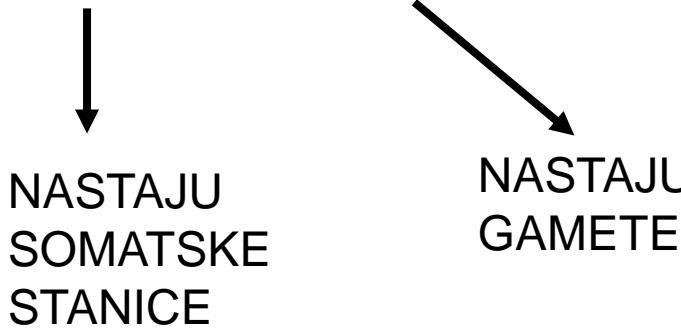
STANICNI CIKLUS



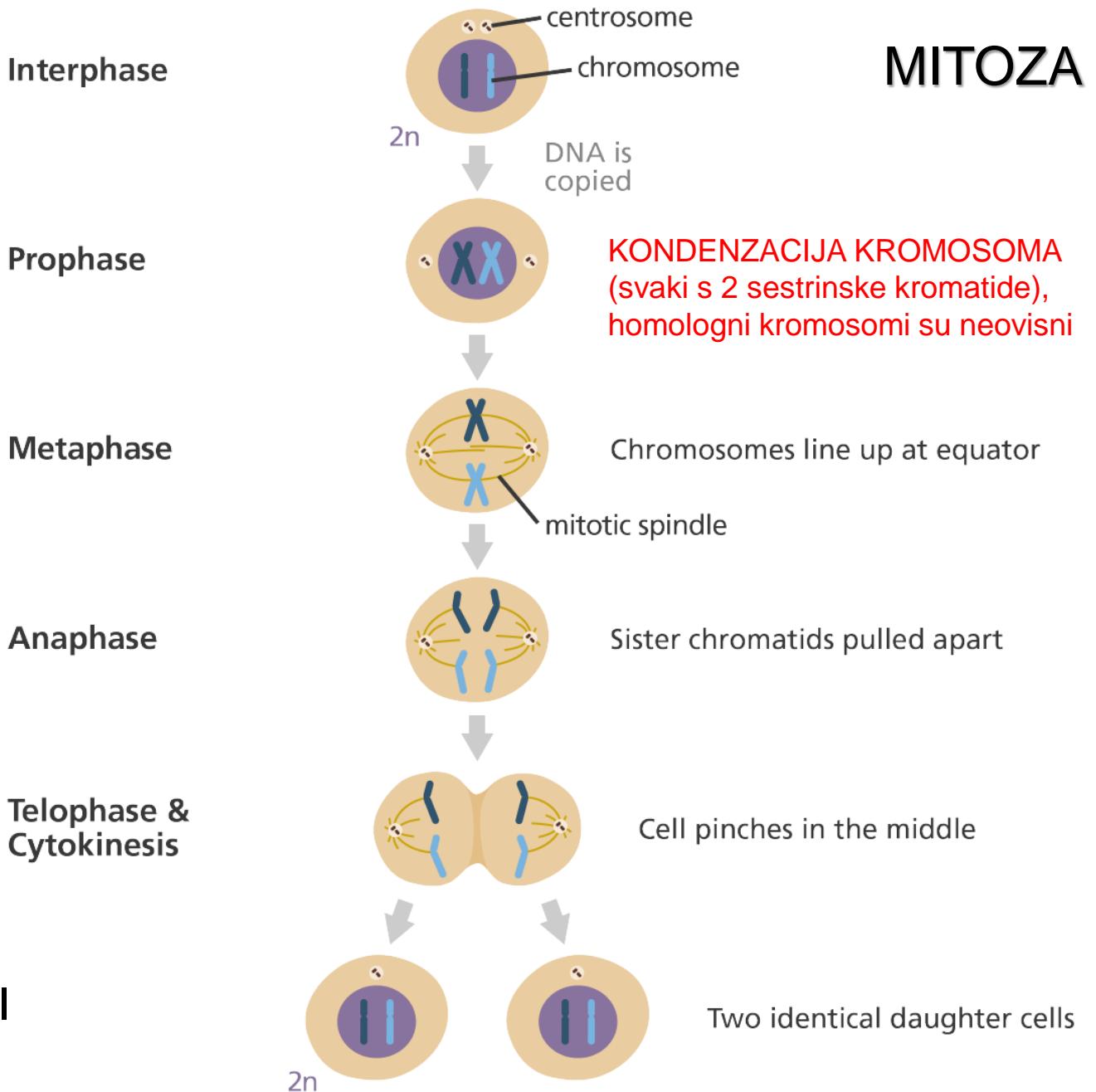
KONDENZACIJA



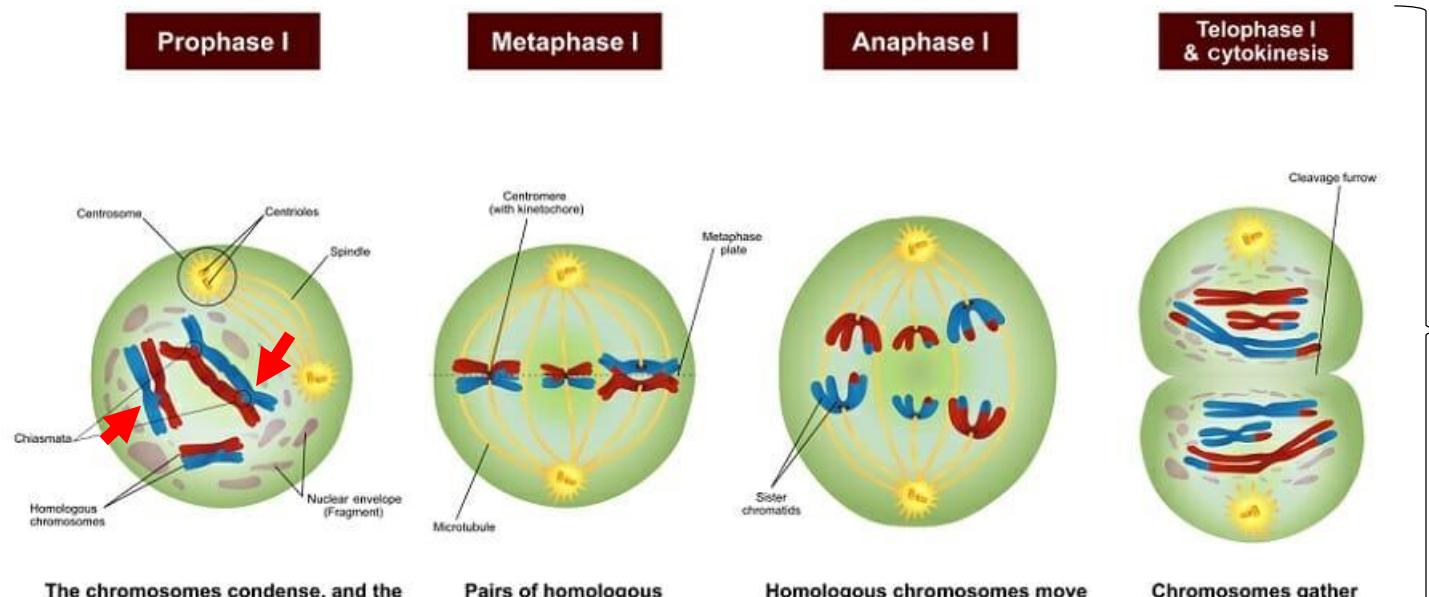
**Rekombinacija alela tijekom
mejoze (gametogeneze) stvara
novu genetičku raznolikost:
mitoza vs. mejoza**



UDVOSTRUČENJE DNA (PRIJE MITOZE)
→ PRAVILNA PODJELA NA STANICE KĆERI
(GENETIČKI IDENTIČNE)



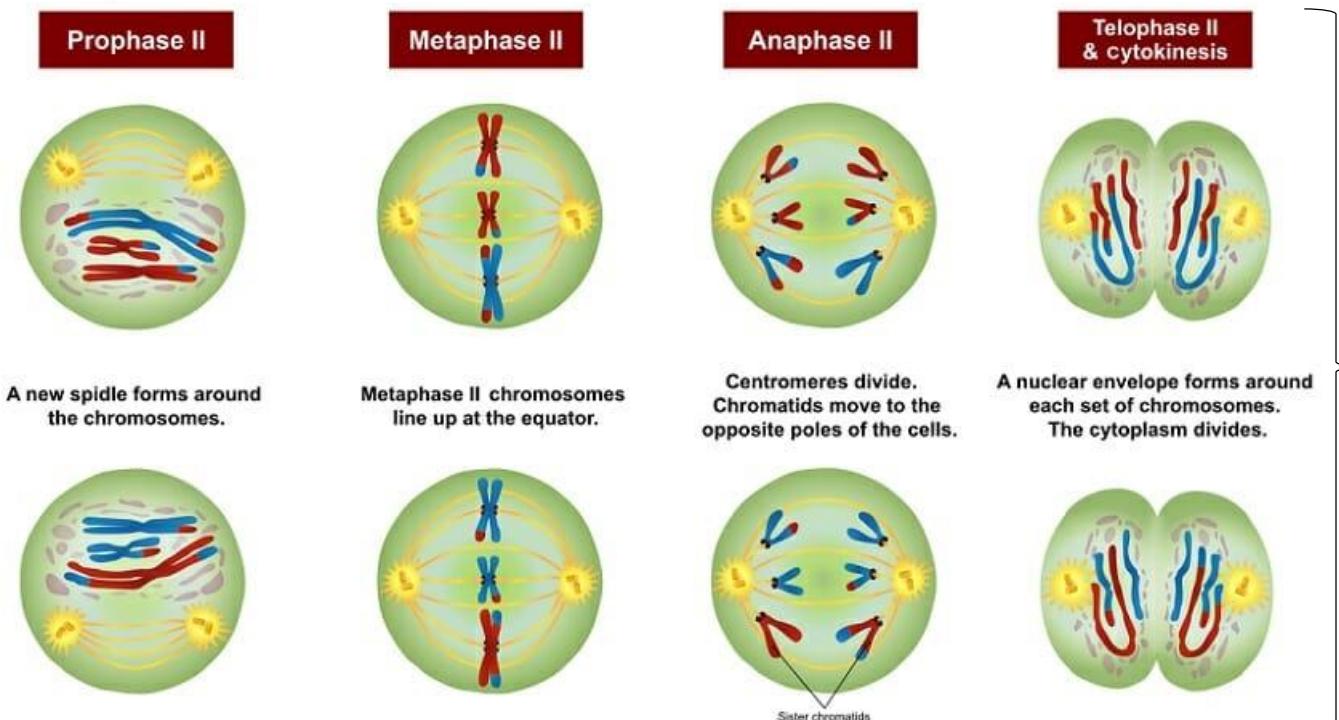
Rekombinacija alela tijekom mejoze (gametogeneze) stvara novu genetičku raznolikost: mitoza vs. mejoza



