

IAEA Training Course RAS5094

Regional Training Course on Plant Mutation Breeding and Molecular Techniques for Crop Improvement

29 April 2024 – 3 April 2024

Trainers:

Ryohei Terauchi Lab. Crop Evolution, Graduate School of Agriculture, **Kyoto University**, Kyoto, Japan
 Iwate Biotechnology Research Center (IBRC) Iwate, Japan

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Self introduction



Ryohei
Terauchi

Kyoto U
IBRC



Toshiyuki
Sakai

Kyoto U

Questions & Comments

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Lab. Information

<http://www.crop-evolution.kais.kyoto-u.ac.jp/index.php/en/>

<https://www.ibrc.or.jp/english-page>

Kyoto



Iwate

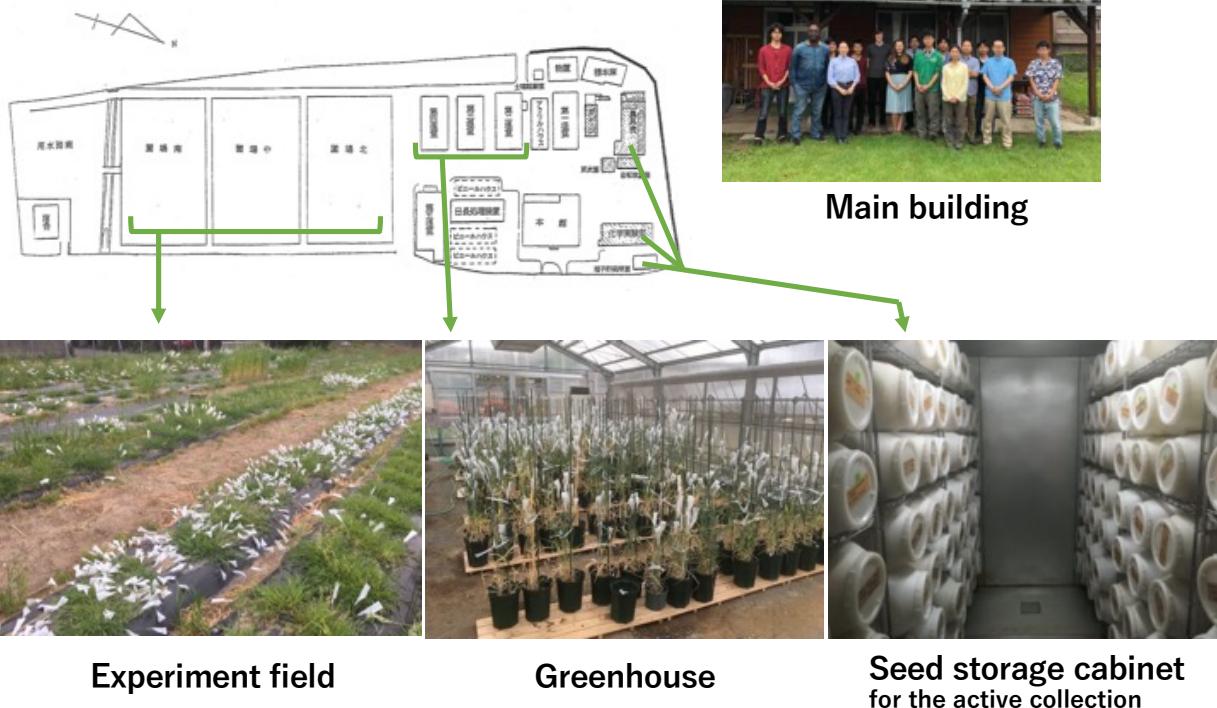


Iwate

Kyoto

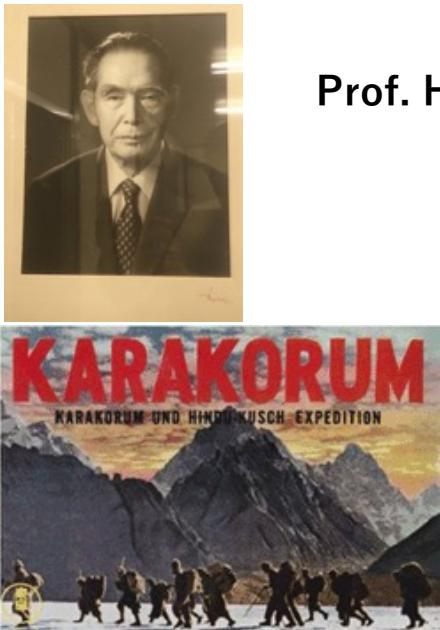
Lab. Crop Evolution, Mozume, Kyoto University

Maintenance
of wheat
genetic
resources



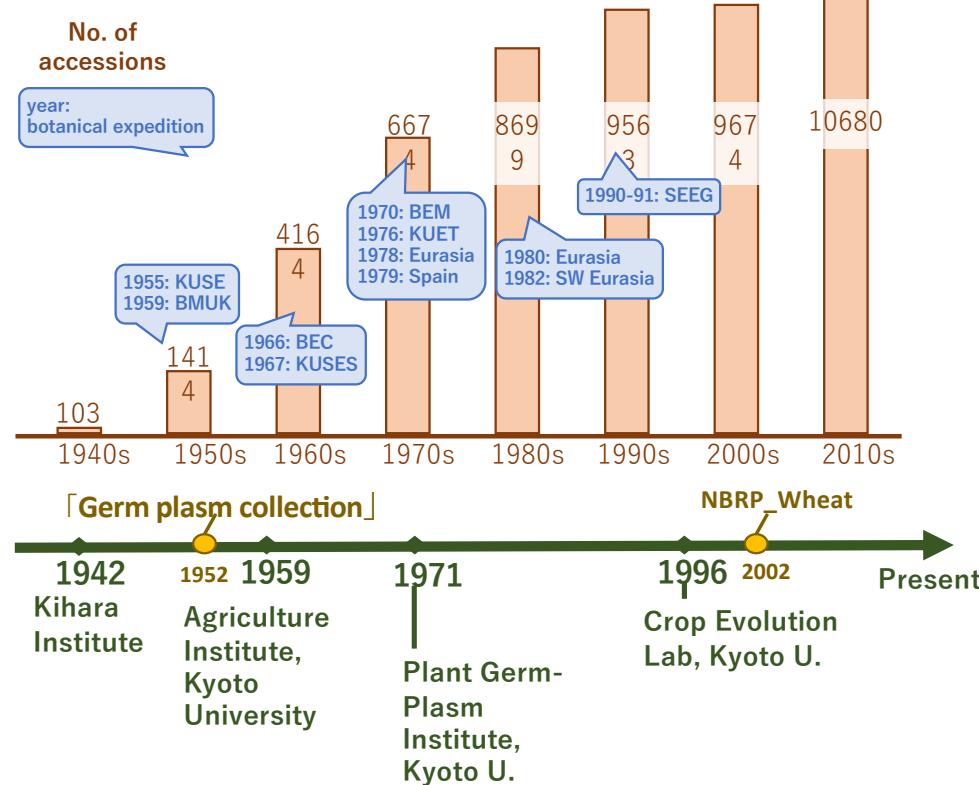
- Use genomics to study crop diversity and evolution
- Contribute to world food security by crop improvement

Genetic resources of wheat and wild relatives preserved in Mozume



Kihara with *Aegilops tauschii*

Prof. Hitoshi Kihara (1893-1986)

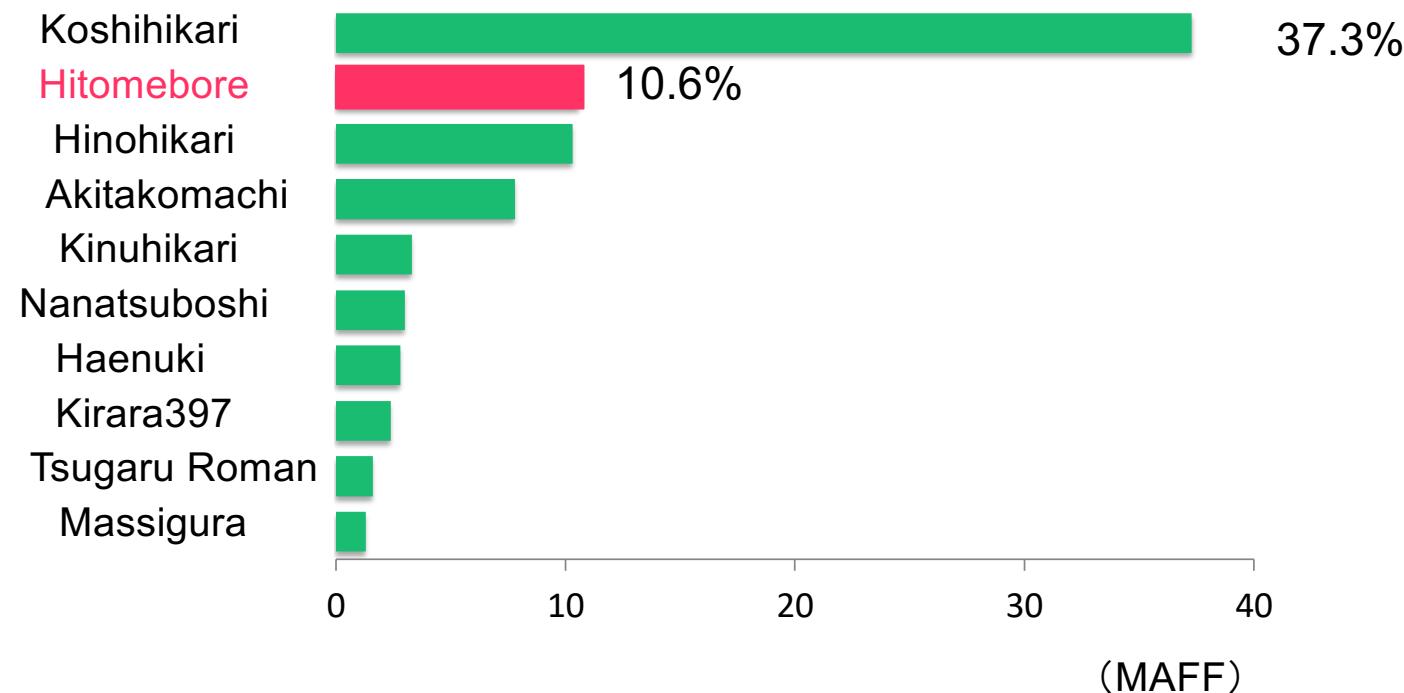


~12,000 *Triticum* and *Aegilops* accessions preserved (NBRP project)

Iwate Prefecture, Japan = Agriculture



Cultivation area of rice cultivars (Japan)



Hitomebore: Major elite cultivar in Miyagi, Iwate, Fukushima

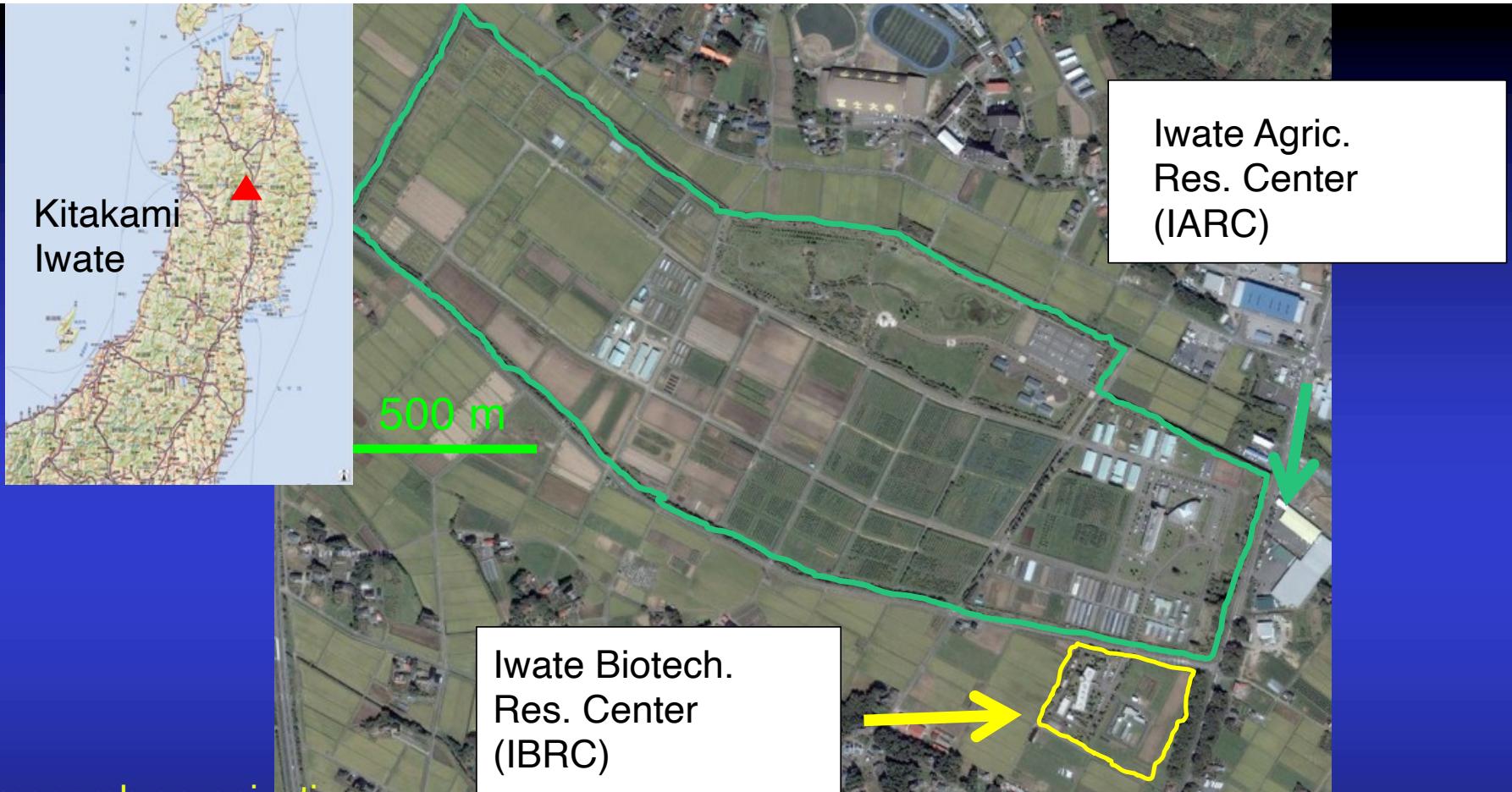
Rice cultivar “Hitomebore”

Major elite cultivar of Tohoku region

Improvements needed:

- Blast disease resistance
- Cold tolerance
- Eating quality
- Seedling vigor
- Yield

→ Deliver **safer** and
more **competitive** cultivars to farmers



Non-profit research organization
Funded by Iwate Local Government
40 researchers
Major mandates: rice
gentian

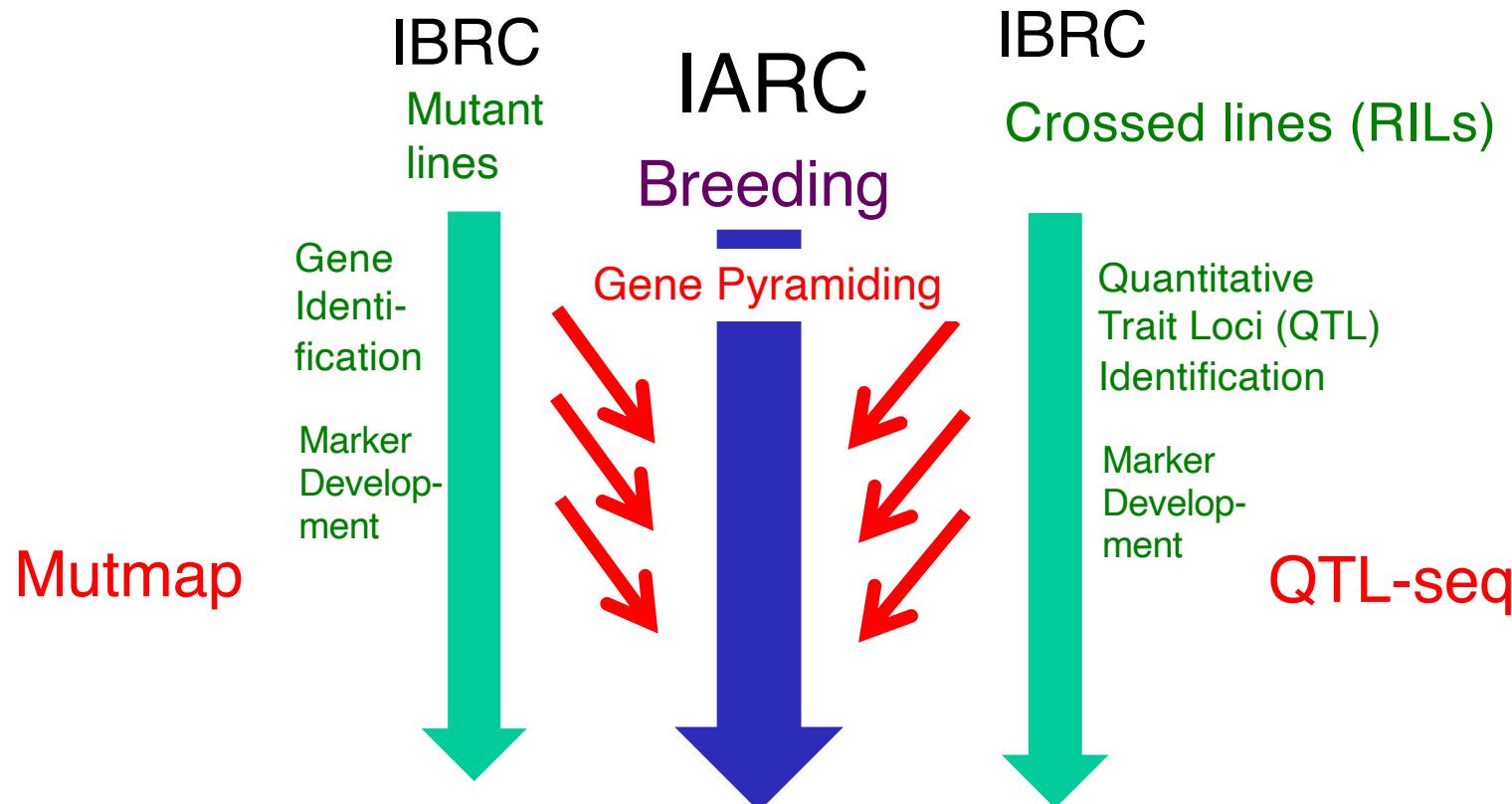


Rice Breeding in Iwate

Elite cultivar
“Hitomebore”