MEJOZA
MEJOZA I: REDUKCIJSKA DIOBA ($2n \rightarrow n$)

Rezultat mejoze:

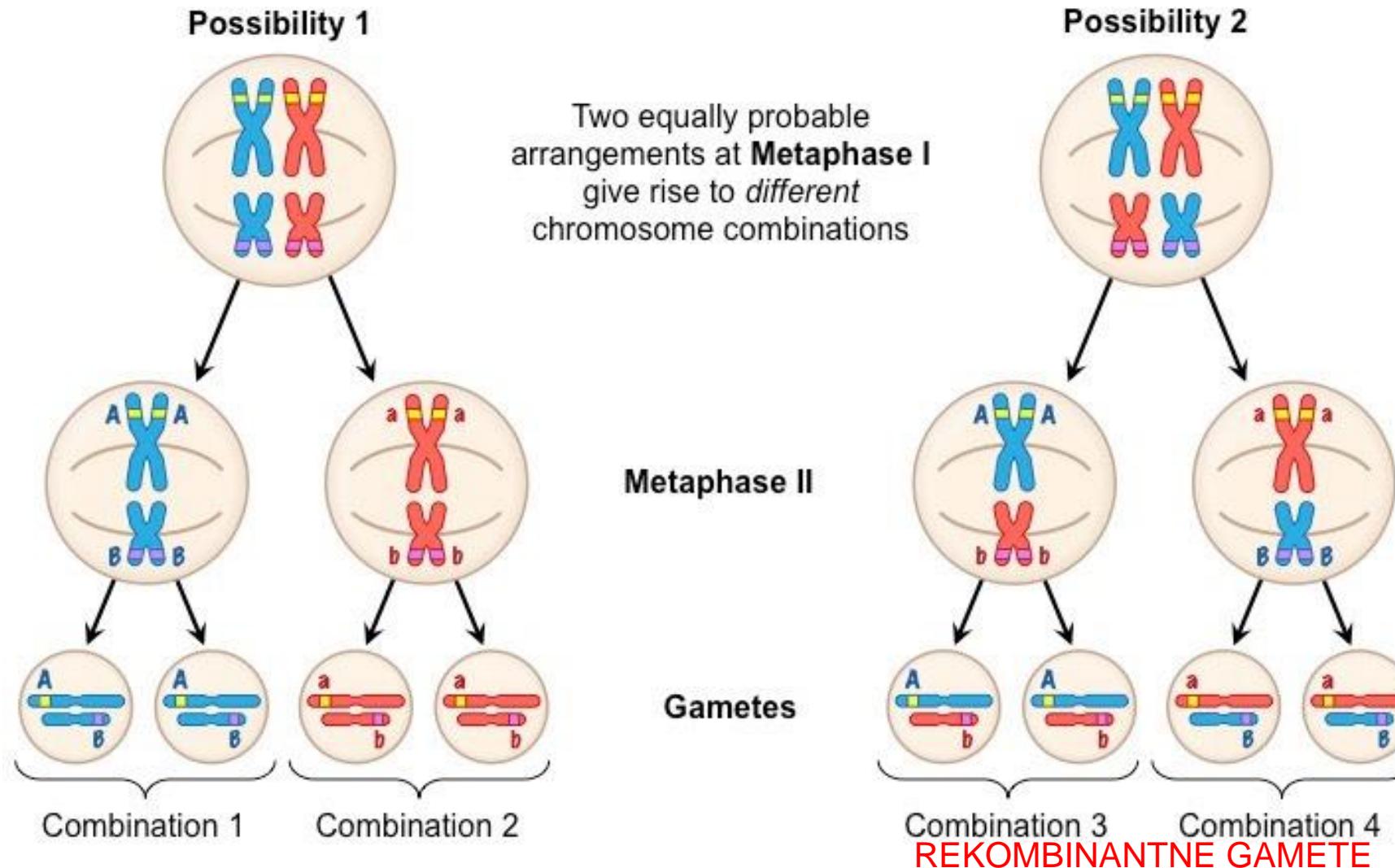
- 4 haplodne gamete
- 2 roditeljske
- 2 rekombinante
(50% rekomb. gameta)



MEJOZA II: EKVACIJSKA DIOBA ($n \rightarrow n$)

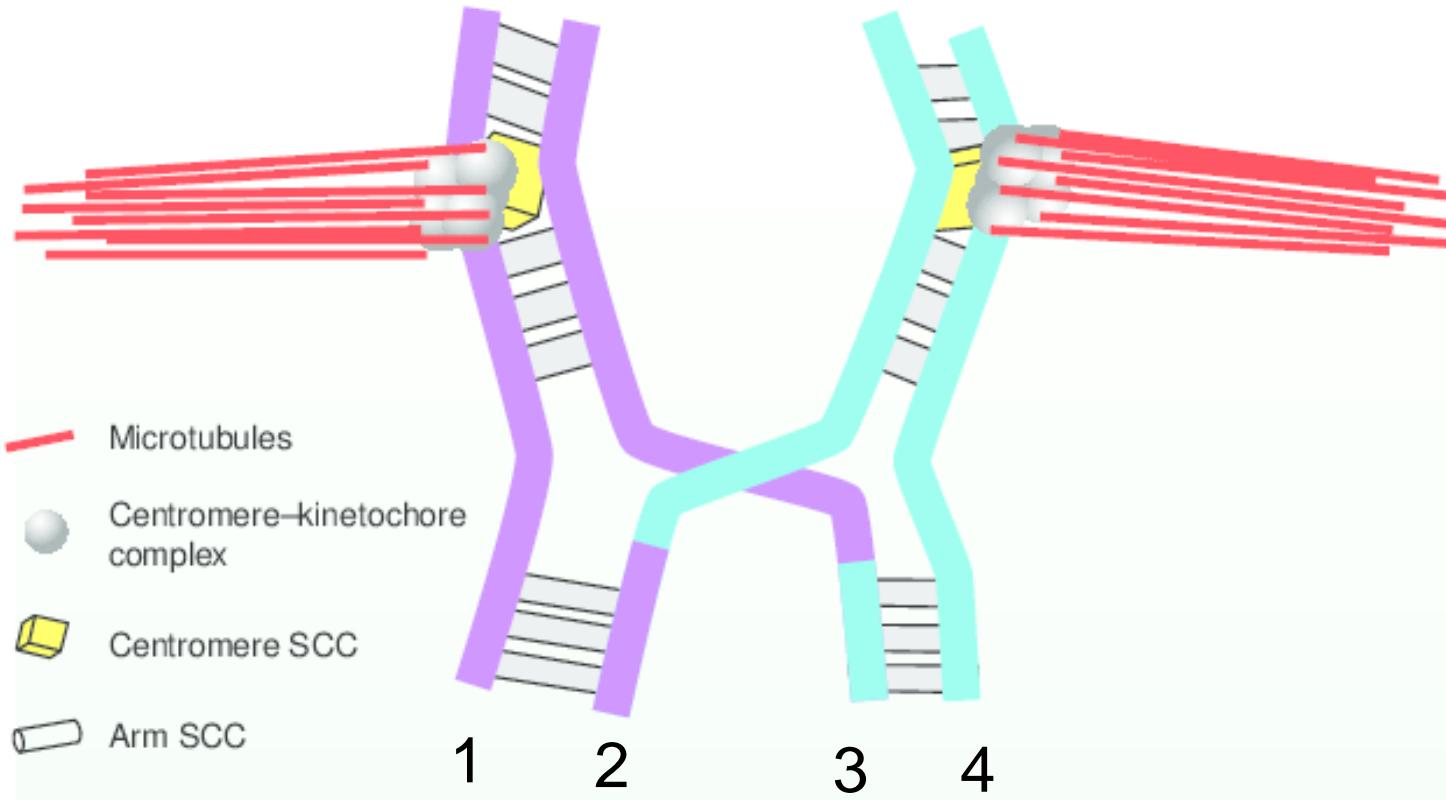
Rekombinacija alela u mejozi je posljedica:

1. Nasumične orientacije homolognih kromosoma tijekom metafaze I (vrijedi za alele lokusa na različitim kromosomima)



Rekombinacija alela u mejozi je posljedica:

2. *Crossing-over-a* (prekapčanja) između ne-sestrinskih kromatida homolognog para kromosoma (vrijedi za alele lokusa na istom kromosomu)



Ako geni nisu blizu jedan kraj drugog na kromosomu, i dalje ima 50% roditeljskih, a 50% rekombinantnih gameta.

Učestalost rekombinacije je proporcionalna je međusobnoj udaljenosti gena na istom kromosomu:

$$\frac{\text{rekombinantni potomci}}{\text{ukupni broj potomaka}} = \% \text{ rekombinanata} = \text{cM (centimorgan)}$$

- aleli gena na različitim kromosomima nasljeđuju se nezavisno (50% rekombinantnih gameta tj. 50% rekombinantnih potomaka)
- aleli gena na istom kromosomu također se mogu naslijedivati nezavisno (uvijek se dogodi *crossing-over* između dva gena → 50% rekombinanata)
- aleli bliskih gena na istom kromosomu ne pokazuju nezavisnu segregaciju tj. među njima je *crossing-over* rjeđi (geni pokazuju vezanost)
- što su geni fizički bliže, manje je rekombinantnih gameta/rekombinanata

F_2 :

	ABD							
ABD	AABBDD							
ABd	AABBdd							
AbD	AABbDD	AABbDd	AAAbDD	AAAbDd	AaBbDD	AaBbDd	AabbDD	AabbDd
Abd	AABbDd	AABbdd	AAAbDd	AAAbdd	AaBbDd	AaBbdd	AabbDd	Aabbdd
aBD	AaBBDD							
aBd	AaBBDd							
abD	AaBbDD							
abd	AaBbDd							

FENOTIPSKI OMJER:



$n=3$

$\text{Fenotipske klase } (2^n) = 2^3 = 8$

$\text{Genotipske klase } (3^n) = 3^3 = 27$

$\text{Udio trostrukih recessivnih homozigota } 1/(2^n)^2 = 1/(2^3)^2 = 1/64$

Trihibridno križanje:

P: AABBCC x aabbcc



Gamete: ABC

abc

 F_1 : AaBbCc x AaBbCc

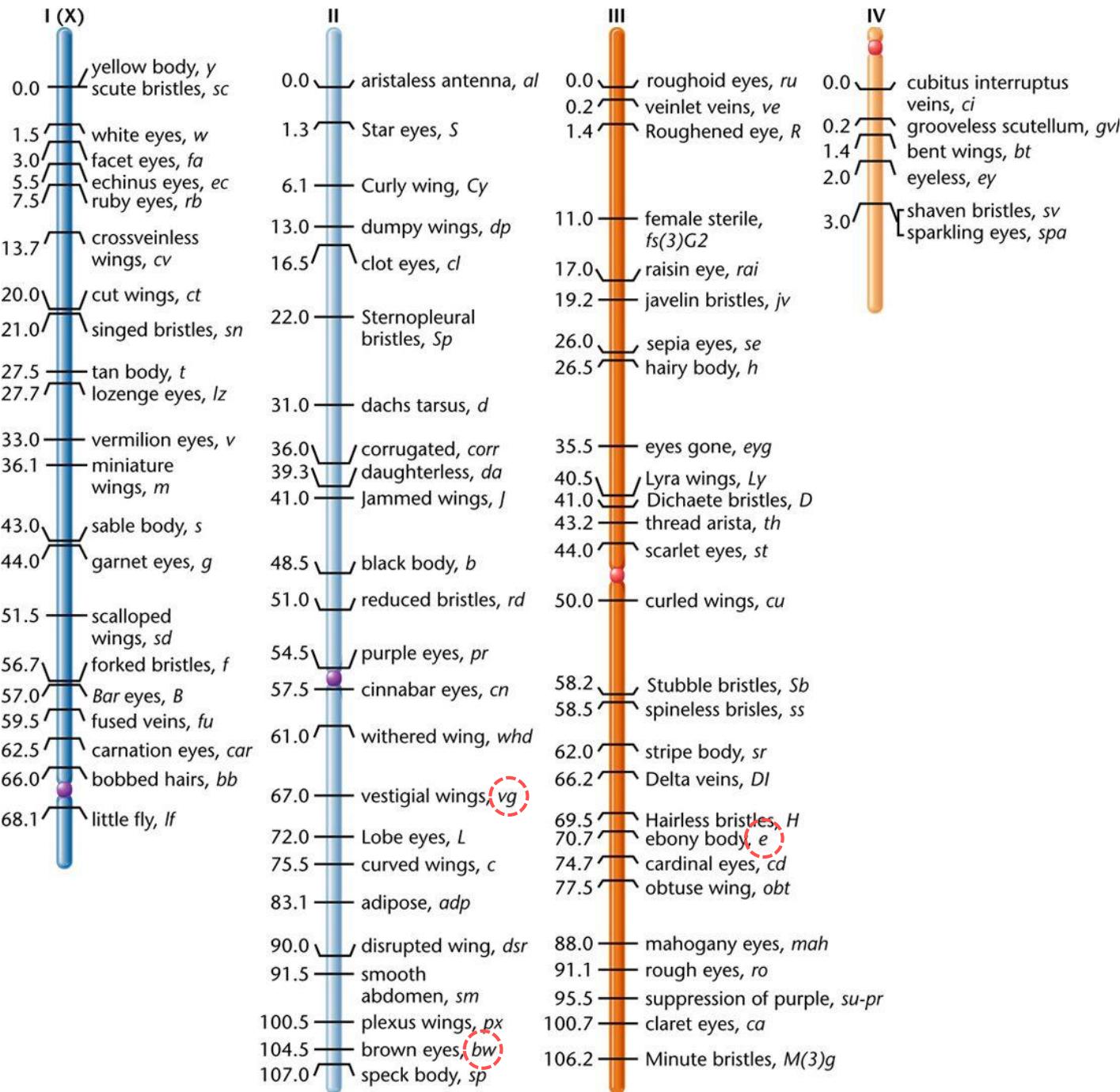
VINSKA MUŠICA

D R

- boja očiju (crvena/smeđa)
- boja tijela (siva/crna)
- oblik krila (velika/zakržljala)

Genetička mapa vinske mušice: $2n = 8$

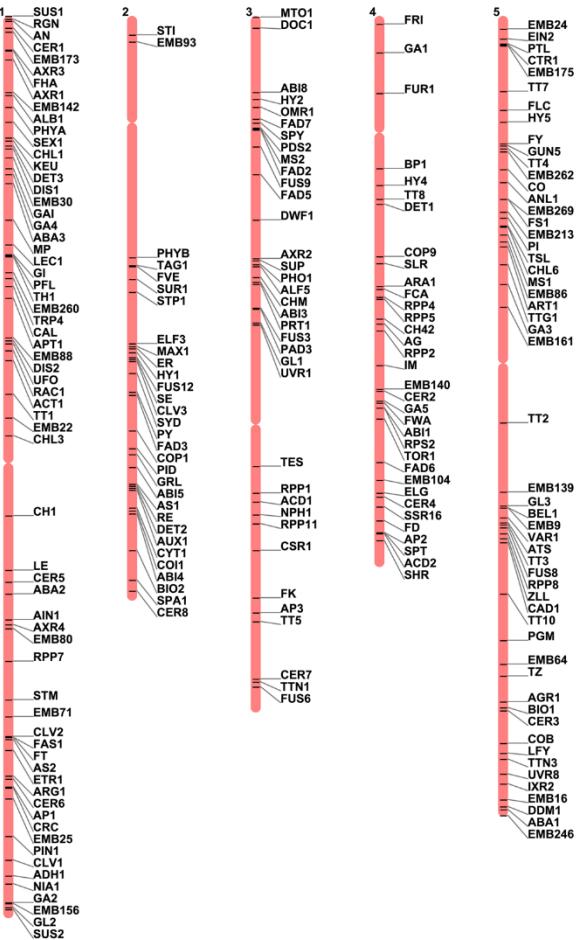
- boja očiju (crvena/smeđa) ***bw***
- boja tijela (siva/crna) ***e***
- oblik krila (velika/zakržljala) ***vg***



Genetička mapa prikazuje relativne udaljenosti gena na kromosomu u centimorganima (cM).

Genetička mapa (cM) vs. fizička mapa (pb/bp)

RELATIVNA
UDALJENOST
GENA (cM)



APSOLUTNA
UDALJENOST
GENA (pb)

Arabidopsis thaliana (uročnjak): $2n = 10$

parovi baza / broj nukleotida (eng. base pairs)

Genom *A.thaliana*: 125 Mbp (~25.000 gena)