Target Traits: Blast resistance, Cold tolerance
Eating quality, Seedling vigor, Yield

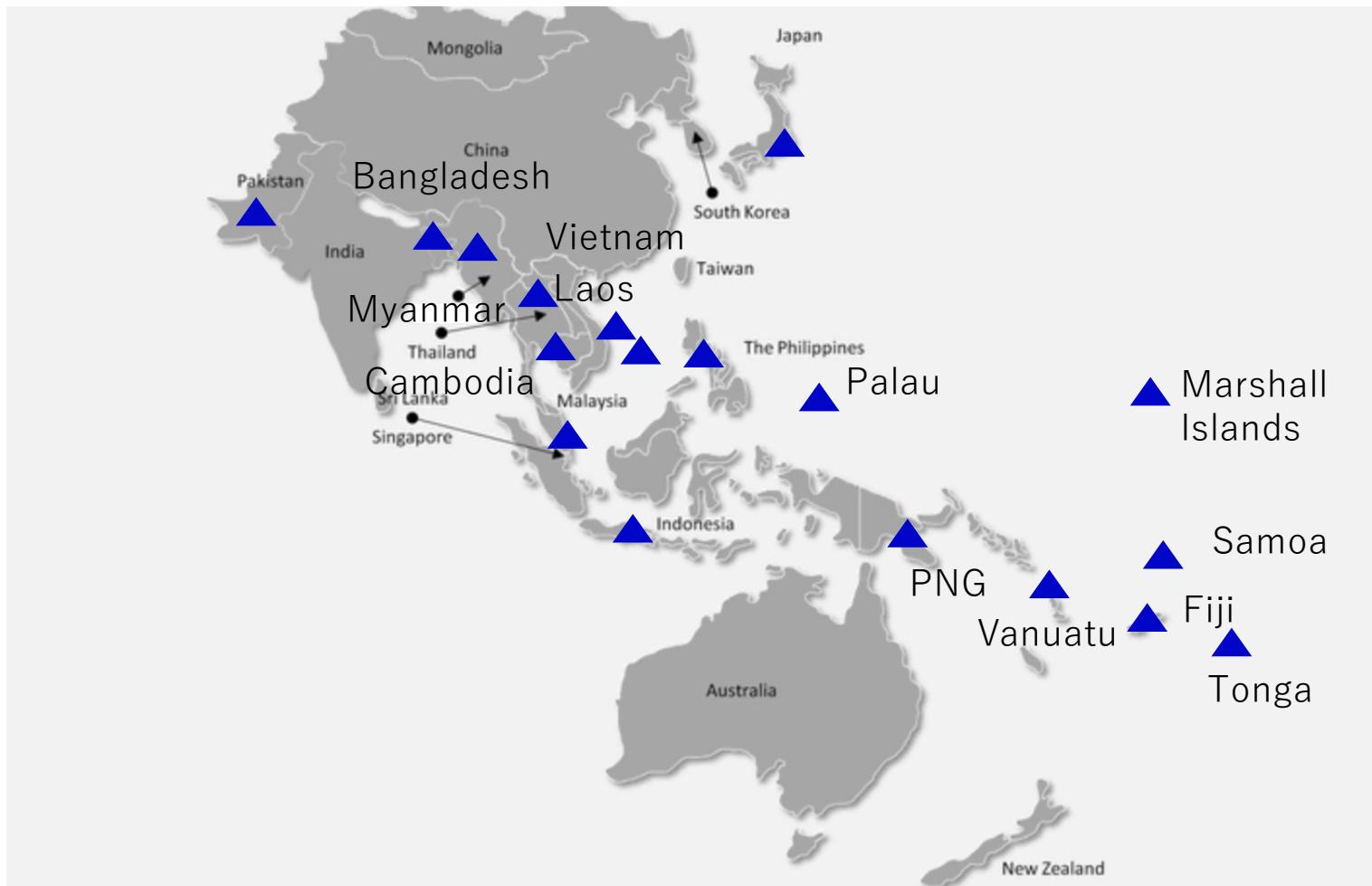
Non transgenic



Course Participants

4. BGD_Mr. Abu Sayeed MD Hasibuzzaman
5. BGD_Ms. Shova Anjuman SHAMMY
6. KAM_Mr. Dara Oudom LENG
7. INS_Ms. Winda PUSPITASARI
8. INS_Ms. Indrastuti RUMANTI
9. LAO_Mr. Vanhna PEUAKKEO
10. LAO_Mr. Khamphanh Xayyalat
11. MAL_Mr. Faiz Bin AHMAD
12. MAL_Ms. Wan Dalila Binti WAN CHIK
13. MYN_Ms. Than Than AYE
14. PAK_Ms. Maria Ghaffar AWAN
15. PAK_Ms. Ayesha WADOOD
16. PHI_Mr. Arvin DIMAANO
19. VIE_Ms. Loan HA
20. VIE_Ms. Thi Hue HOANG
21. FIJ_Mr. Guo Fu LUO
22. Marshall_Mr. Vincent ENRIQUEZ
23. Palau_Mr. Mc Martinus ASSITO
24. Papua New Guinea_Ms. Janiella AIDABOE
25. Samoa_Ms. Ferila FITI SAMUELU
26. Tonga_Mr. Eniselika Taani Akuola Siupelikoula MATALAVE
27. Vanuatu_Ms. Floriane LAWAC

Asia Pacific region



Course contents

29 April (Mon) Introduction to crop genetics and breeding
 Linkage mapping, markers

30 April (Tue) Mapping populations
 DNA sequencing

1 May (Wed) Mutmap, QTL-seq

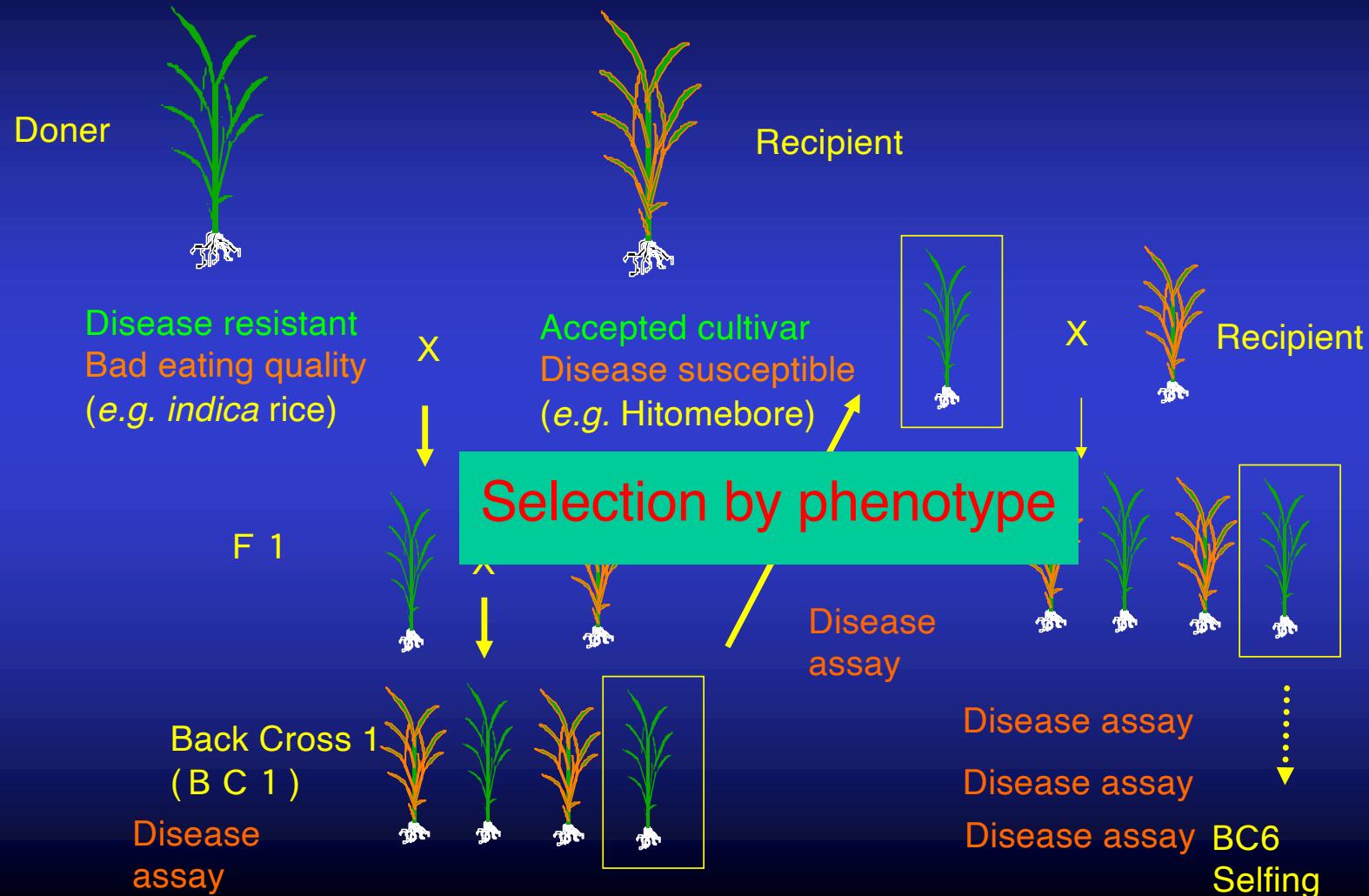
2 May (Thu) Excursion

3 May (Fri) Genomic Prediction

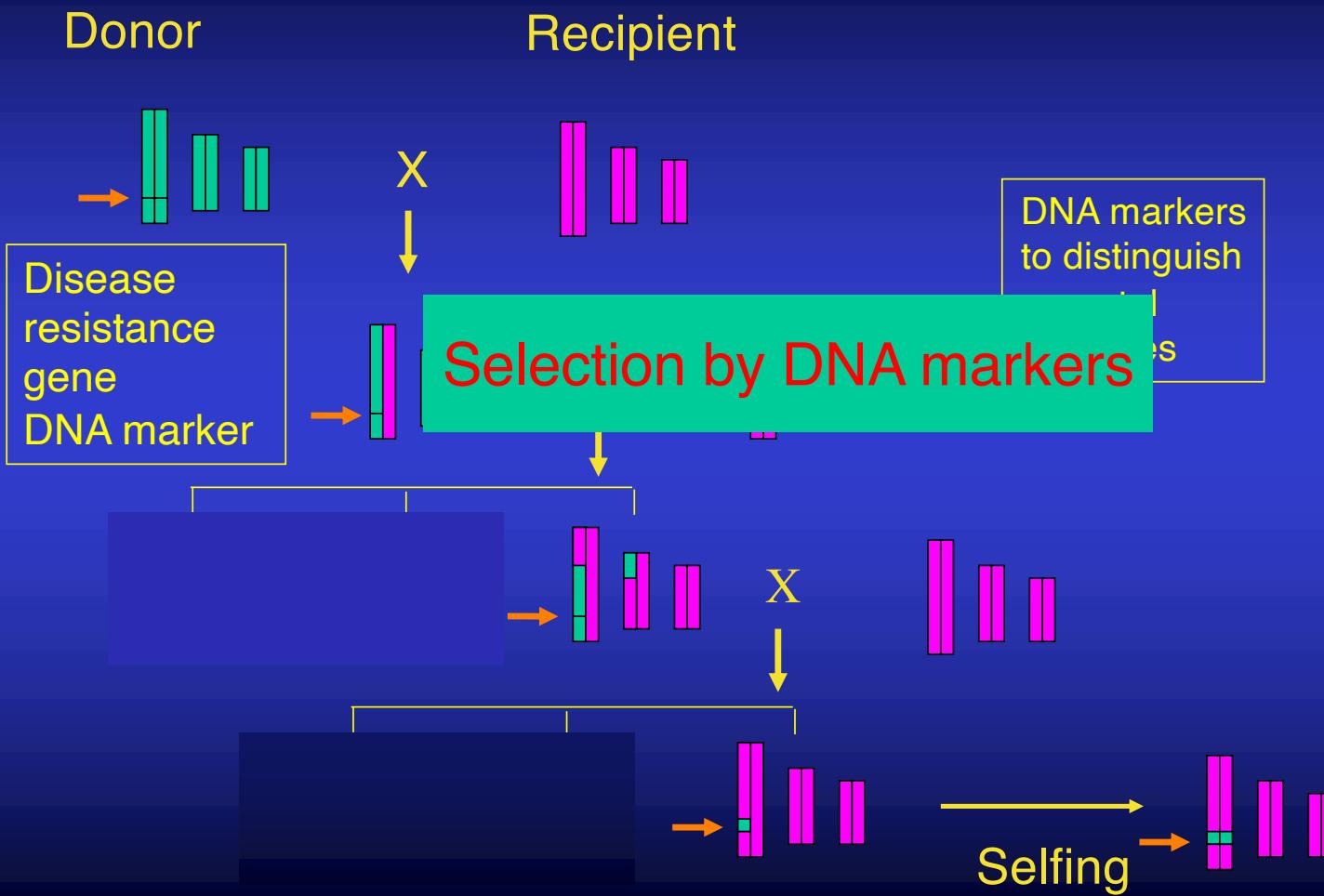
29 April Introduction to crop genetics and breeding
Markers for linkage mapping

- Introduction to Plant Genetics and Breeding
- Principle of linkage mapping
- Molecular markers
- QTL mapping

Conventional breeding: time consuming and labor intensive



Modern breeding: DNA marker assisted selection



Introduction to Genetics

Forward Genetics: Phenotype → Genotype

Reverse Genetics: Genotype → Phenotype
(Gene Editing)

Mendel's law

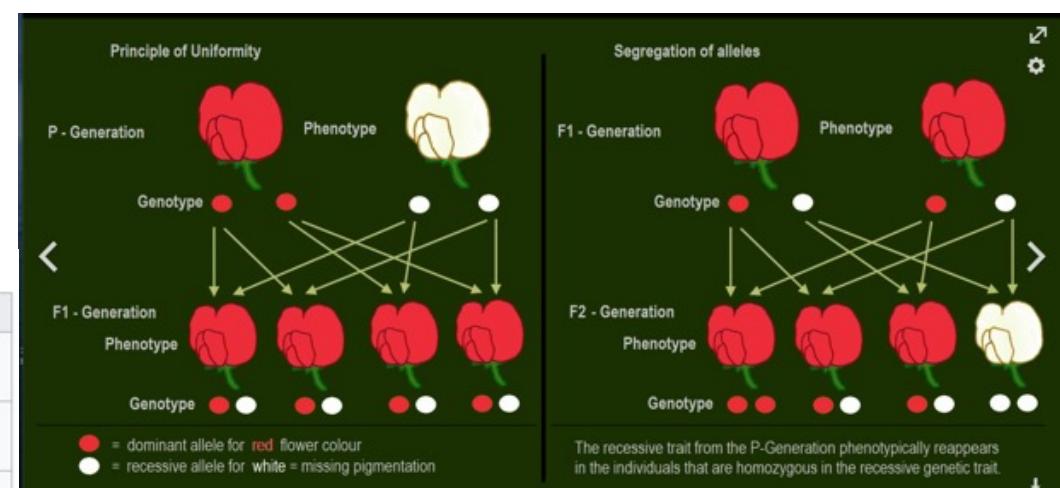


Gregory
Mendel
(1822-1884)

Brno
Czech Republic

Law	Definition
Law of dominance and uniformity	Some alleles are dominant while others are recessive; an organism with at least one dominant allele will display the effect of the dominant allele. ^[18]
Law of segregation	During gamete formation, the alleles for each gene segregate from each other so that each gamete carries only one allele for each gene.
Law of independent assortment	Genes of different traits can segregate independently during the formation of gametes.