GTCCGGCTAGCGACTGCGTACGACGTTACGACTACTGCATGACGCCGACTAGCTAGCATCG
ACAGTCATCGACTCGCCTCTGCCGTATATAGCCTCTCTCTTTTTATATAGAGAGCT
TCGTGTGGGTATCAGATCGCATACTGATCGTTGACCGATGCAACGCTGCATTGATGAAAAA
ATCAGACTGCTACGTACGACGATCGATTCTCTGACATGTGAATATGGTCGGCGCTATGCTA
CCCGCATATACTGTATCGACATGTCGCGCGATATAATATCCAGACTCTGCTGACATAACG
ATATACTACGATGACCGATGATGTAAGCTACAGACGCACTGAAAGAGCGCCTCTATACG
ATCTATATCTGCATGCTACGACACGTACCGTATATGCTGCTATGCAAGCCGTCACTAGCGCAA
CCGACTGATGACTAACCGCCTACTGCGCTACTGACTCACTATGCCGCCGCGCCGTGGGGATA
TACGCTGATCGTACGCCGATATCGGGATCTGCGCTCATATCGCATCGTATCTACGCATA
TACAGATCATGCCGTAATAGTACTATGATTATAATCGTACAGCTAAAGCTCGATCAGATC
GATAAGACTTATTACGAAGGCCTAATATCGTAGCAAACCTATGATTAGCAGGGTCGATAT
ACGATCAATGAATGATACTAATTAACTAATACCGCATAATCGCATAACCGCCTACAGTTA
CCGCACGTATCTATATCGACGCCGATATTCGATACGAGAAAGTCAGTAGCCGCTATCGGGATT
ACACGTACATATACTAACTGACTAATGACTAGCGACTACTGACCTACTAGCTAGCACTATT
TATCATACTGACACTACTCATCAGTCACGACGACATCATTCTAGTGTGATGATATGCTATA
GCTACGTACGACACTCTATCACGATCGCTACGCTACGCTATGCTACTCTGCGTTTACTA
ACTGCGTACACGTACTGACATACTACTCATTACTGACTACTGACTGAATGCCGCCGTAATGCT
CTGACGATATGATATGATTGAAATTGGGGGTCTATCATGATGATATGAAATATGACTACTGA
ACAATCGATCGATCGACGTGACTAGCTAGCATGACGCCGCTAGCGATGCCATGCCGATA
GTCCACATGCATGCATCAACTATACATGATCGTACGCCGCGCTTCCCGATGATGC
ATGCATGCATGCATACTACTGCATGCATACTGCATGACGGGGTGCGATGATCATCAT
GCAGTACGTCACTGACTGCATTTGCATGCTGACTGCATGACTGCATGACTGCATGATGATGCA
TACGTCTGACTGCGTACTGACAAGGTGCATGCCACTGACTGACTACTGATGATGAGAGGGGA
TCGATTGATCGACTGATCGTGCATCGATGCATTGCACTGACTGCATGACTGCATGACTGCATGATGCA
CTGACTGATGAGCTAGCACGTACGGATCGTAGCTAGATATGCTAGCTACGGCGATCGATC
AATATATCGAGAGTCAGTGCATATACCGATAAACAGCGGGCTCTCTCGAGAGAGCTCTT
ATATACGCCGCCGATCAGTCACTACTCCCACTAGCTACAAACGATCACTCGCGCCGCGATA

Jedno od osnovnih pitanja genetike je kauzalni (uzročno-posljedični) odnos slijeda DNA (genotipa) i funkcije (fenotipa).

- koji gen je odgovoran za (mutirani) fenotip koji proučavamo?
- mutacija u kojem genu je odgovorna za neku nasljednu bolest?
- je li moguće mutiranu varijantu gena zamijeniti onom divlјeg tipa?

Prirodne mutacije (varijacije fenotipa) su relativno rijetke (greške tijekom replikacije).

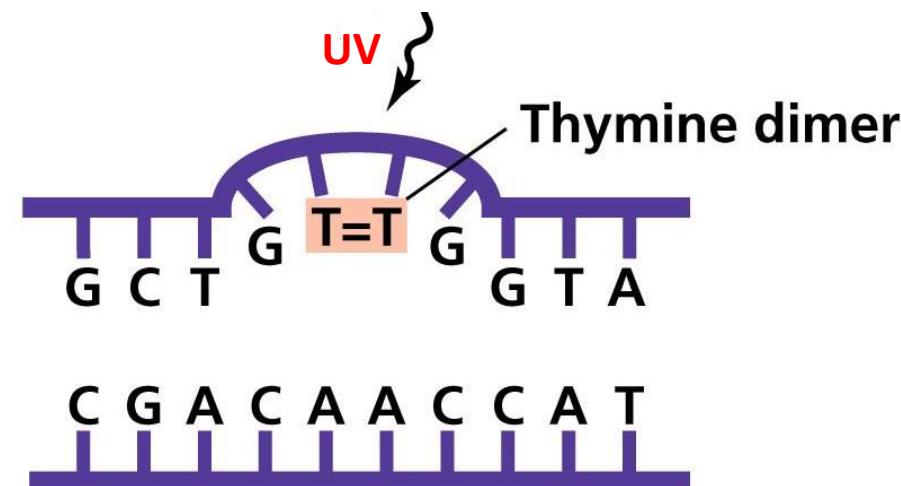
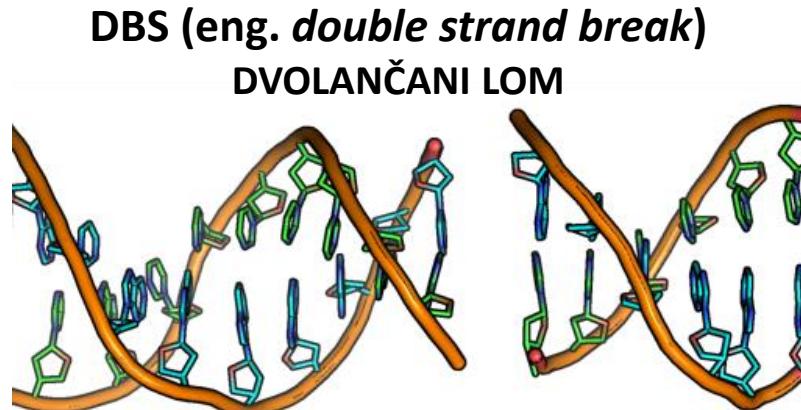
Jedan od glavnih klasičnih alata u genetici je mutageneza (svjesno izazivanje mutacija).

Inducirane (potaknute, izazvane) mutacije:

- nastaju kao posljedica oštećenja DNA različitim fizikalnim ili kemijskim mutagenima

1) zračenje (fizikalni mutagen):

- ionizirajuće → X-zračenje, radioaktivno i kozmičko zračenje
 - velika prodornost: uzrokuje dvolančane lomove DNA
- neionizirajuće → UV zračenje
 - manje je prodorno: uzrokuje pirimidinske dimere



Dobivanje mutanata fizikalnim ili kemijskim mutagenima:



Institute of Radiation Breeding, Japan <https://mvd.iaea.org/#!Home>

Inducirana mutageneza:

- nasumično izazivanje mutacija
- selekcija poželjnog fenotipa
- nepoznata modifikacija na DNA

1) zračenje:

- X-zrake
- γ -zrake
- UV

2) kemijski mutageni:

- etilmetsulfonat (EMS)
- 5-bromouracil
- 2- aminopurin

Table 1. Crops improved through induced mutation and the traits improved.

Crop	Mutagen	Trait alteration	
Rapeseed*	EMS	Increased oleic acid, reduced poly unsat. fats	(Auld et al., 1992)
Rapeseed	EMS	Sylfonyl-urea resistance	(Tonnemacher et al., 1992)
Rice	Gamma	Dwarf, high yield	(Chakrabarti, 1995)
Rice	Gamma	Thermosensitive, genetic, male sterility	(Maruyama et al., 1991)
Flax	EMS	cooking oil quality	(Rowland, 1991)
Sunflower	X-ray	High oleic acid, high palmitic	(Fernández-Martínez et al., 1997)
Apple	Gamma	Skin color	(Brunner and Keppl, 1991)
Pear	Gamma	Disease resistance	(Masuda et al., 1997)
Grapefruit	X-ray	Flesh color, seedlessness	(Hensz, 1991)
Pineapple	In vitro	Spineless	(Lapade, 1995)

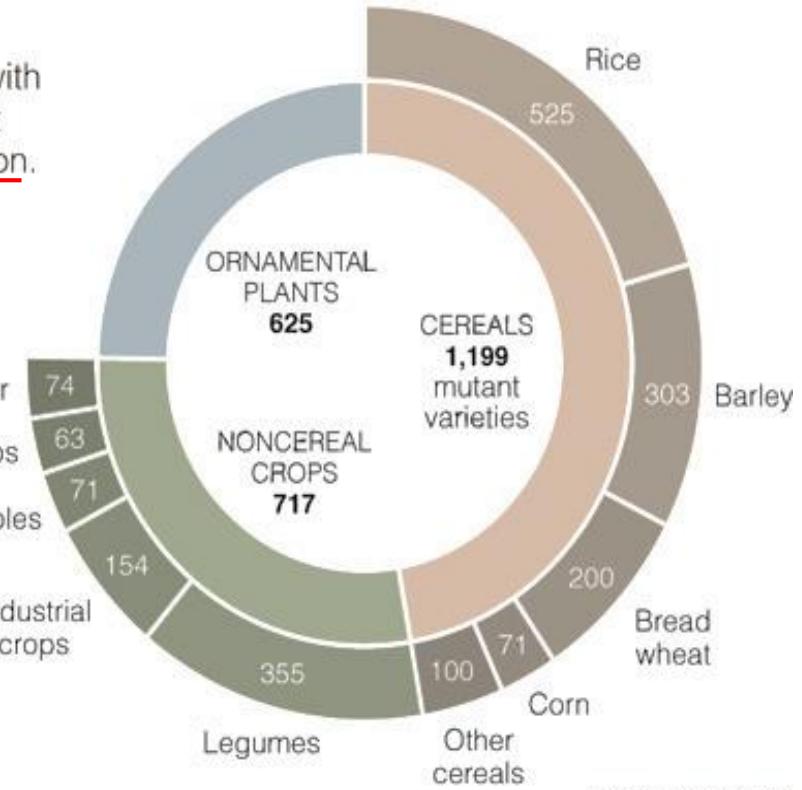
* Largely adapted from Ahloowalia and Maluszynski, 2001

Dobivanje novih sorti zračenjem:

More than 2,500 mutant crop varieties have been officially registered with the United Nations and the International Atomic Energy Agency. About three-quarters of the varieties were directly induced by gamma radiation.



Source: F.A.O./I.A.E.A. Mutant Variety Database



THE NEW YORK TIMES

> 2000 sorti kultiviranih biljaka je dobiveno induciranim mutagenezom

Dobivanje novih sorti zračenjem:

„Star Ruby” crveni grejp



Kruška otporna na bolesti



Ove sorte (mutanti) su u prodaji i nitko ne dovodi u pitanje njihovu sigurnost za zdravlje ljudi i „otpuštanje” u okoliš (za razliku od GM kultura).