Characteristics of pea plants Gregor Mendel used in his inheritance experiments						
Seeds	Flower	Pod	Stem			
form	cotyledons	colour	form	colour	position	size
round roundish	yellow	white	full	yellow	axial	long
wrinkled	green	violet-red	constricted between the seeds	green	terminal	short

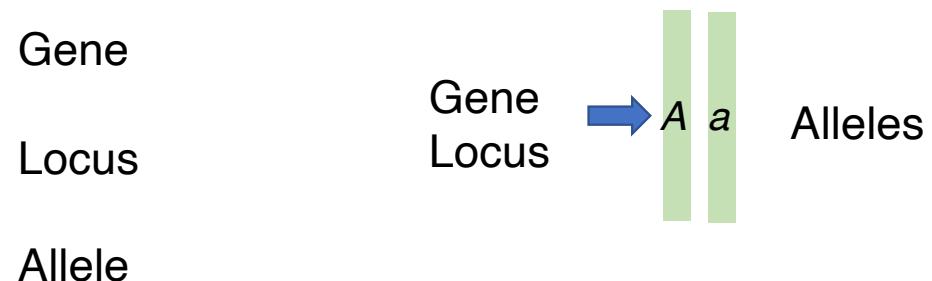


Terms: dominant / recessive

Source: Wikipedia

"Versuche über Pflanzenhybriden" 1865

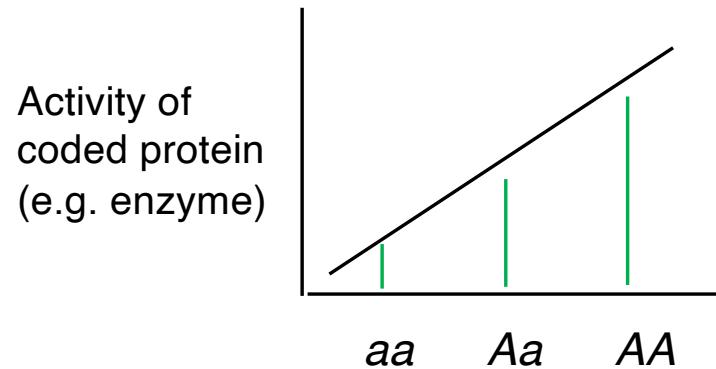
Terminology



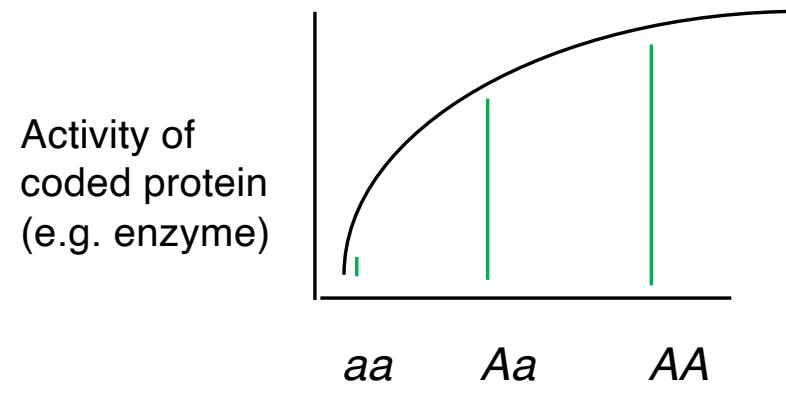
Convention: genes, alleles are described in *italic* : *Aa*

coded proteins are described in Roman : Aa

Possible reason of dominance of alleles



Semi-dominant



dominant

Law of segregation

Parent (P) $AA \times aa$



Filial 1 (F1) Aa

$Aa \times Aa$



F2 $AA = 1, Aa = 2, aa = 1$

Punnett square

	Male gamete	A	a
Female gamete		1	1
A	1	$AA = 1$	$Aa = 1$
a	1	$aA = 1$	$Aa = 1$

Rediscovery of Mendel's works (1900; 35 years after Mendel)

Hugo de Vries ForMemRS HonFRSE ^[1]	
	
De Vries, c. 1907	
Born	Hugo Marie de Vries 16 February 1848 Haarlem , Netherlands
Died	21 May 1935 (aged 87) Lunteren , Netherlands
Scientific career	
Fields	Botany
Institutions	Leiden University

Carl Correns	
	
Carl Correns in the 1910s	
Born	19 September 1864 ^[1] Munich , Kingdom of Bavaria
Died	14 February 1933 (aged 68) Berlin , Germany
Scientific career	
Institutions	Kaiser Wilhelm Institute for Biology

Erich von Tschermak	
	
Erich Tschermak-Seysenegg	
Born	15 November 1871 Vienna , Austrian Empire
Died	11 October 1962 (aged 90) Vienna , Austria
Nationality	Austria
Scientific career	
Fields	Agronomy
Doctoral advisor	Karl Wilhelm von Nägeli

Source: Wikipedia

Hugo de Vries

ForMemRS HonFRSE^[1]



De Vries, c. 1907

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Source: Wikipedia

Definition of the gene [edit]

In 1889, De Vries published his book *Intracellular Pangenesis*,^[4] in which, based on a modified version of Charles Darwin's theory of *Pangenesis* of 1868, he postulated that different characters have different hereditary carriers. He specifically postulated that inheritance of specific traits in organisms comes in particles. He called these units *pangenes*, a term 20 years later to be shortened to *genes* by Wilhelm Johannsen.

Term: pangene → gene

To support his theory of pangenes, which was not widely noticed at the time, De Vries conducted a series of experiments hybridising varieties of multiple plant species in the 1890s. Unaware of Mendel's work, De Vries used the laws of dominance and recessiveness, segregation, and independent assortment to explain the 3:1 ratio of phenotypes in the second generation.^[5] His observations also confirmed his hypothesis that inheritance of specific traits in organisms comes in particles.

Oenothera glazioviana



Evening primrose

Oenothera lamarckiana

Carl Correns



Carl Correns in the 1910s

Born	19 September 1864 ^[1] Munich, Kingdom of Bavaria
Died	14 February 1933 (aged 68) Berlin, Germany
Scientific career	
Institutions	Kaiser Wilhelm Institute for Biology

Key experiments and findings [edit]

Carl Correns conducted much of the foundational work for the field of genetics at the turn of the 20th century. He rediscovered and independently verified the work of Mendel in a separate [model organism](#). He also discovered [cytoplasmic inheritance](#), an important extension of Mendel's theories, which demonstrated the existence of extra-chromosomal factors on phenotype. Some of his unpublished work and most of his lab books were destroyed in the Berlin bombings of 1945.

Rediscovery of Mendel [edit]

In 1892, while at the [University of Tübingen](#), Correns began to experiment with trait inheritance in plants. Correns published his first paper on 25 January 1900, which cited both [Charles Darwin](#) and Mendel, recognising the relevance of [genetics](#) to Darwin's ideas. In Correns' paper, "G. Mendel's Law Concerning the Behavior of the Progeny of Racial Hybrids", he restated Mendel's results as the 'law of segregation' and introduced a new 'law of independent assortment'.^{[3][4][5][6][7][8][9][10]}

Cytoplasmic inheritance [edit]

After rediscovering Mendel's laws of heredity, which can be explained with [chromosomal inheritance](#), he undertook experiments with the four o'clock plant *Mirabilis jalapa* to investigate apparent counterexamples to Mendel's laws in the heredity of variegated (green and white mottled) leaf color. Correns found that, while Mendelian traits behave independently of the sex of the source parent, leaf color depended greatly on which parent had which trait. For instance, pollinating an [ovule](#) from a white branch with pollen from another white area resulted in white progeny, the predicted result for a [recessive gene](#). Green pollen used on a green stigma resulted in all green progeny, the expected result for a [dominant gene](#). However, if green pollen fertilized a white stigma, the progeny were white, but if the sexes of the donors were reversed (white pollen on a green stigma), the progeny were green.



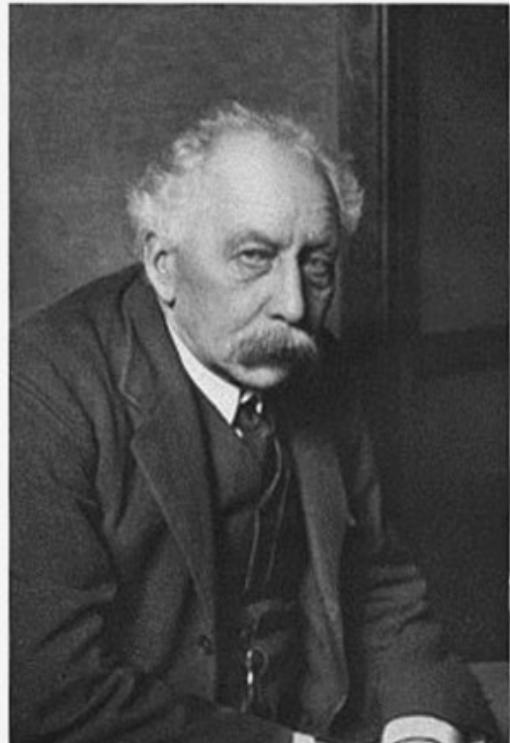
Carl Correns

Mirabilis jalapa



Source: Wikipedia

William Bateson and Genetics



W. Bateson

1861-1926

Source: Wikipedia

Founding the discipline of genetics [edit]

Further information: [Mutationism](#)

Bateson became famous as the outspoken Mendelian antagonist of [Walter Raphael Weldon](#), his former teacher, and of [Karl Pearson](#) who led the biometric school of thinking. The debate^[when?] centred on [saltationism](#) versus [gradualism](#) (Darwin had represented gradualism, but Bateson was a saltationist).^[27] Later, [Ronald Fisher](#) and [J.B.S. Haldane](#) showed that discrete mutations were compatible with gradual evolution, helping to bring about the [modern evolutionary synthesis](#).

Bateson first suggested using the word "genetics" (from the Greek *gennō*, γέννω; "to give birth") to describe the study of inheritance and the science of variation in a personal letter to [Adam Sedgwick](#) (1854–1913, zoologist at Cambridge, not the [Adam Sedgwick](#) (1785–1873) who had been Darwin's professor), dated 18 April 1905.^[28] Bateson first used the term "genetics" publicly at the Third International Conference on Plant Hybridization in London in 1906.^{[29][30]} Although this was three years before [Wilhelm Johannsen](#) used the word "gene" to describe the units of hereditary information, De Vries had introduced the word "pangene" for the same concept already in 1889, and etymologically the word *genetics* has parallels with Darwin's concept of [pangenesis](#). Bateson and [Edith Saunders](#) also coined the word "allelomorph" ("other form"), which was later shortened to [allele](#).^[31]

Bateson co-discovered [genetic linkage](#) with [Reginald Punnett](#) and [Edith Saunders](#), and he and Punnett founded the [Journal of Genetics](#) in 1910. Bateson also coined the term "[epistasis](#)" to describe the genetic interaction of two independent [loci](#).

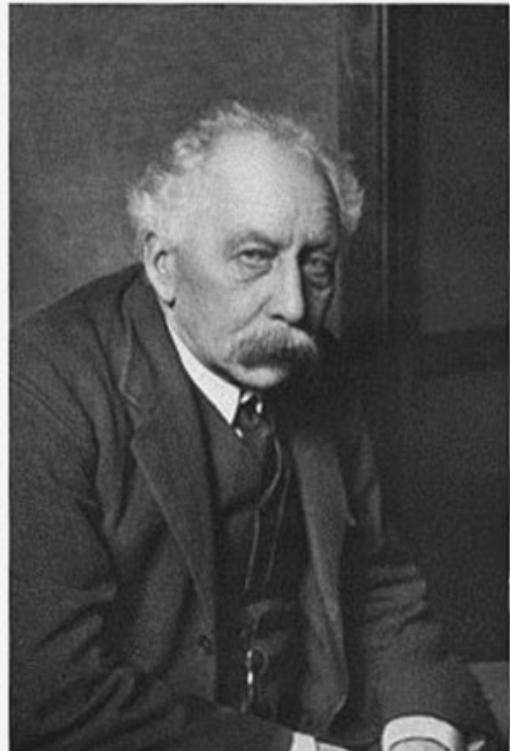
The [John Innes Centre](#) holds a [Bateson Lecture](#) in his honour at the annual John Innes Symposium.^[32]

Terms: genetics, allelomorphs (alleles)

Mentor of Nicolai Vavilov
Coworker of J.B.S. Haldane
Father of Gregory Bateson

William Bateson and Genetics

William Bateson



W. Bateson

1861-1926

If the Proceedings of the Brunn Natural History Society had been a little rarer I suppose that Bateson would now be lying in Westminster Abbey. For we have only to read between the lines of the first report to the Evolution Committee of the Royal Society by himself and Miss E. Saunders, published in 1902, to realize that when Mendel's paper in the Brunn Society's Journal was discovered in 1900, Bateson had already hit upon the atomic theory of heredity, which goes by the name of Mendelism. It was characteristic of him that no hint of this fact is to be found in his published work. His classical explosion of the subject is entitled Mendel's Principles of Heredity. Copernicus, if he admitted Aristarchus's Priority, did not write on "Aristarchus's principles of astronomy". But Mendel's and Bateson's discovery was as fundamental as that of Copernicus, and of much greater practical importance.

In: Possible Worlds J. B. S. Haldane 1927

Fruit fly

Drosophila

Thomas Hunt Morgan's and sex linkage of eye color

Thomas Hunt Morgan

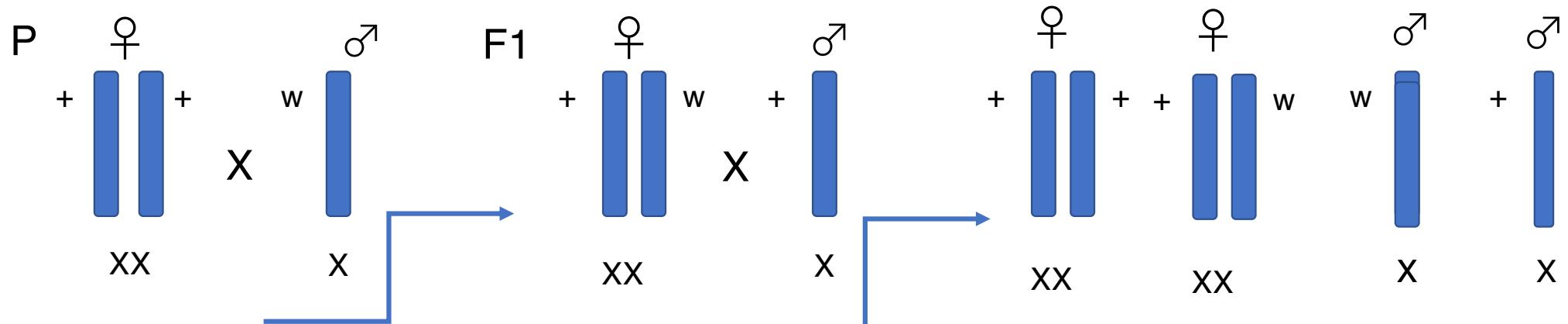


1866-1945

Red: +
White: w

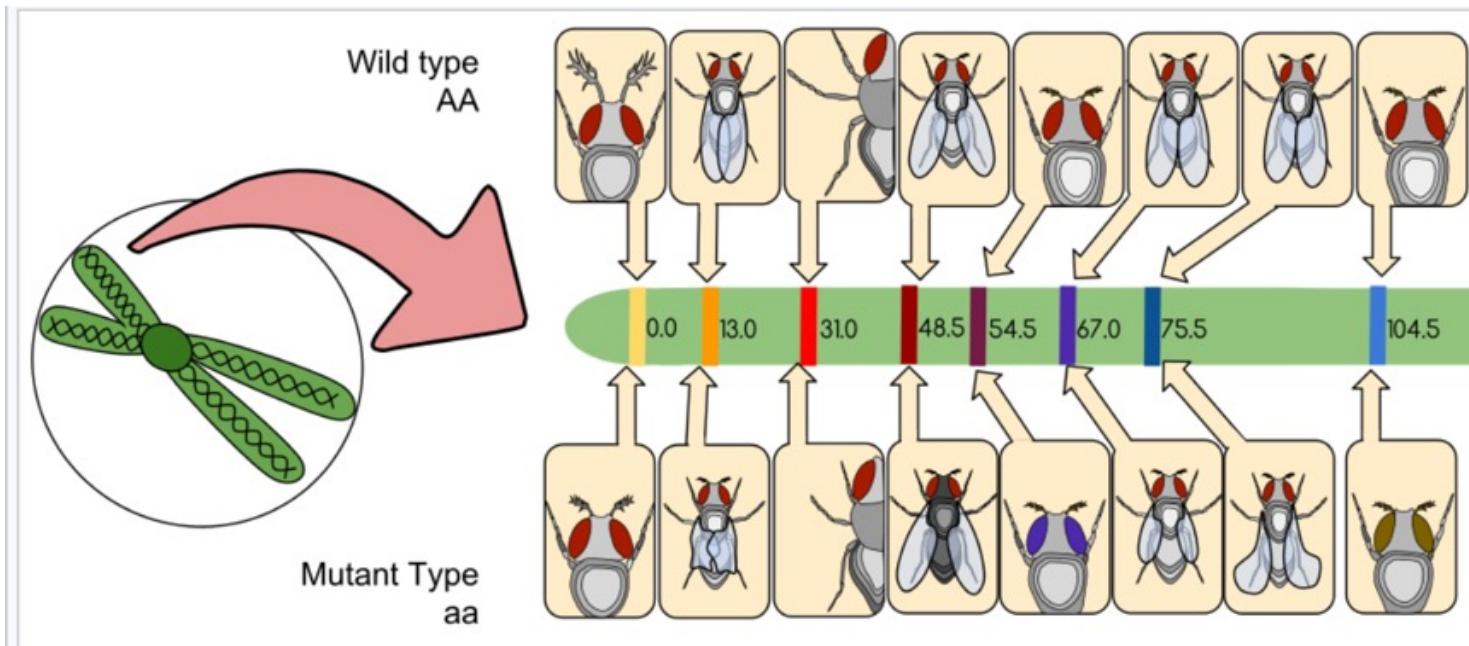
Cross	Outcome	
	Expected Phenotypes	Observed Phenotypes
P ₁ Red ♀ × P ₁ White ♂	F ₁ = All Red	F ₁ = All Red*
F ₁ Red ♀ × F ₁ Red ♂	75% Red ♀ and ♂ 25% White ♀ and ♂	50% Red ♀ 25% Red ♂ 25% White ♂

*Morgan did observe 3 white-eyed males in the F₁ generation. His original paper suggested that these white-eyed males were evidence of "further sporting."



Source: Miko, I. (2008) Thomas Hunt Morgan and sex linkage. *Nature Education* 1(1):143

Genetic Linkage



Thomas Hunt Morgan's *Drosophila melanogaster* genetic linkage map. This was the first successful gene mapping work and provides important evidence for the chromosome theory of inheritance. The map shows the relative positions of alleles on the second Drosophila chromosome. The distances between the genes (centimorgans) are equal to the percentages of chromosomal crossover events that occur between different alleles.^[5]

Law of independent association (segregation of two loci)

Locus A
Locus B

		BB	Bb	bb
		1	2	1
AA	1	1	2	1
Aa	2	2	4	2
aa	1	1	2	1

$$AABB = 1, AABb = 2, Aabb = 1$$

$$AaBB = 2, AaBb = 4, Aabb = 1$$

$$aaBB = 1, aaBb = 2, aabb = 1$$

$$\begin{aligned} A-B- &= 9 & - : \text{either } A \text{ or } a \\ A-bb &= 3 \\ aaB- &= 3 \\ aabb &= 1 \end{aligned}$$

$- : \text{either } B \text{ or } b$

Genetic linkage in plants

Bateson, Saunders and Punnett (1905)

$$PPLL \times ppll \rightarrow PpLI$$

$$PpLI \times PpLI$$

Bateson and Punnett experiment

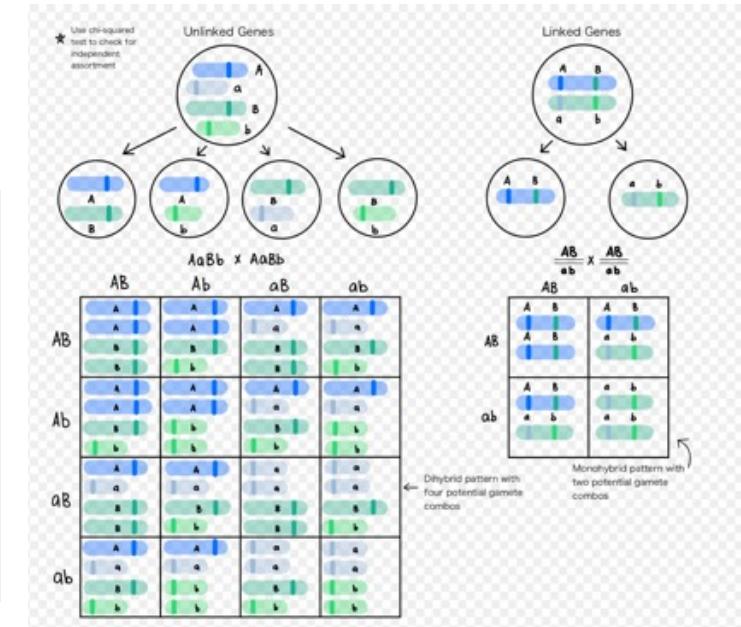
Phenotype and genotype	Observed	Expected from 9:3:3:1 ratio
Purple, long ($P_L_$)	284	216
Purple, round ($P_l l$)	21	72
Red, long ($p p L_$)	21	72
Red, round ($p p l l$)	55	24

Sweet pea experiment

P and L (and p and l) are **coupled**

\Leftrightarrow **repulsion**

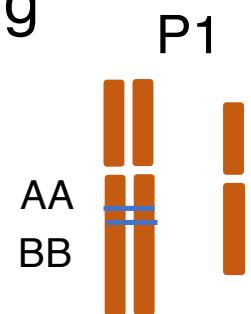
Mendel's "Law of independent assortment" violated



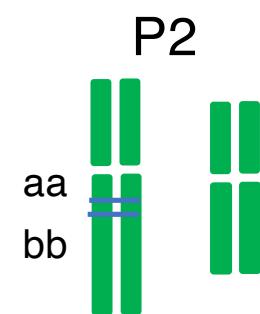
https://en.wikipedia.org/wiki/Genetic_linkage

Linkage mapping

Test cross
(Back cross)



X
↓

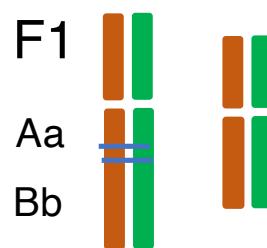


A and B tightly linked

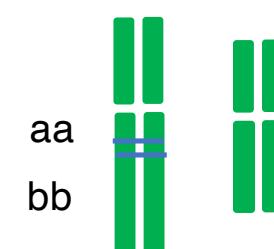
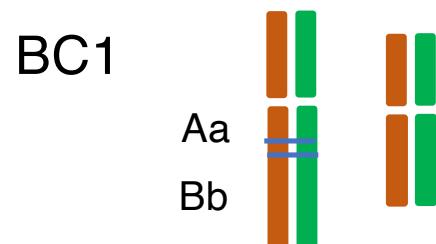
$AABB \times aabb$

♀ ♂

No recombination



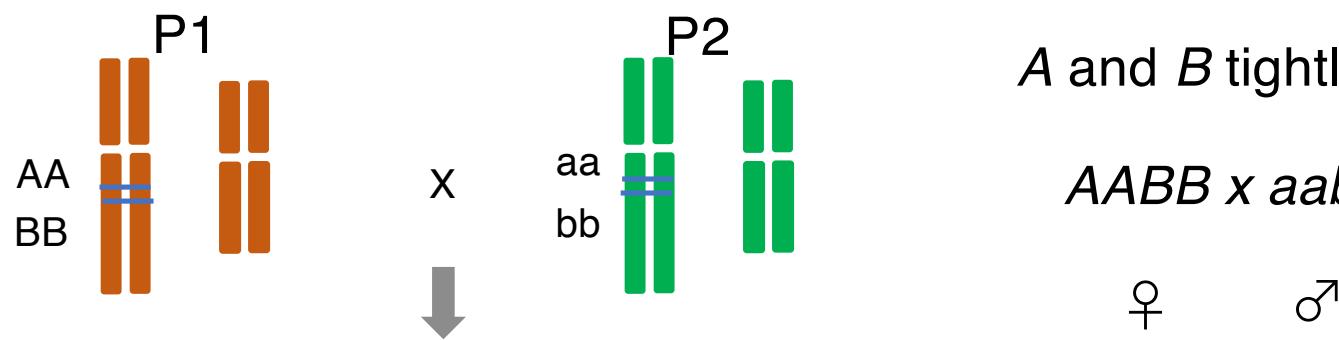
Convention:
Female x Male



$AaBb : aabb \rightarrow 1 : 1$

Linkage mapping

Test cross
(Back cross)

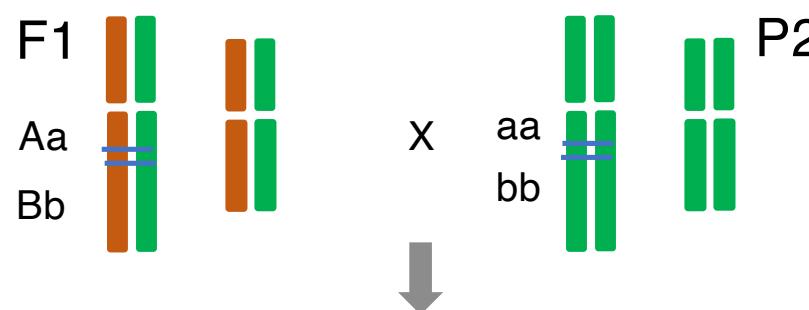


A and *B* tightly linked

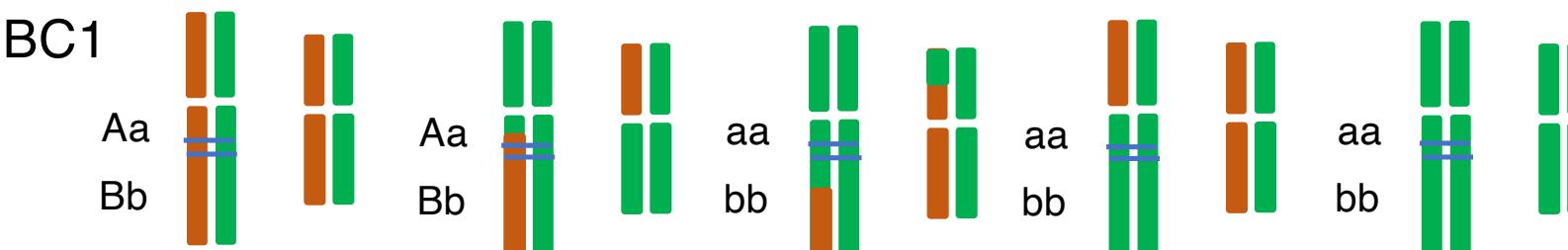
AABB x *aabb*

♀ ♂

With recombination



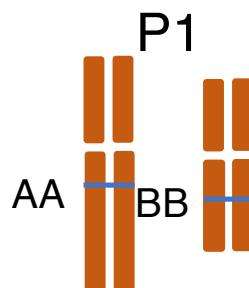
BC1



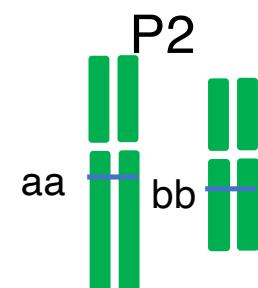
AaBb : *aabb* → 1 : 1

Linkage mapping

Test cross
(Back cross)



X

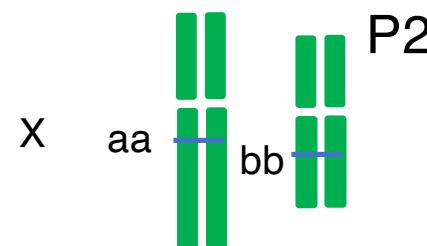
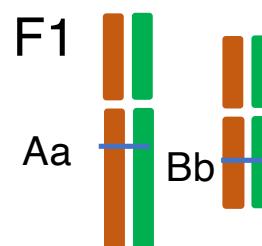


A and *B* unlinked

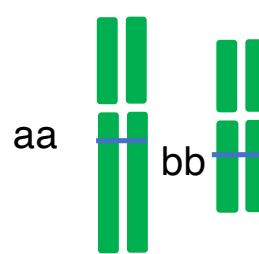
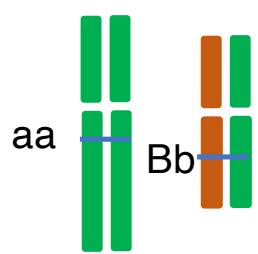
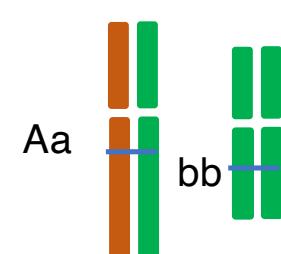
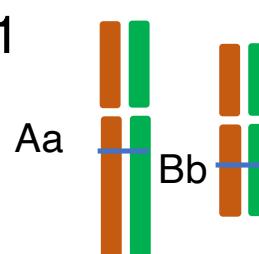
AABB x *aabb*

♀

♂



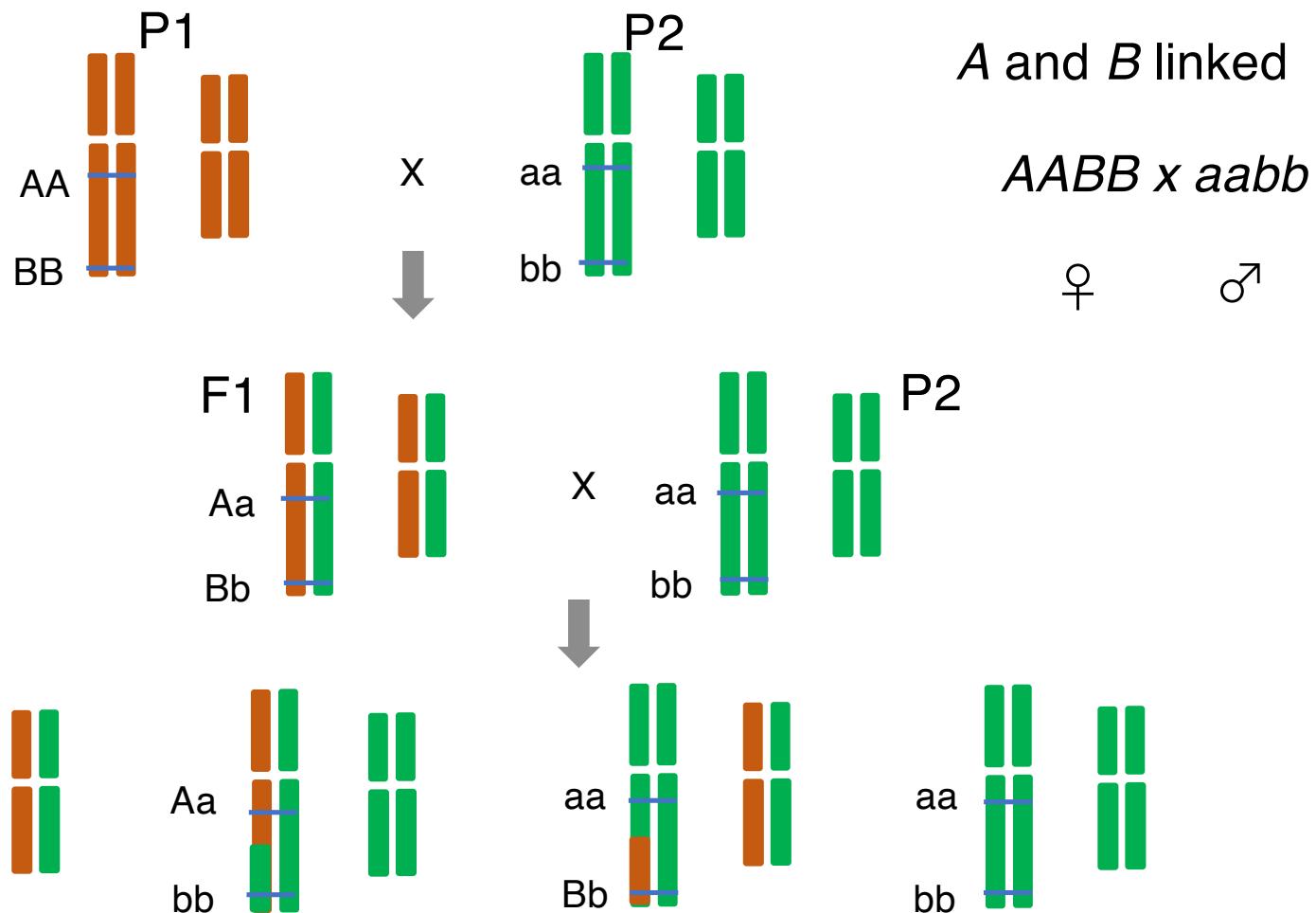
BC1



AaBb : Aabb: aaBB: aabb → 1 : 1 : 1 : 1

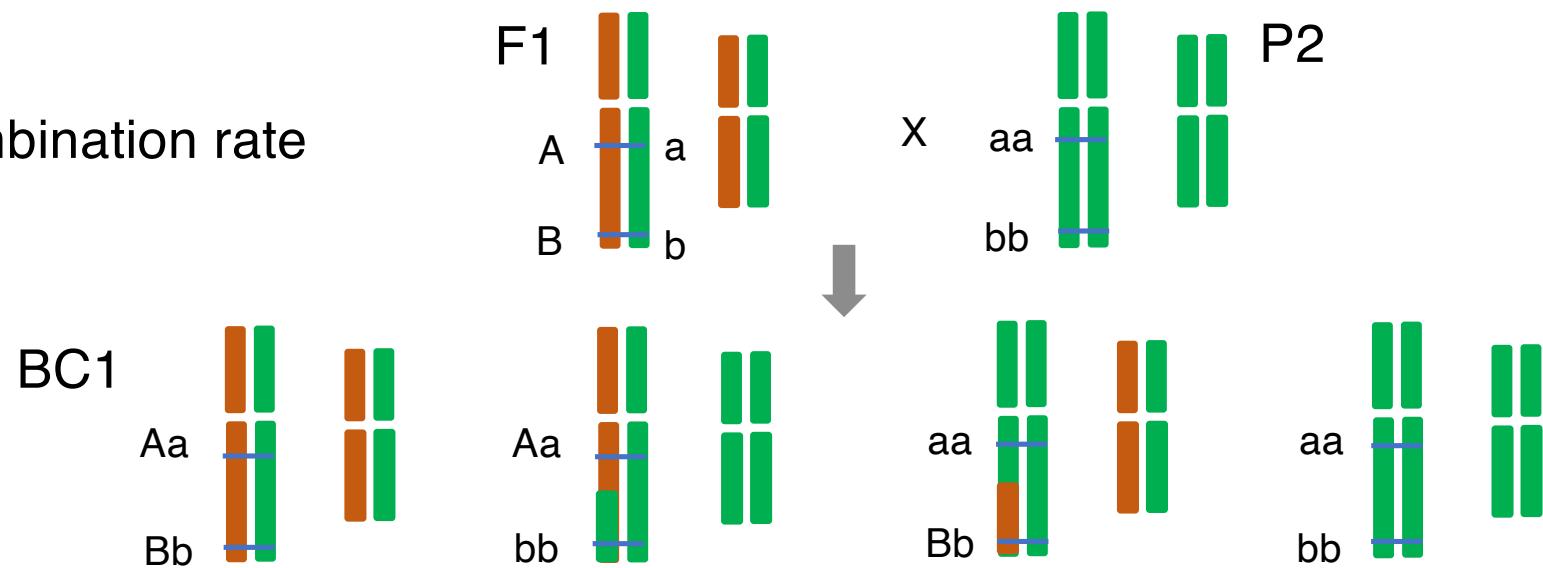
Linkage mapping

Test cross
(Back cross)