Patuljasta papaja (mali plod)

A
T
O
M
I
C

F
R
U
I
T
S

Pusa Nanha

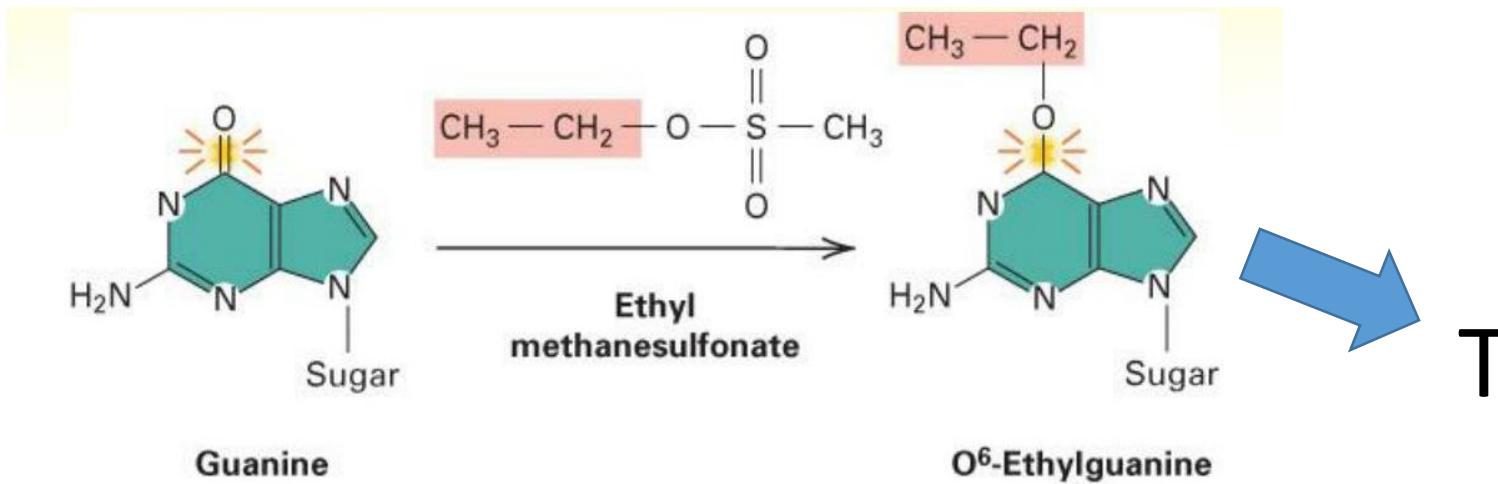


„Golden Promise” ječam tolerantan na slana tla



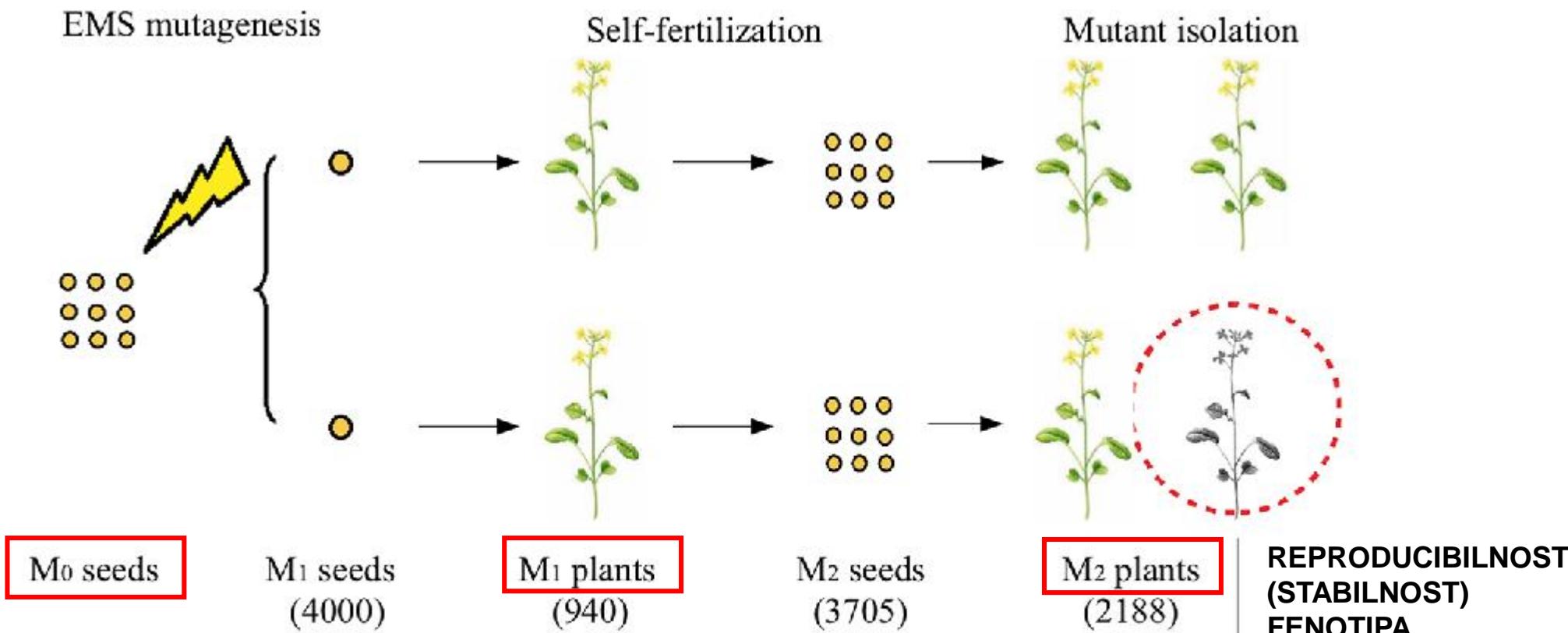
Djelovanje kemijskog mutagena EMS (etilmetsansulfonat):

- EMS uzrokuje nasumične „točkaste” mutacije (promjenu jednog nukleotida)



EMS uzrokuje zamjenu nukleotidnog para**GC** → **AT**.

Kemijska mutageneza u biljaka: EMS



LINIJA HOMOZIGOTNA SAMO ZA
MUTACIJU OD INTERESA (NEMA VIŠE
DRUGIH MUTACIJA)

5-6 povratnih križanja
s divljim tipom...

...+ selekcija fenotipa od
interesa u svakoj generaciji

REPRODUCIBILNOST
(STABILNOST)
FENOTIPA

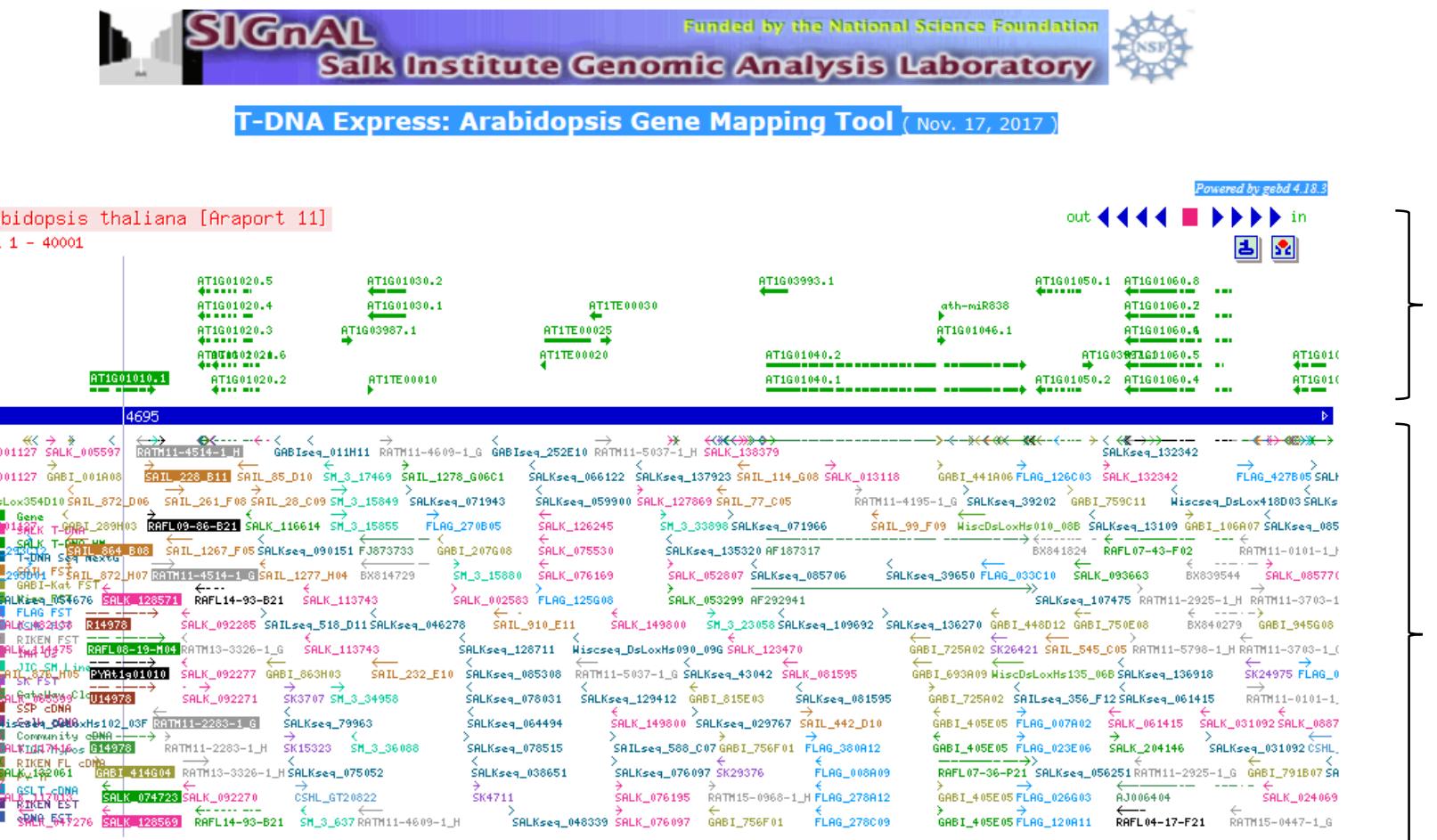
OVA BILJKA JE HOMOZIGOTNA ZA MUTACIJU
KOJA UZROKUJE FENOTIP OD INTERESA + IMA
VELIKI BROJ DRUGIH MUTACIJA

• Recheck at M₃ plants (200)

Inducirane mutacije:

3) „biološki“ mutageni → insercijska mutageneza:

- nasumična ugradnja T-DNA/transpozona u genom (*Arabidopsis thaliana*):



Koja mutacija je odgovorna za gubitak bodlji divljeg kestena?