$$AaBb : Aabb : aaBb : aabb \rightarrow 1 : <1 : <1 : 1$$

Calculation of recombination rate

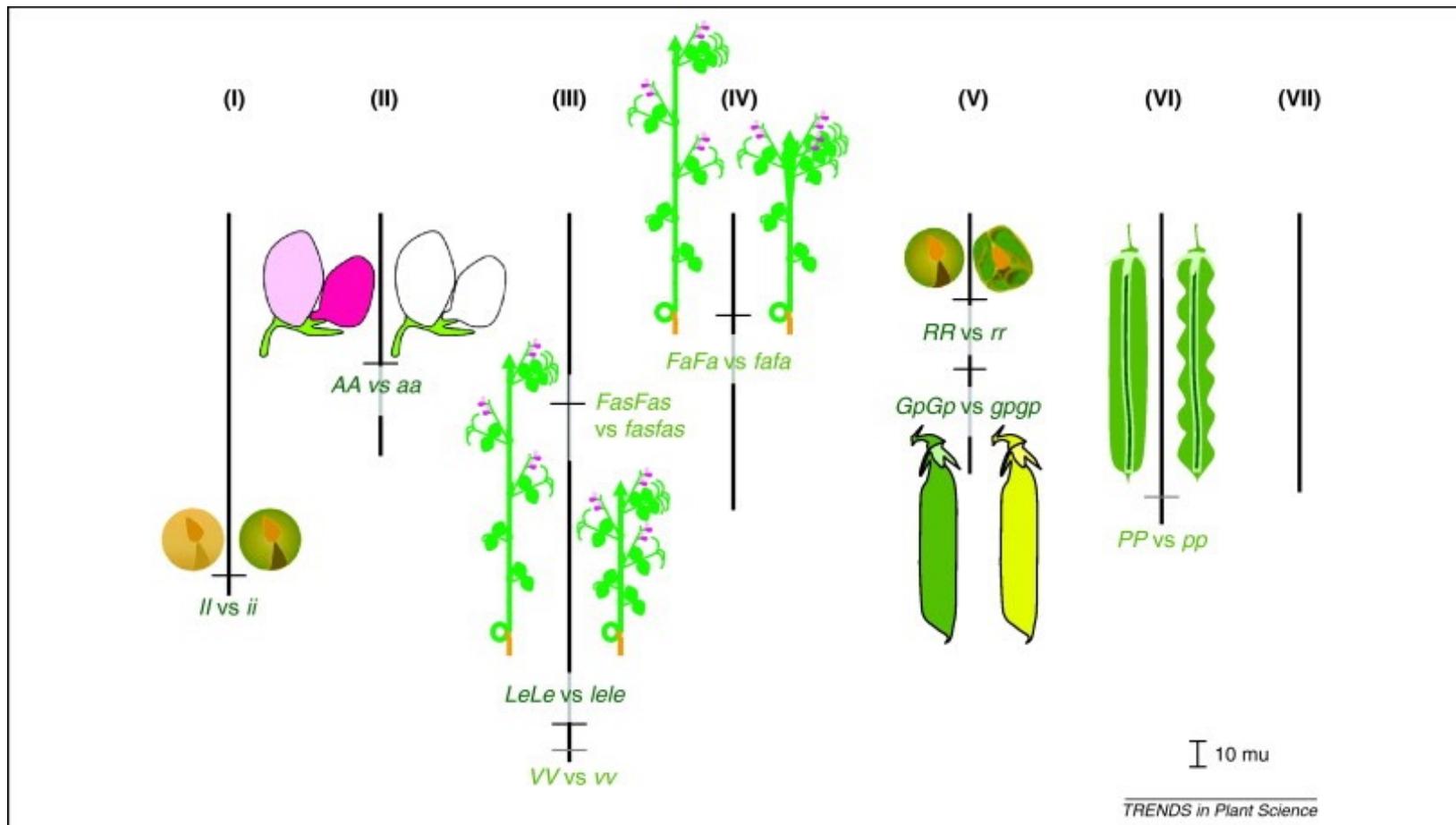


Female gamete	AB (1-r)/2	Ab r/2	aB r/2	ab (1-r)/2
Male gamete	Zygote			
ab	AaBb (1-r)/2	Aabb r/2	aaBb r/2	aabb (1-r)/2

$r = 0 \rightarrow$ Complete linkage

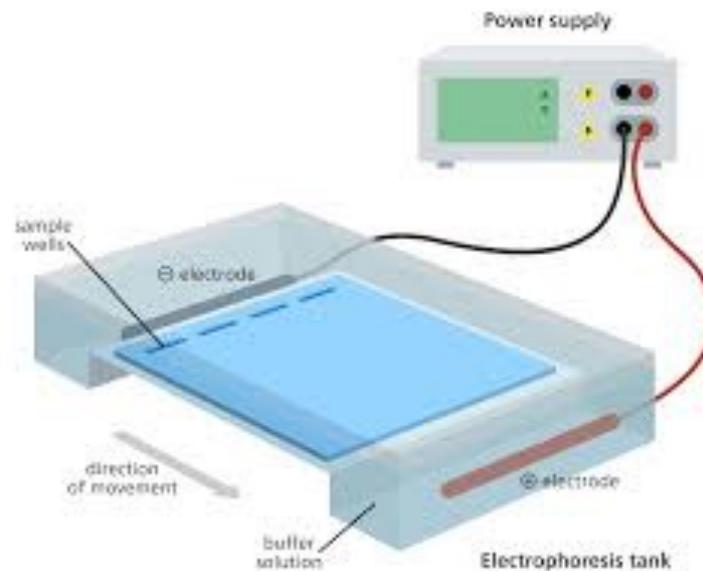
$r = 0.5 \rightarrow$ Independent segregation

Morphological markers



Source: https://link.springer.com/chapter/10.1007%2F978-981-13-7119-6_2

Gel electrophoresis



Agarose

Acrylamide

Starch

An example of isozyme markers

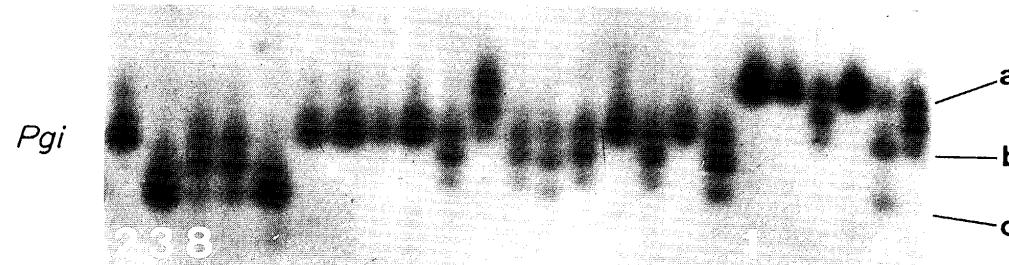
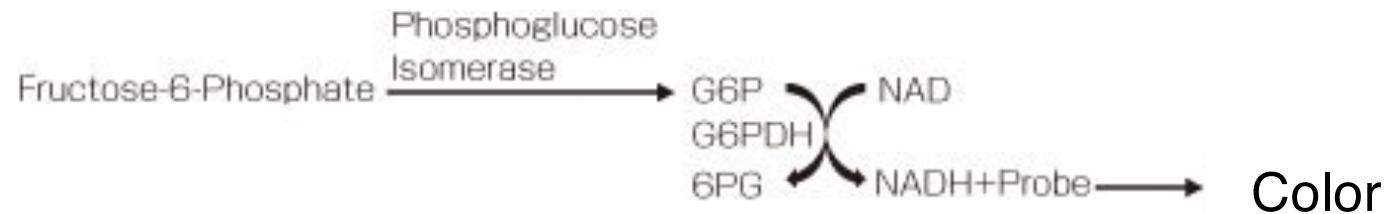


Fig. 2. Electrophoretic patterns of EST (upper) and PGI (lower) allozymes of *D. tokoro*. Alphabets and numbers indicate alleles and phenotypes, respectively.

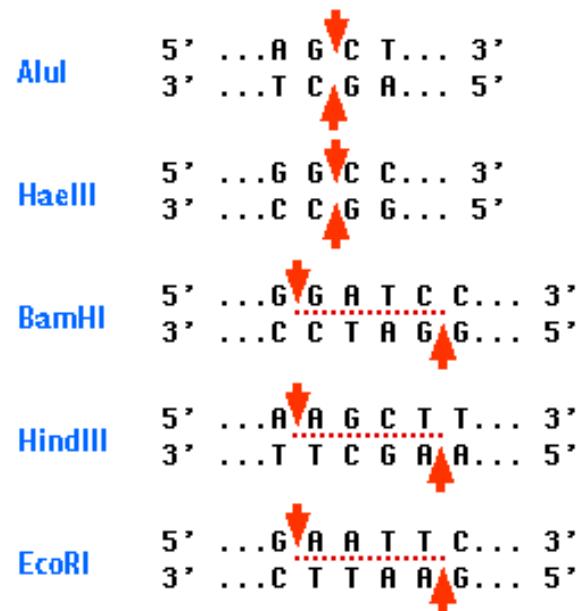


Dioscorea tokoro : phosphoglucone isomerase (PGI)

Terauchi 1990

DNA markers

Restriction enzymes (Restriction endonucleases)



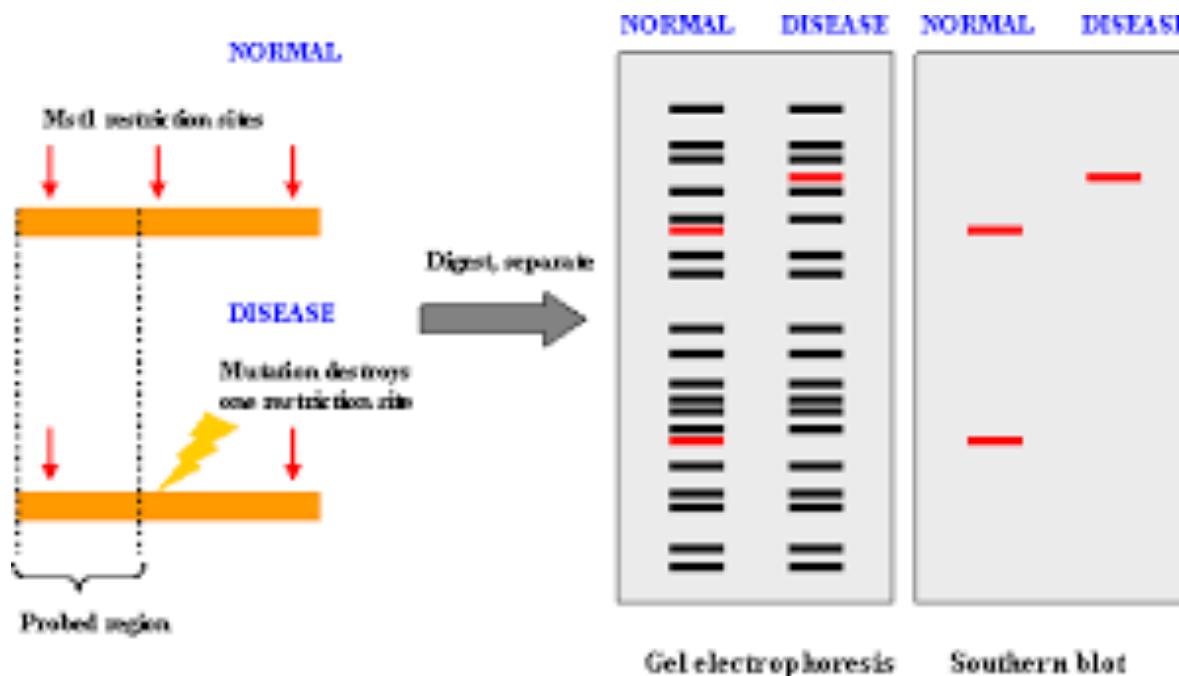
Alul and **HaeIII** produce blunt ends

BamHI **HindIII** and **EcoRI** produce "sticky" ends

Source: <https://www.biology-pages.info/R/RestrictionEnzymes.html>

DNA markers

RFLP (Restriction Fragment Length Polymorphism)

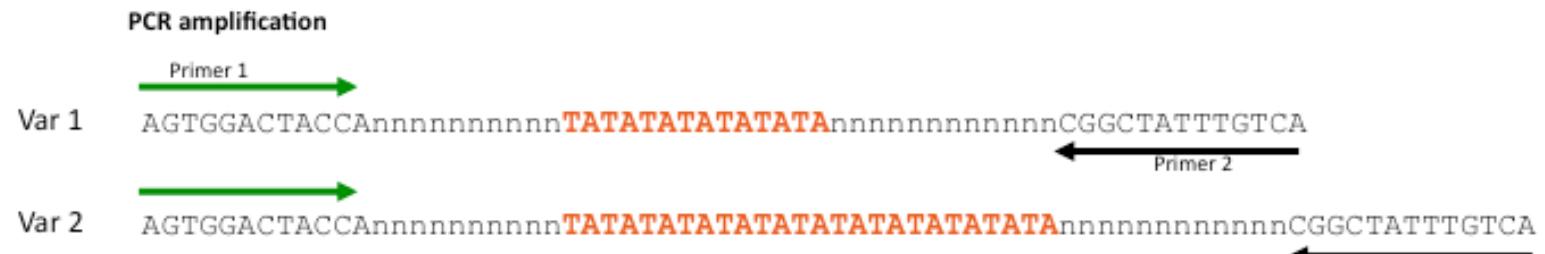


Source: [https://www.news-medical.net/life-sciences/Restriction-Fragment-Length-Polymorphism-\(RFLP\)-Technique.aspx](https://www.news-medical.net/life-sciences/Restriction-Fragment-Length-Polymorphism-(RFLP)-Technique.aspx)

DNA markers

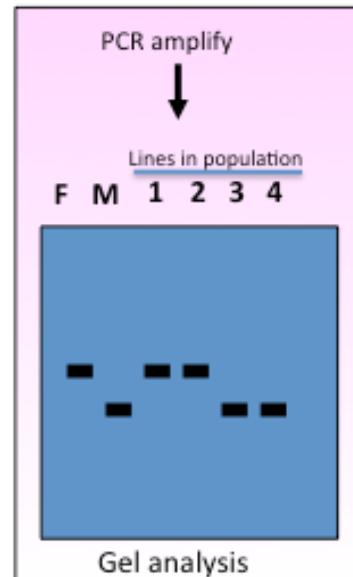
SSR assays

- Short sequences of nucleotides (typically 2 to 5) that are repeated multiple times in tandem
- Often particularly polymorphic



Simple Sequence
Repeats (SSR) markers

Microsatellite markers



Pros

- Highly polymorphic
- Reproducible
- Co-dominant
- Have multiple alleles
- Sequence tagged
- Amenable to high throughput and multiplexing
- Easily transferable between labs

Cons

- Fluorescent labels are expensive
- SSR discovery time consuming

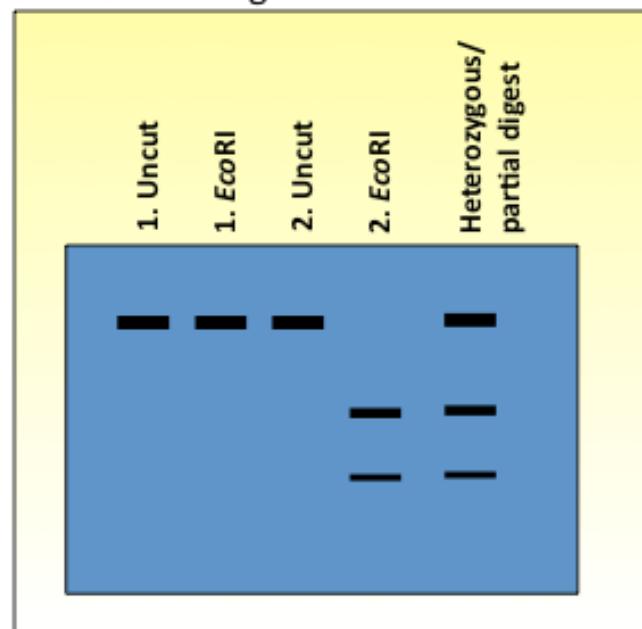
DNA markers

CAPS assay



1. PCR amplify
2. Cut with *Eco*RI
3. Resolve in gel

Cleaved Amplified Polymorphic Sequence (CAPS) markers



Pros:

- Easy to develop by inspecting sequence data
- Generally cheap
- No special equipment required
- Good for mapping specific genes
- Co-dominant

Cons:

- Multi-step assay not so amenable to HT
- Cannot always find a suitable enzyme
- Some restriction enzymes are more obscure/expensive

Codominant vs. dominant markers

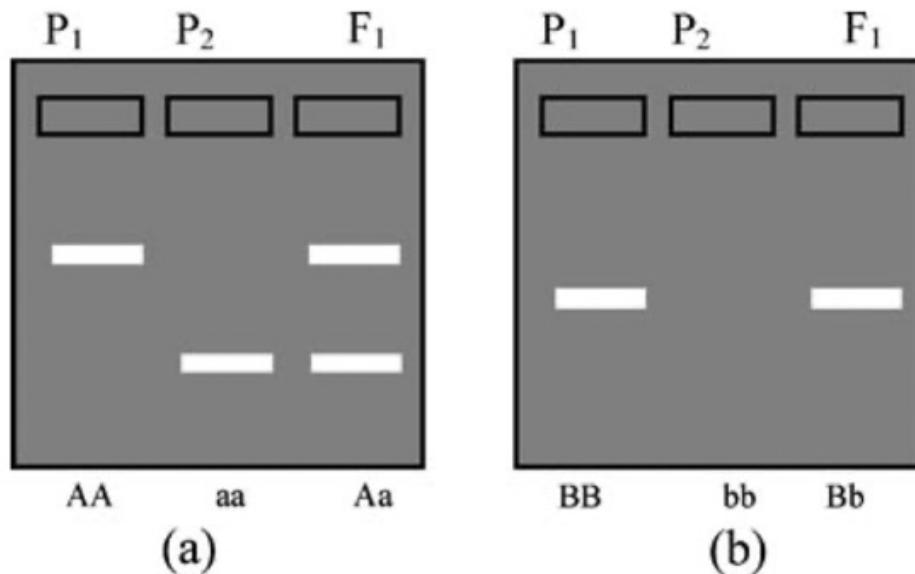


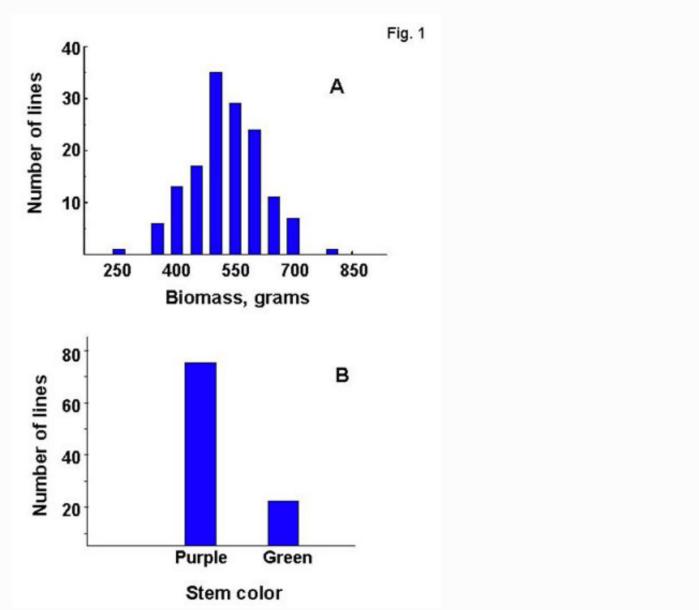
Figure 2. Comparison between (a) codominant and (b) dominant markers. Codominant markers can clearly discriminate between homozygotes and heterozygotes whereas dominant markers do not. Genotypes at two marker loci (A and B) are indicated below the gel diagrams.

Collard et al. 2005 *Euphytica* 142:169

Quantitative trait loci (QTL)

Introduction

Many traits important to crop production, such as yield potential, end-use quality characteristics, and stress tolerance, are quantitatively inherited, i.e., they are controlled by many genes acting together to produce the desired plant type. These quantitative traits are characterized by continuous variation, in contrast to qualitative traits, which show discrete variation and are controlled by one or two major genes (Fig. 1). Because of the often subtle differences among individuals for quantitative traits, they are evaluated by precise measurement rather than by classification.

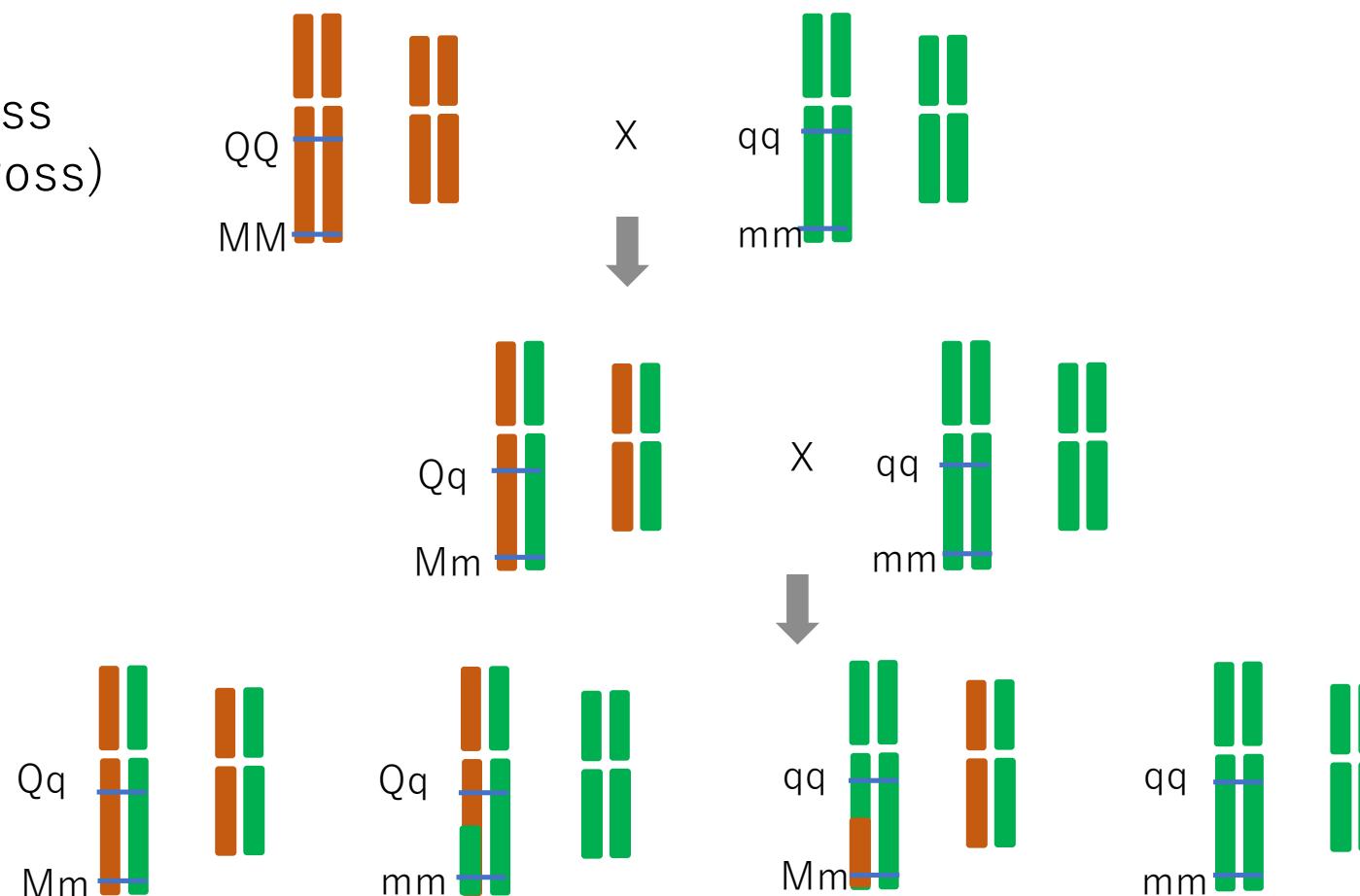


Yield
Plant height
Oil content
etc.

Source: <https://passel2.unl.edu/view/lesson/f1e5f56fc023/3>

QTL mapping

Test cross
(Back cross)



Simple ANOVA for QTL detection

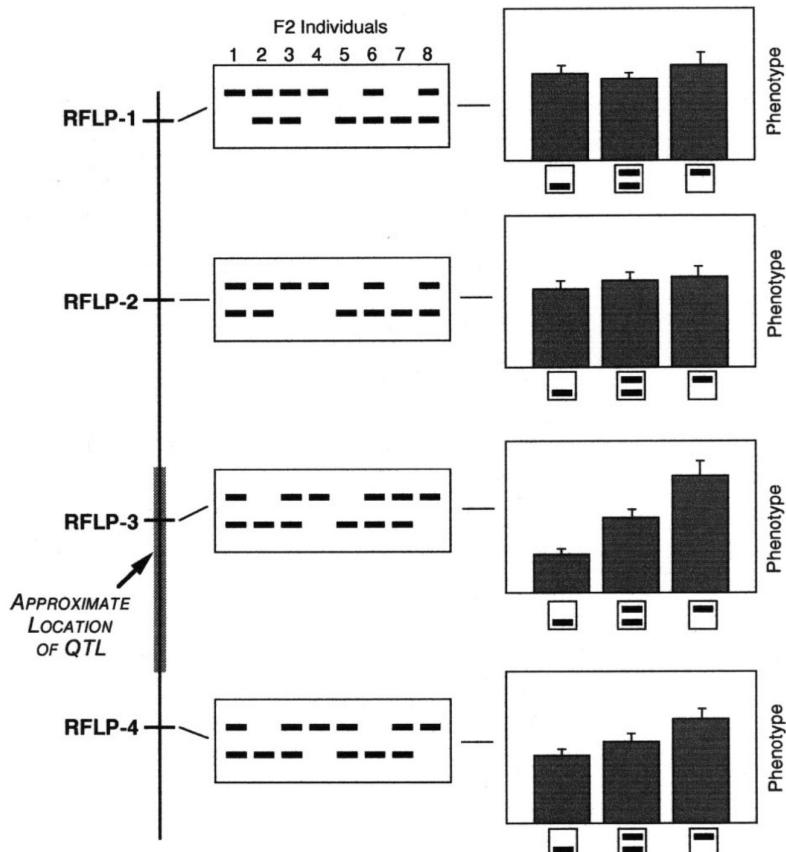
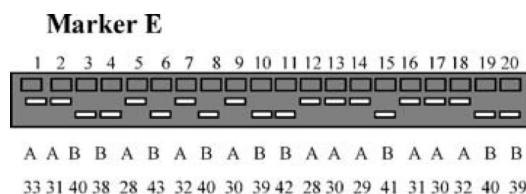


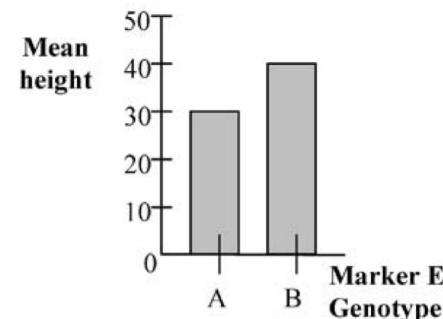
Figure 1 Conceptual basis of QTL mapping in an F₂ population. DNA markers throughout the

Young 1996
QTL mapping and
Quantitative disease resistance in plants.
Annu. Rev. Phytopathol. 34:479

Trait-Marker association

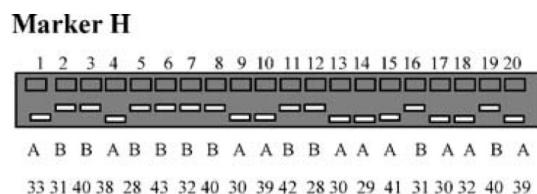


Group 1 (progeny with fragment A) Group 2 (progeny with fragment B)

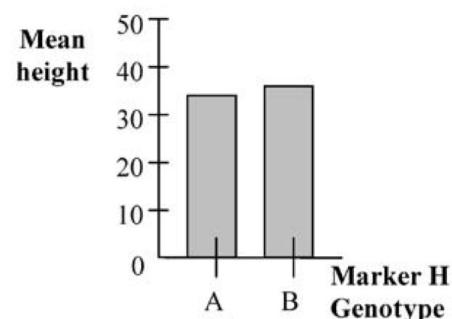


P value <0.0001 = significant

Conclusion: marker is linked to a QTL



Group 1 (progeny with fragment A) Group 2 (progeny with fragment B)



P value 0.5701 = not significant

Conclusion: marker is unlinked to a QTL

Table 3. Single-marker analysis of markers associated with QTLs using QGene (Nelson, 1997)

Marker	Chromosome or linkage group	P value	R ²
E	2	<0.0001	91
F	2	0.0001	58
G	2	0.0230	26
H	2	0.5701	2

LOD: logarithm of odd

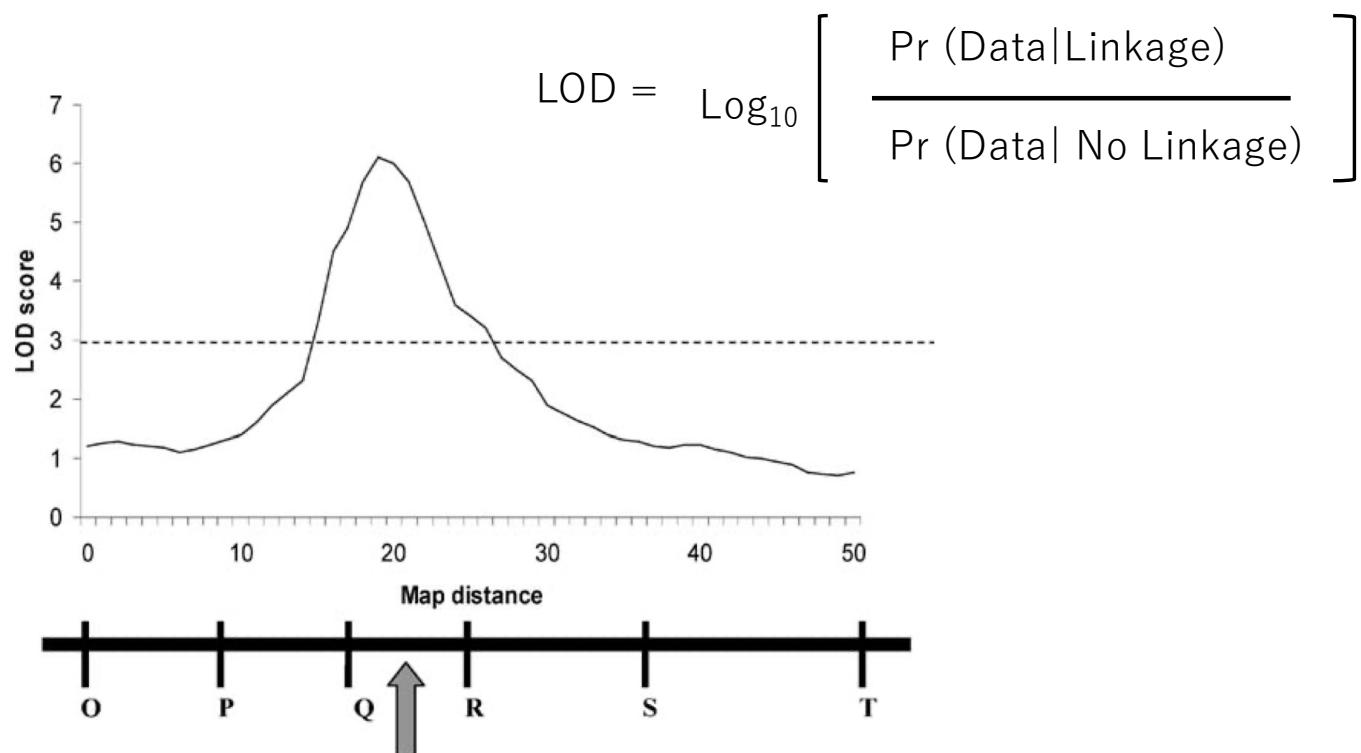
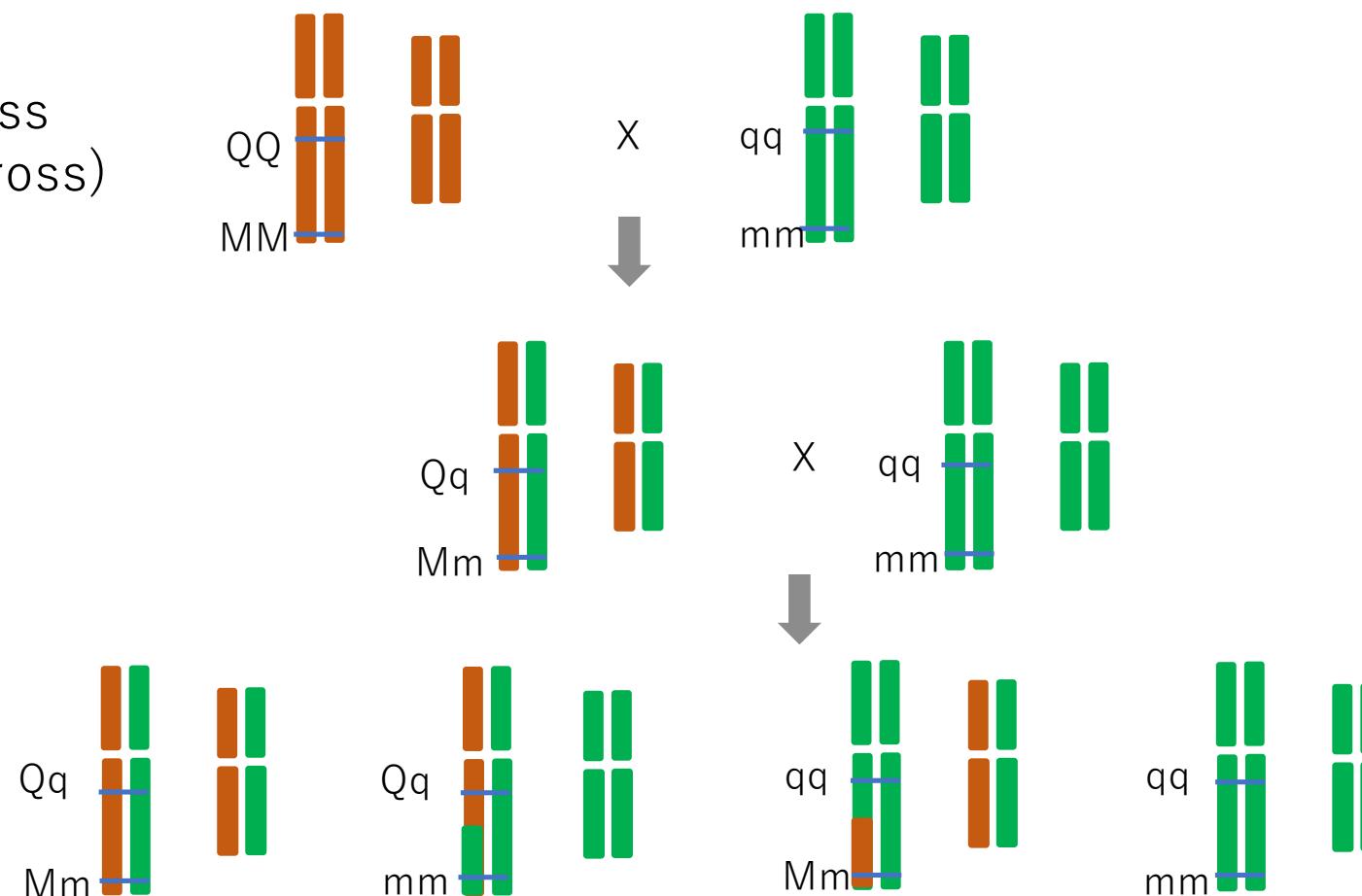


Figure 10. Hypothetical output showing a LOD profile for chromosome 4. The dotted line represents the significance threshold determined by permutation tests. The output indicates that the most likely position for the QTL is near marker Q (indicated by an arrow). The best flanking markers for this QTL would be Q and R.