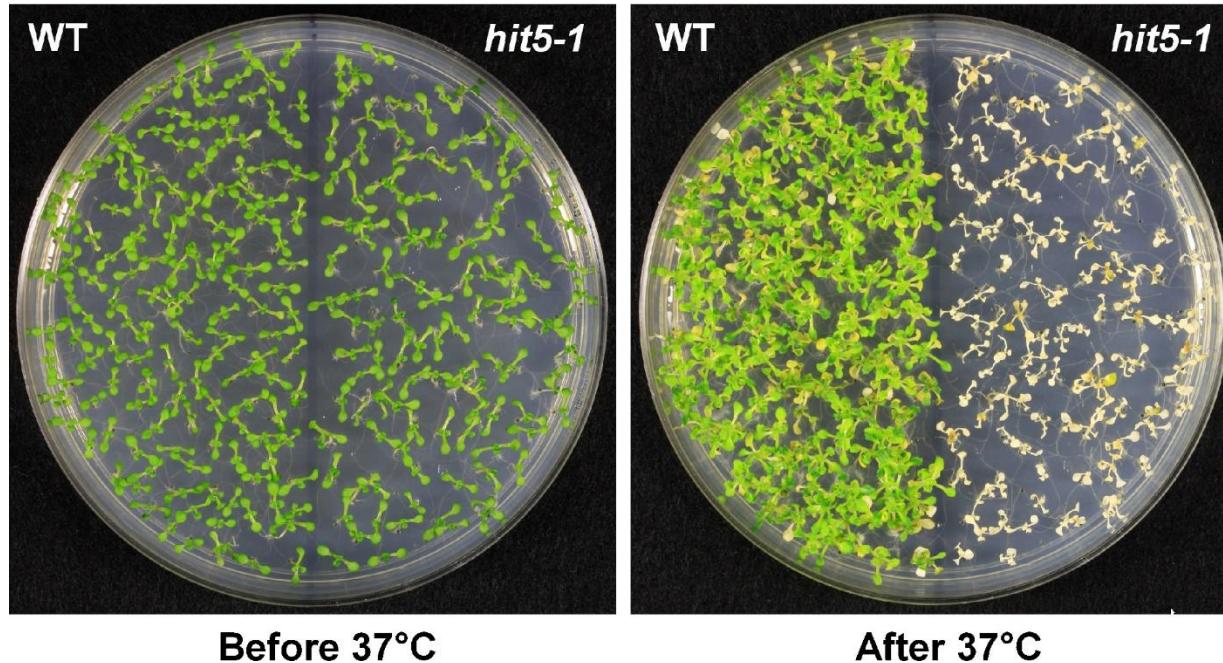
- jedna ili više mutacija? → 3:1?
- postoji li genetička mapa (DNA markeri na svim kromosomima)?
- je li genom sekvenciran (fizička mapa)?



Forward (klasična) & reverse (reverzna, obrnuta) genetika:

Klasična genetika: fenotip → genotip

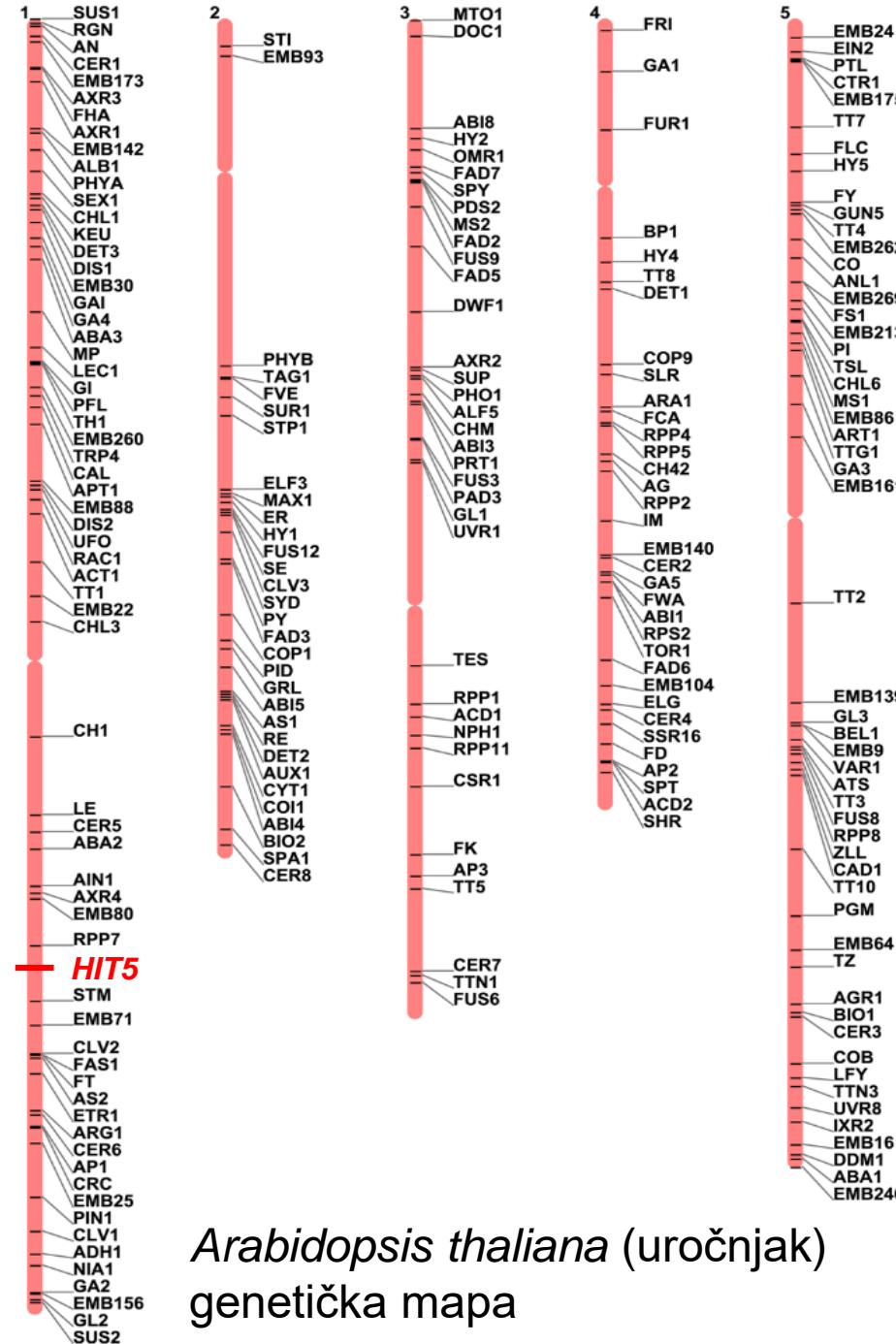
MUTAGENEZA → FENOTIP
(spontana ili inducirana) (u selektivnim uvjetima)



GEN / SEKVENCA
(lociranje gena na
genetičkoj mapi
pomoću
molekularnih
markera)

hit5 -heat intolerant 5
(β podjedinica
farneziltransferaze)