Collard et al. 2005 Euphytica 142:169

QTL mapping

Test cross
(Back cross)



QTL-mapping

Maximum Likelihood Estimate (MLE) of linkage Lander and Botstein, 1989

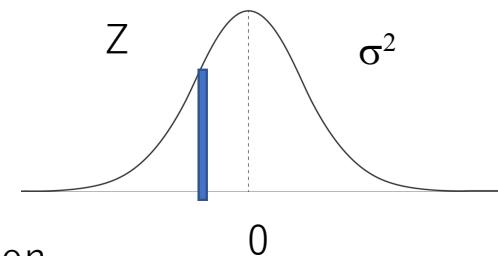
$$P_i = M + \alpha g_i + \epsilon$$

P_i : phenotypic value

M : mean value

α : effect of allele replacement

$g_i = 0$ when mm , $g_i = 1$ when Mm



$$L(M, \alpha, \sigma^2) = \prod_i z(P_i - (M + \alpha g_i), \sigma^2)$$

Where $z(x, \sigma^2)$ is the probability density of the normal distribution with mean = 0 and variance = σ^2

LOD: Logarithm of Odd Score

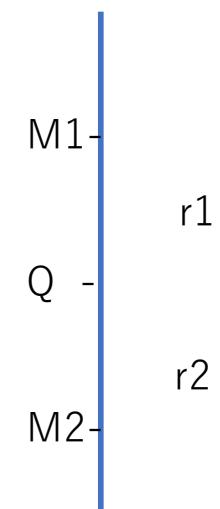
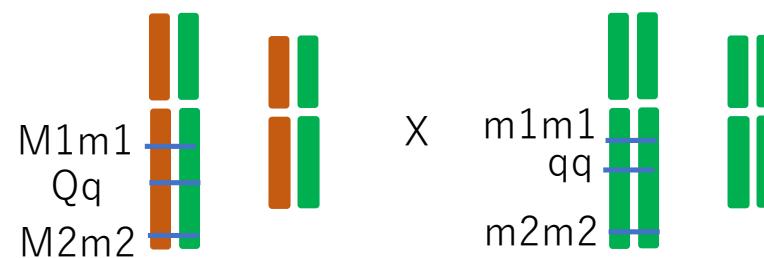
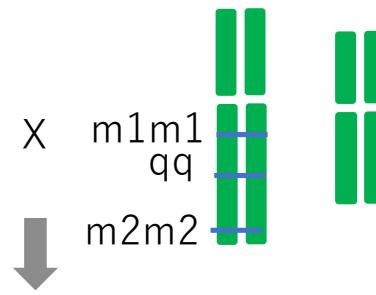
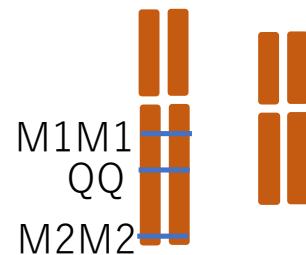
$$\text{LOD} = \frac{\log_{10} [L(M, \alpha, \sigma^2) \text{ with QTL}]}{\log_{10} [L(M, \alpha, \sigma^2) \text{ without QTL}]} = \frac{\log_{10} L(M, \alpha, \sigma^2)}{\log_{10} L(M, 0, \sigma^2)}$$

Interval Mapping of QTL

Simple Interval Mapping (SIM)
Composite Interval Mapping (CIM)

QTL mapping: Simple Interval Mapping (SIM)

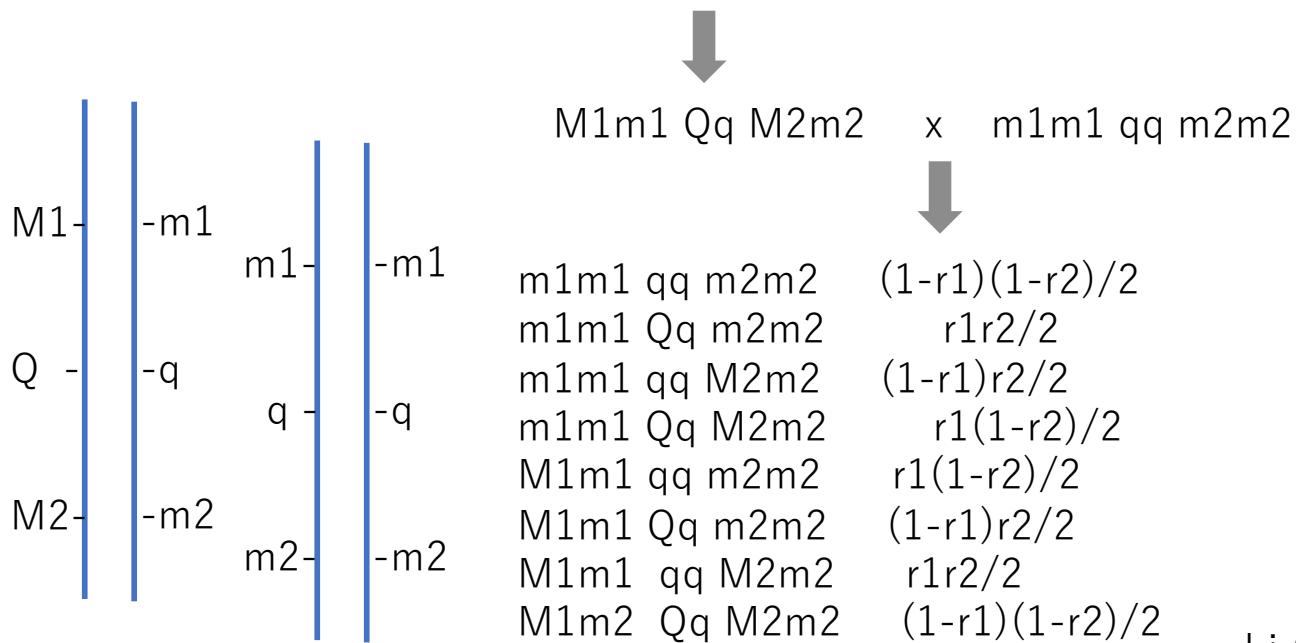
Test cross
(Back cross)



QTL mapping: Simple Interval Mapping (SIM)

M1M1 QQ M2M2 x m1m1 qq m2m2

Lander and Botstein, 1989



Genotype qq Qq
Effect 0 α

$$L_i(x) = z(P_i - (M + \alpha x), \sigma^2)$$

$$L(M, \alpha, \sigma^2) = \prod_i [G_i(0)L_i(0) + G_i(1)L_i(1)]$$

$$\text{LOD} = \frac{\log_{10} [L(M, \alpha, \sigma^2) \text{ with QTL}]}{\log_{10} [L(M, \alpha, \sigma^2) \text{ without QTL}]}$$

$G_i(x)$ is the probability that the genotype for the QTL is $x = 0$ for qq and $x=1$ for Qq conditioned to the markers observed in the individual and their frequencies

QTL mapping software

R/qtl: A QTL mapping environment

Software for mapping quantitative trait loci in experimental crosses

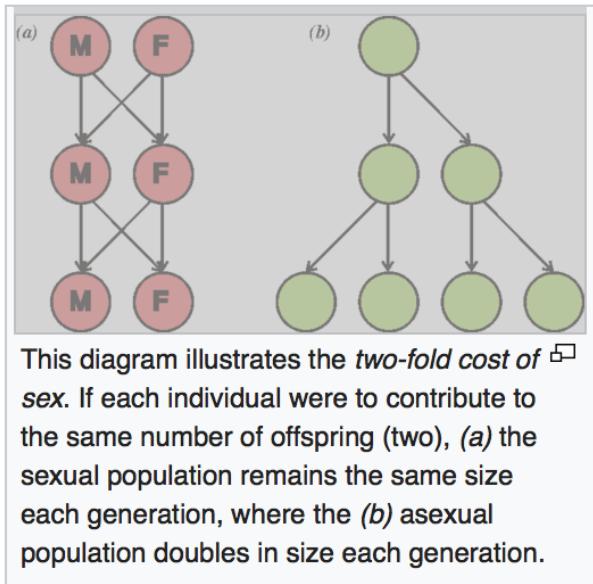
Current version: 1.52 (2022-07-09)

QTL Cartographer

is a suite of programs to map quantitative traits using a map of molecular markers. The programs are available via an anonymous ftp [server](#). See the [README](#) for more information. You will also want a copy of Gnuplot to display plots made by QTL Cartographer. Gnuplot is freely available on the web. Do a search to find the latest version for your operating system.

Why recombination matters?

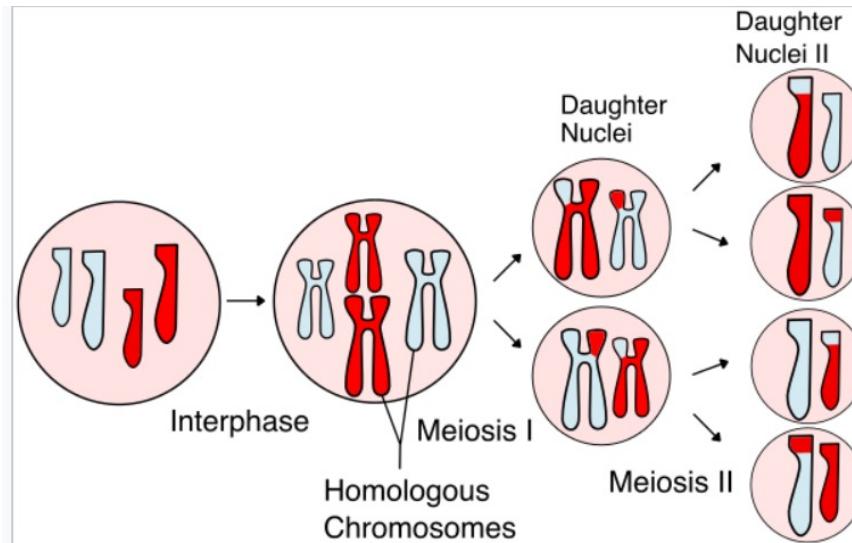
Two-fold cost of sex



- Fisher Muller hypothesis
- Red Queen hypothesis
- Hill-Robertson effect

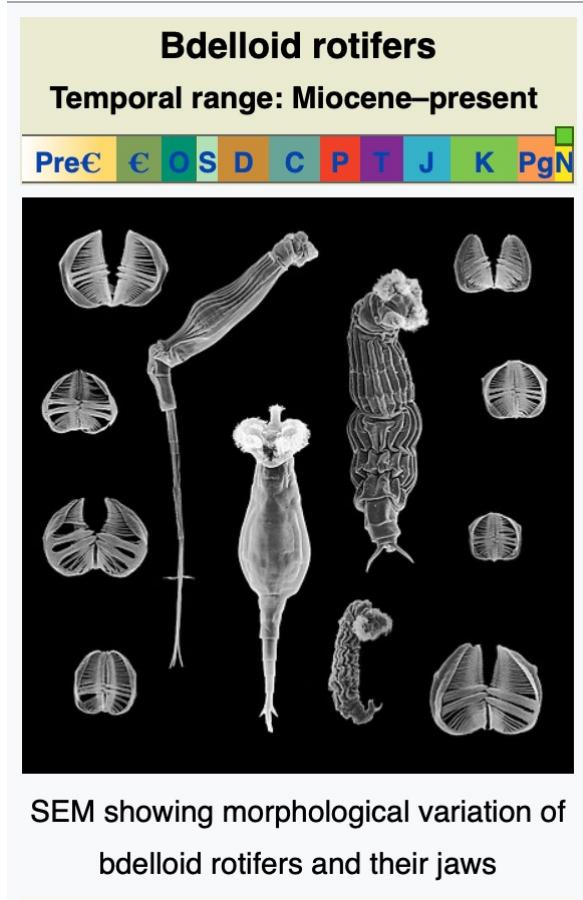
Maynard-Smith, 1971

Meiosis (Wikipedia)



In meiosis, the chromosome or chromosomes duplicate (during [interphase](#)) and [homologous chromosomes](#) exchange genetic information ([chromosomal crossover](#)) during the first division, called meiosis I. The daughter cells divide again in meiosis II, splitting up [sister chromatids](#) to form haploid [gametes](#). Two gametes fuse during [fertilization](#), forming a diploid cell with a complete set of paired chromosomes.

Asexual species are rare: an example of bdelloid rotifers



Reported to propagate by sexual reproduction (parthenogenesis) for many millions of years

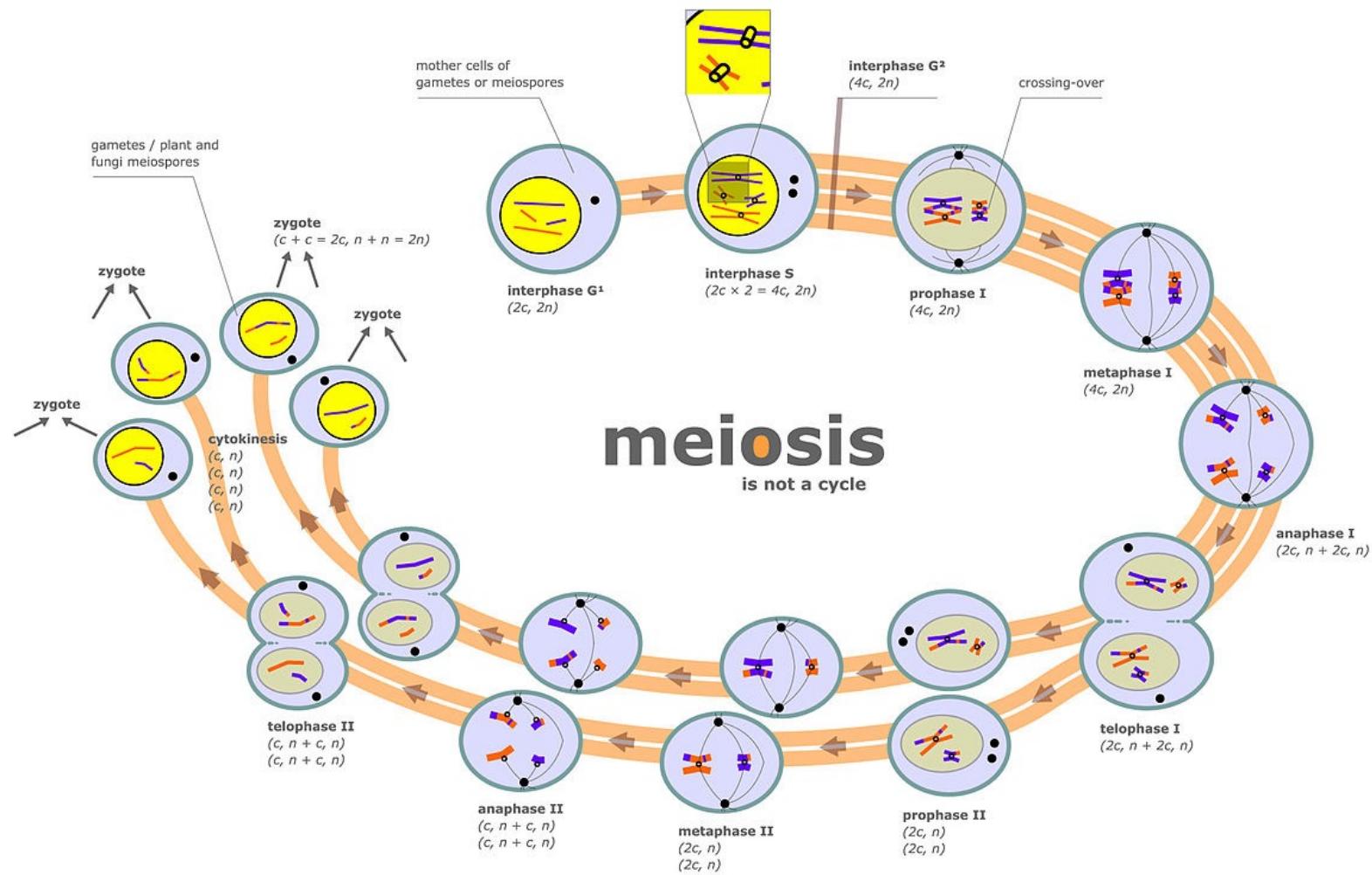
Bdelloidea

From Wikipedia, the free encyclopedia

For the mite superfamily, see [Bdelloidea \(mite\)](#).