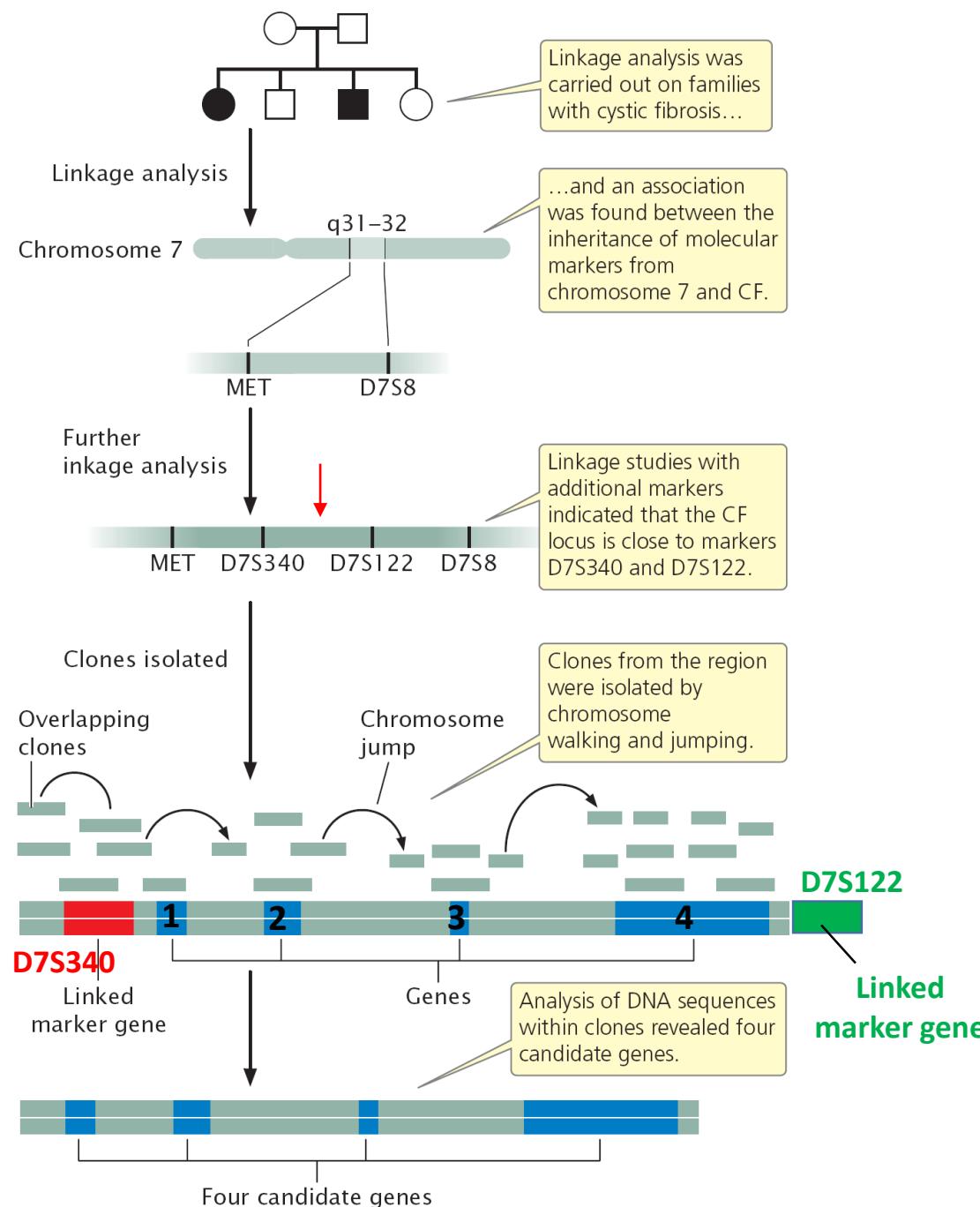
Fenotip mutanta i sekvenca mutiranog lokusa mapiranog molekularnim markerima (vezani geni!) ukazuju na funkciju gena.



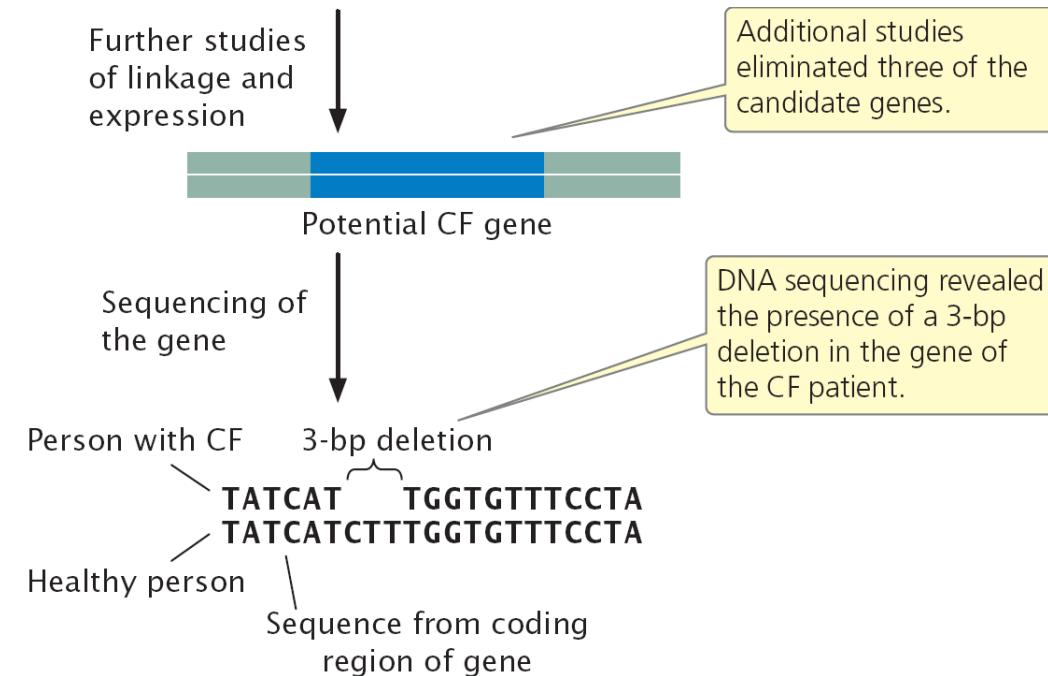
Koji gen je odgovoran za mutaciju *hit5*?

MAPIRANJE NA GENETIČKOJ MAPI/KARTI (POZICIJSKO KLONIRANJE)

1. praćenje kosegregacije fenotipa *hit5* i poznatih markera (vezani geni; < 50% rekombinanata)
2. pozicioniranje *hit5* između poznatih markera koji ne daju rekombinante (jer su jako blizu genu odgovornom za *hit5* fenotip)
3. sekvenciranje svih gena između ta dva poznata markera → lista gena kandidata
4. usporedba sekvenci svih gena kandidata između mutanta *hit5* i divljeg tipa
5. gen koji pokazuje razliku u sekvenci je kandidat gen za *hit5*
6. Dodavanje (transformacija) gena kandidata divljeg tipa u *hit5* mutantu → komplementacija



Izolacija gena za cističnu fibrozu: „pozicijsko kloniranje” na genetičkoj mapi/karti:

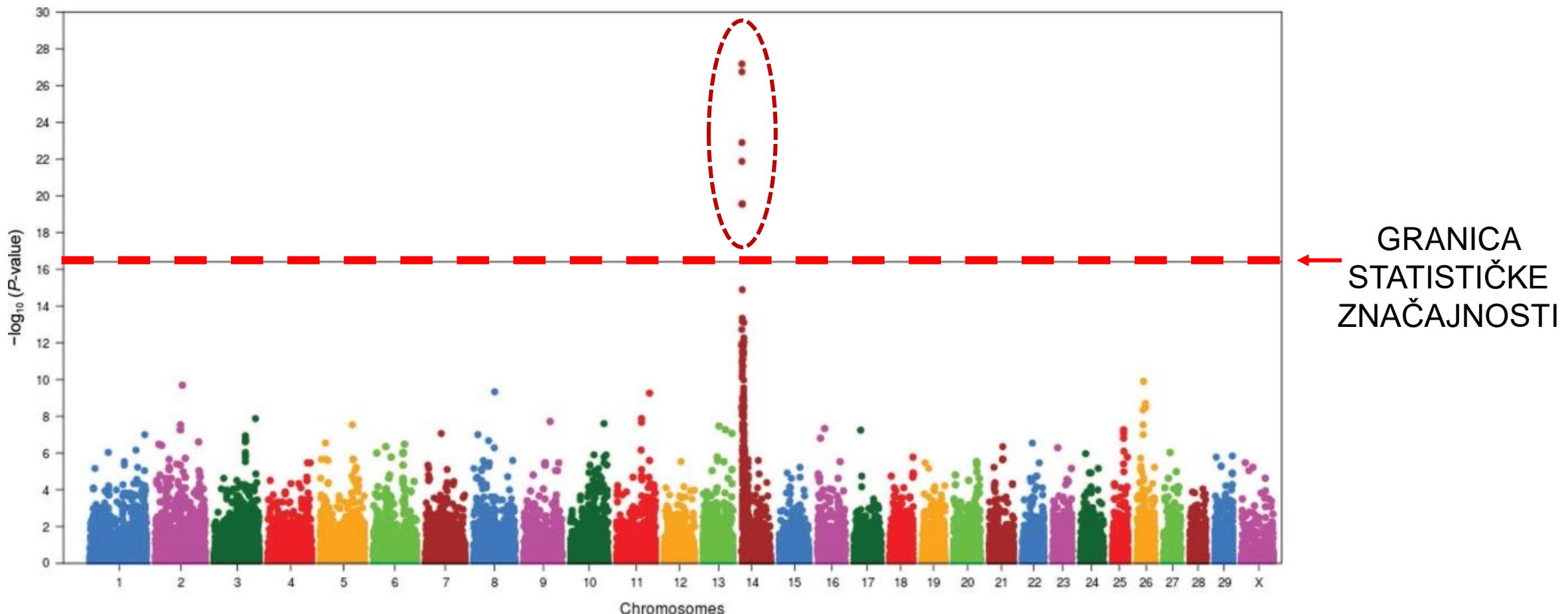


Danas se ova metoda pokušava zamijeniti metodom **GWAS** (genome-wide association study).

GWAS analiza: statistička korelacija genotipa i fenotipa

(*genome-wide association study*)

- sekvenciraju se cijeli genomi pacijenata i zdravih kontrola
- koji polimorfizmi i na kojem kromosomu se javljaju samo kod pacijenata?



Forward (klasična) & reverse (reverzna, obrnuta) genetika:

Reverzna („obrnuta“) genetika: genotip → fenotip

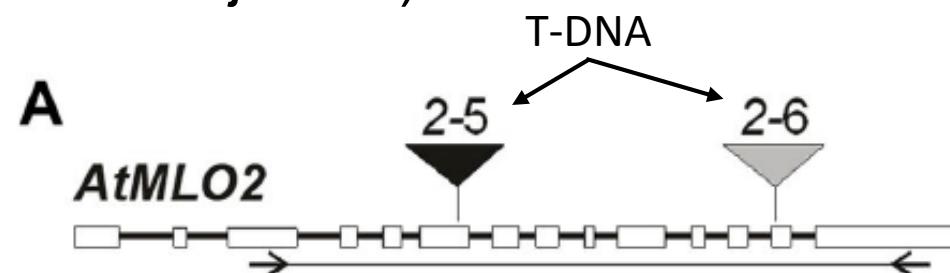
SEKVENCA / GEN

(nepoznate funkcije za koji postoji insercijski mutant;
kolekcija T-DNA ili
transpozonskih linija)

FENOTIP

(u selektivnim uvjetima)

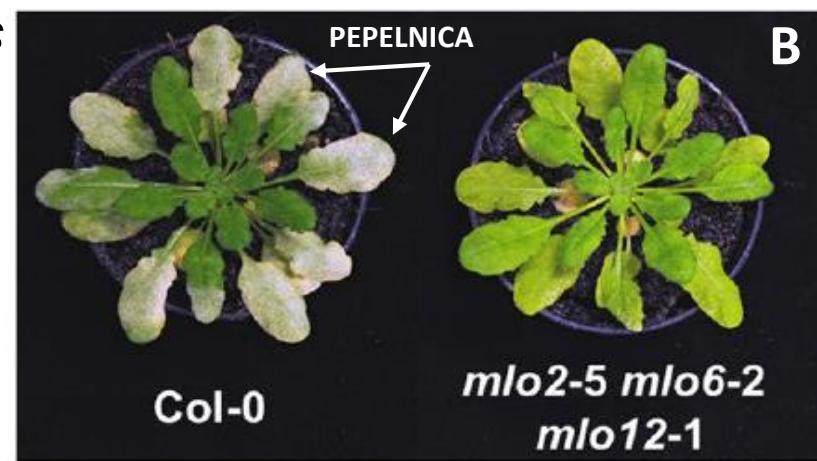
FUNKCIJA



mlo2 - mildew resistance locus

(integralni transmembranski protein
nepoznate biokemijske funkcije)

Sekvenca lokusa ukazivala je da je riječ o transmembranskom proteinu, a funkcija mu je dodijeljena na temelju fenotipa mutanta.



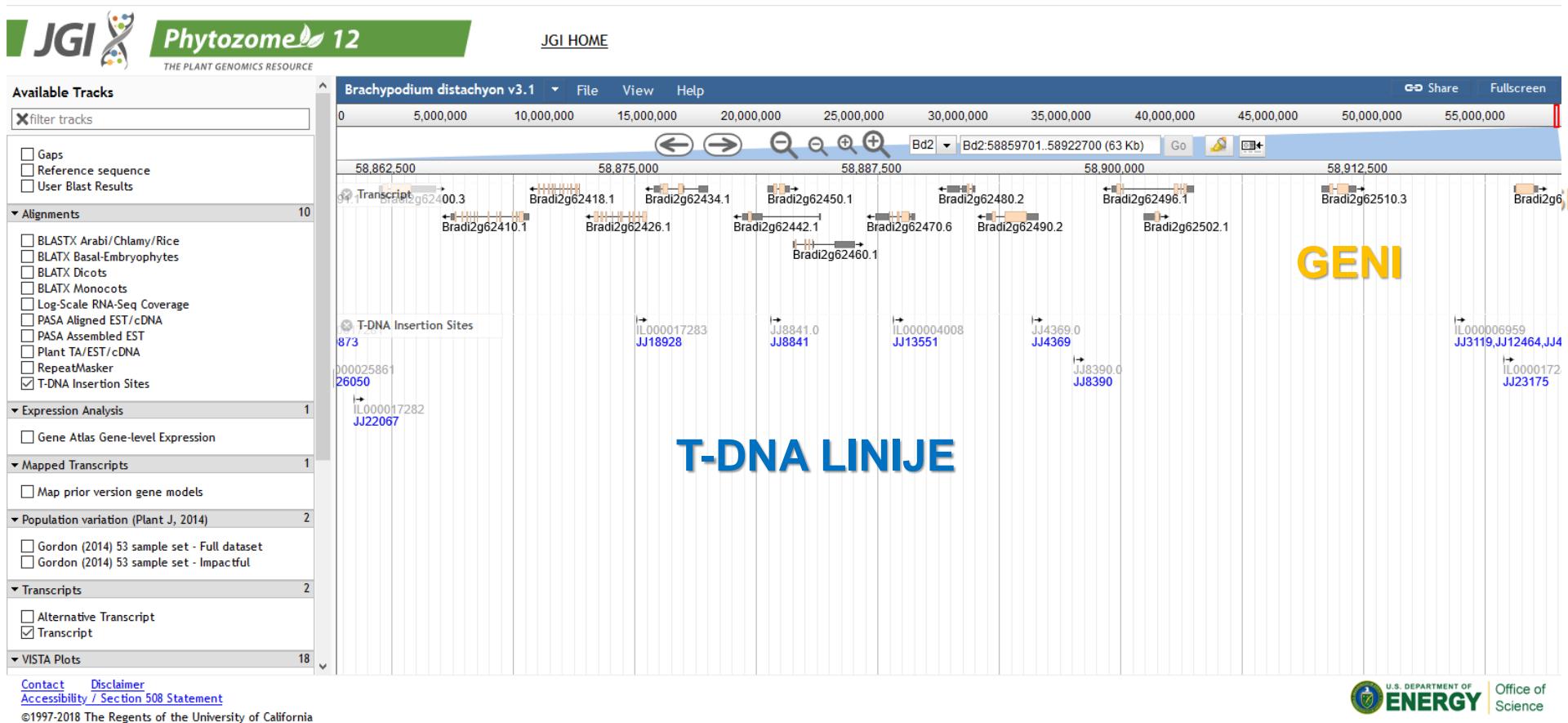
Acevedo-Garcia et al. 2017.

1. *Brachypodium distachyon* (~355 Mb)

- 34.310 gena (*protein-coding*)
 - broj T-DNA linija u kolekciji: 23.649
 - jedinstvena mjesta insercije T-DNA: 25.977



ŽITARICA-MODEL

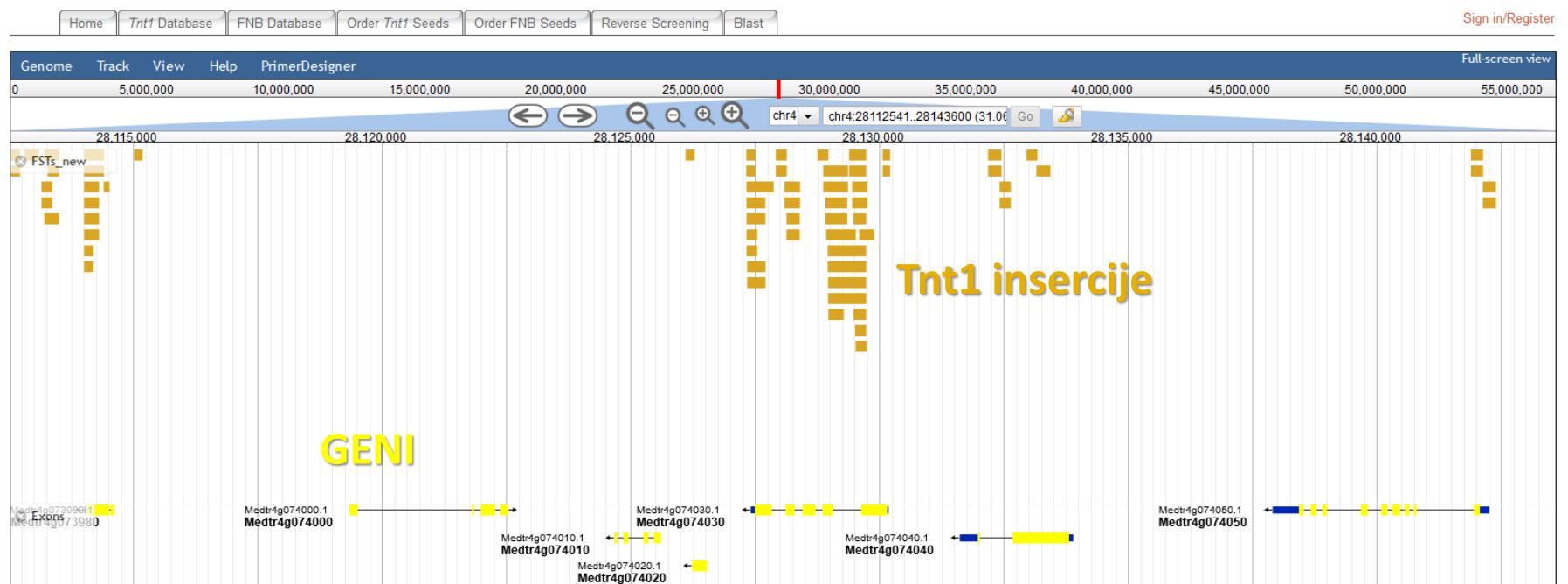


2. *Medicago truncatula* (buretasta lucerna)

- kolekcija ima ~ 21.700 linija s ukupno 500.000 *Tnt1* insercija
- kolekcija mutanata dobivena insercijskom mutagenezom s retrotranspozonom duhana *Tnt1*
- kolekcija insercijskih mutnata „pokriva” 90% gena lucerne



LEGUMINOZA-MODEL



Ušli smo u „zlatno doba” genetike (genetičkog inženjerstva):

Jeftina sinteza DNA
(sinteza gena *de novo*)



**Jeftino sekveciranje
gena i cijelih genoma**
(*Illumina, PacBio i Oxford
Nanopore*)

**Alat za preciznu
modifikaciju
gena i genoma**
(CRISPR-Cas9 i izvedenice)

HVALA NA PAŽNJI!