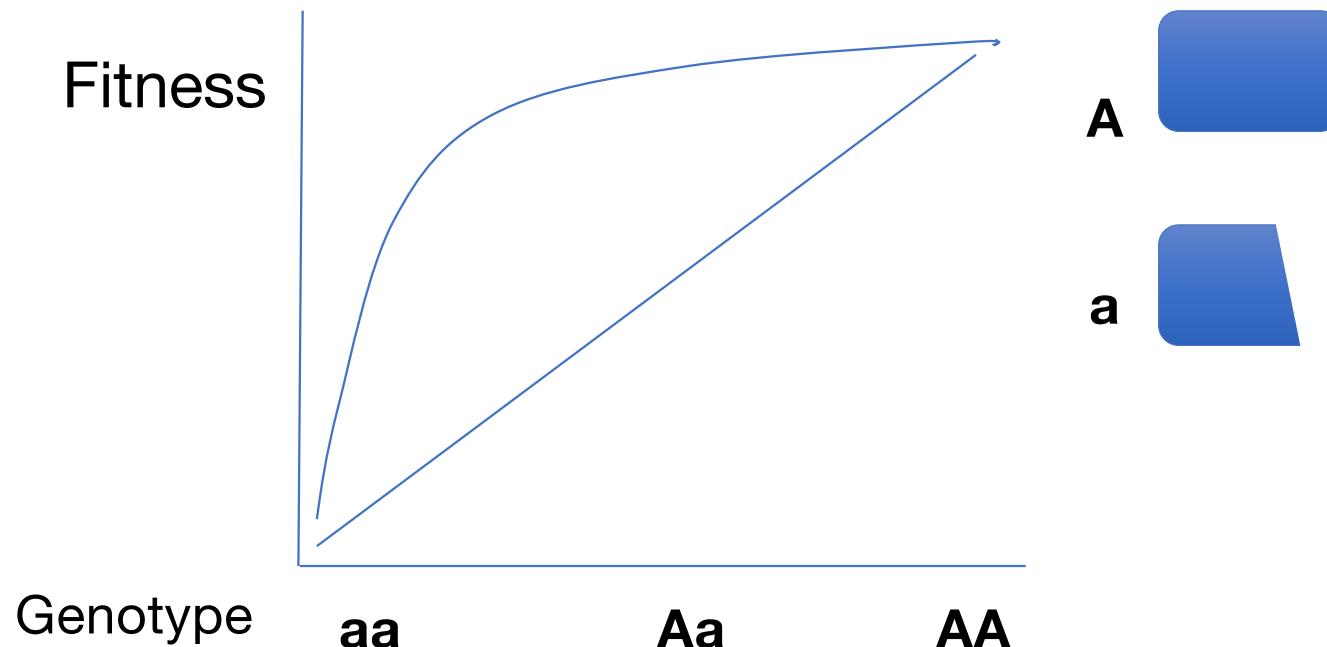
Bdelloidea /bdəlōdēə/ (Greek βδέλλα, *bdella*, "leech") is a class of rotifers found in freshwater habitats all over the world. There are over 450 described species of bdelloid rotifers (or 'bdelloids'),^[1] distinguished from each other mainly on the basis of morphology.^[2] The main characteristics that distinguish bdelloids from related groups of rotifers are exclusively parthenogenetic reproduction and the ability to survive in dry, harsh environments by entering a state of desiccation-induced dormancy (anhydrobiosis) at any life stage.^[3] They are often referred to as "ancient asexuals" due to their unique asexual history that spans back to over 25 million years ago through fossil evidence.^[4] Bdelloid rotifers are microscopic organisms, typically between 150 and 700 µm in length.^[3] Most are slightly too small to be seen with

減数分裂(Meiosis)の模式図



Source: https://en.wikipedia.org/wiki/Chromosome_segregation

Inbreeding depression hypothesis



Frequency of A: p , frequency of a: q , $p+q=1$

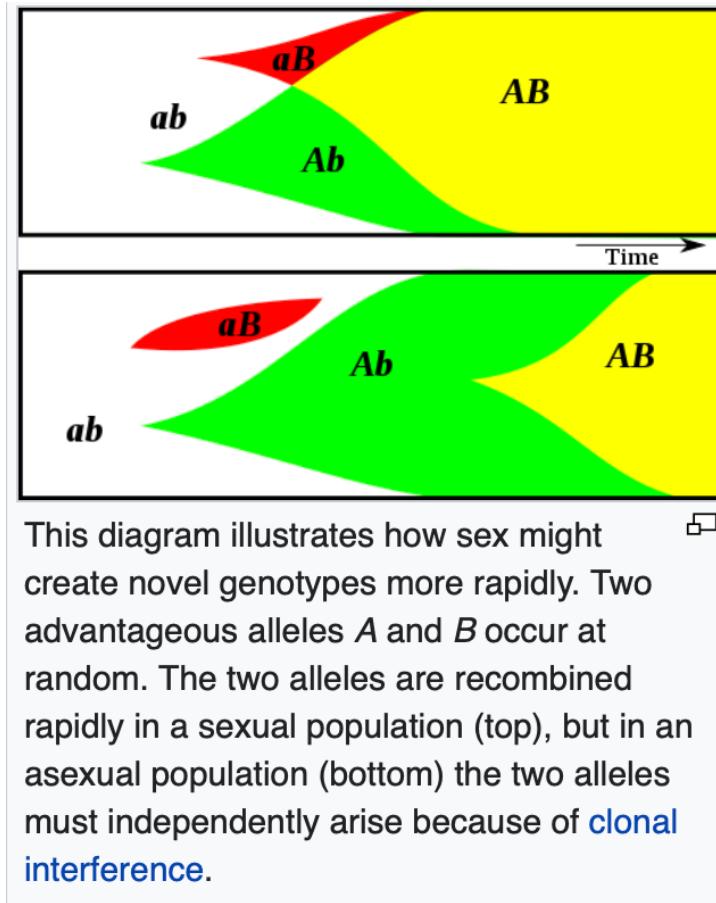
Outcrossing species: $P(aa) = q \times q$

Inbreeding species: $P(aa) = q$

Since $q < 1$, $q \times q < q$

Production of new genotypes

Fisher-Muller hypothesis



Sexual reproduction

Asexual reproduction

Source: Wikipedia

Red Queen hypothesis

REPORTS

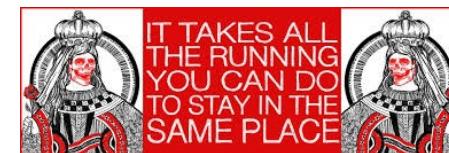
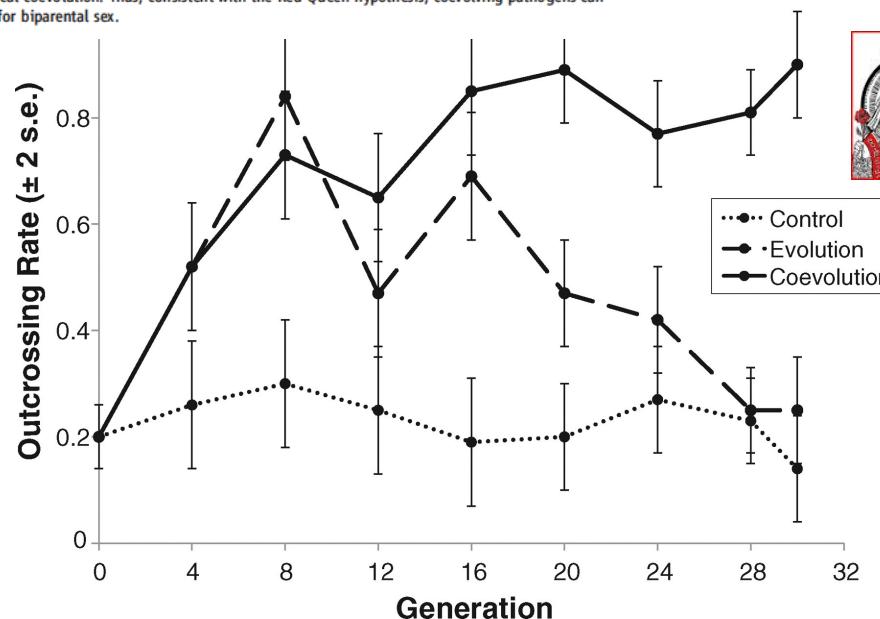
Running with the Red Queen: Host-Parasite Coevolution Selects for Biparental Sex

Levi T. Morran,* Olivia G. Schmidt, Ian A. Gelarden, Raymond C. Parrish II, Curtis M. Lively

Most organisms reproduce through outcrossing, even though it comes with substantial costs. The Red Queen hypothesis proposes that selection from coevolving pathogens facilitates the persistence of outcrossing despite these costs. We used experimental coevolution to test the Red Queen hypothesis and found that coevolution with a bacterial pathogen (*Serratia marcescens*) resulted in significantly more outcrossing in mixed mating experimental populations of the nematode *Caenorhabditis elegans*. Furthermore, we found that coevolution with the pathogen rapidly drove obligately selfing populations to extinction, whereas outcrossing populations persisted through reciprocal coevolution. Thus, consistent with the Red Queen hypothesis, coevolving pathogens can select for biparental sex.



Hamilton's
hypothesis



Levi T. Morran et al. Science 2011;333:216-218

Published by AAAS

Science
AAAS

Sexual reproduction as an adaptation to resist parasites (A Review)

(evolution/recombination/population genetics/evolutionary genetics/disease resistance)

WILLIAM D. HAMILTON*, ROBERT AXELROD^{†‡}, AND REIKO TANESE^{§¶}

*Department of Zoology, Oxford University, South Parks Road, Oxford, OX1 3PS, United Kingdom; and [†]Institute of Public Policy Studies and [§]Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI 48109

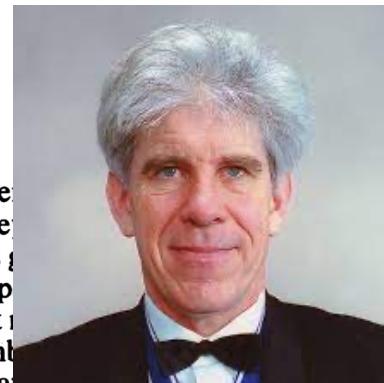
Contributed by Robert Axelrod, December 26, 1989

ABSTRACT Darwinian theory has yet to explain adequately the fact of sex. If males provide little or no aid to offspring, a high (up to 2-fold) extra average fitness has to emerge as a property of a sexual parentage if sex is to be stable. The advantage must presumably come from recombination but has been hard to identify. It may well lie in the necessity to recombine defenses to defeat numerous parasites. A model demonstrating this works best for contesting hosts whose defense polymorphisms are constrained to low mutation rates. A review of the literature shows that the predictions of parasite coevolution fit well with the known ecology of sex. Moreover, parasite coevolution is superior to previous models of the evolution of sex by supporting the stability of sex under the following challenging conditions: very low fecundity, realistic patterns of genotype fitness and changing environment, and frequent mutation to parthenogenesis, even while sex pays the full 2-fold cost.

Parasites and Sex

Parasites are ubiquitous. They are found in all organisms, from small to have parasites. The parasite population is well prepared to their hosts, and this gives them a selective advantage over host populations. Thus antiparasitic resistance is a common phenomenon, leading to constant obsolescence. To resist parasites, host populations continually change gene combinations. This contradicts the assumption of the mutation theory (12, 13), that most species needs to preserve not one ideal genotype but rather an array. In the course of this preservation, selective changes in the midrange of gene frequency must be common. Because single and multiple heterozygosity is maximal for genes in the midrange, the effectiveness of events of recombination in uncoupling these changes is great, thus providing power to the model that we now describe.

We simulated a host population of 200 individuals that are either sexual hermaphroditic or else all-female and parthe-



Population geneticists

R. A. Fisher
(1890-1962)



J.B.S. Haldane
(1892-1964)



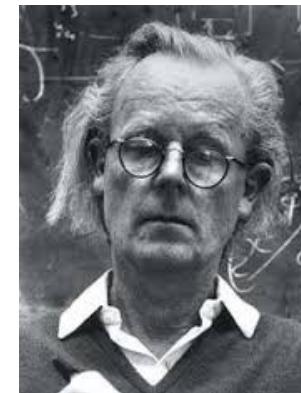
Sewell Wright
(1889-1988)



Motoo Kimura
(1924-1994)



Maynard Smith
(1920-2004)



Recommended books and papers:

Genetics

- Weir B. 1996. Genetic Data Analysis II (Sinauer)
Hartl D. 2018. Essential Genetics and Genomics, (Jones& Bartlett Learning)
Hahn MW. 2019. Molecular Population Genetics (Sinauer)
Caballero A. 2017. Quantitative Genetics (Cambridge)

Statistics and probability

- Feller W. 1991. An Introduction to Probability Theory and Its Applications, Volume 2, 2nd Edition
Dobson AJ. Barnett AG. 2018 An introduction to Generalized Linear Models, CRC press.
Kruschke JK. 2015. Doing Bayesian Data Analysis. Academic Press.

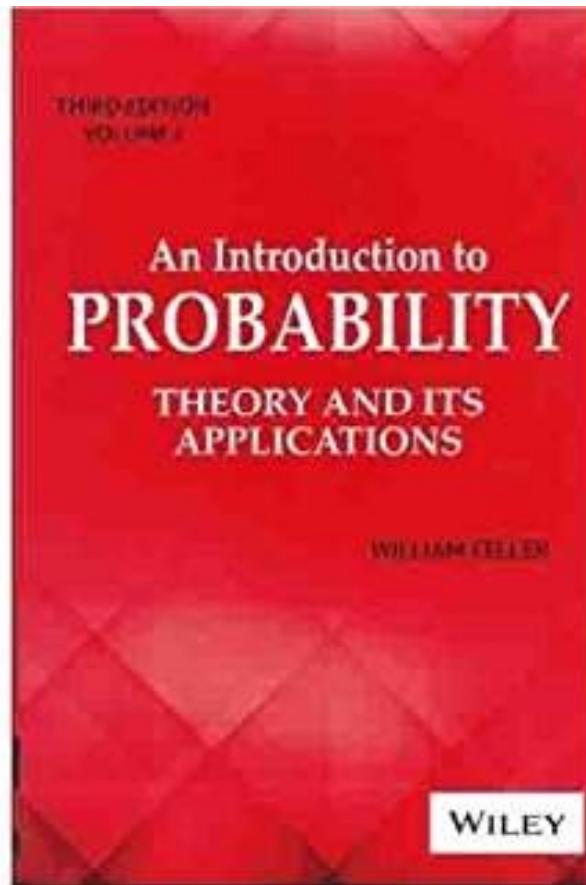
MutMap + QTL-seq

- Abe A et al. 2012. Genome sequencing reveals agronomically important loci in rice using MutMap. *Nature Biotechnol.* 20:174.
Fekih R. et al. 2013. MutMap+: Genetic mapping and mutant identification without crossing in rice. *PLoS One* 8(7): e68529
Takagi H. et al. 2013. MutMap-Gap: whole-genome resequencing of mutant F2 progeny bulk combined with de novo assembly of gap regions identifies the rice blast resistance gene Pi*i*. *New Phytol.* <https://doi.org/10.1111/nph.12369>
Takagi H. et al. 2013. QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *Plant J.* 74:174.
Sugihara Y. et al. 2022. High-performance pipeline for MutMap and QTL-seq. *Peer J* 10:e13179

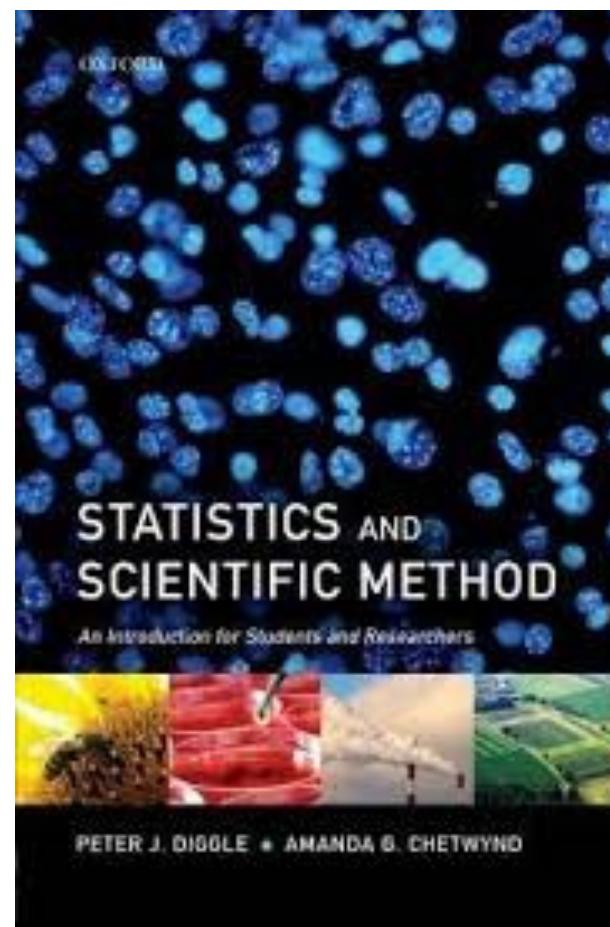
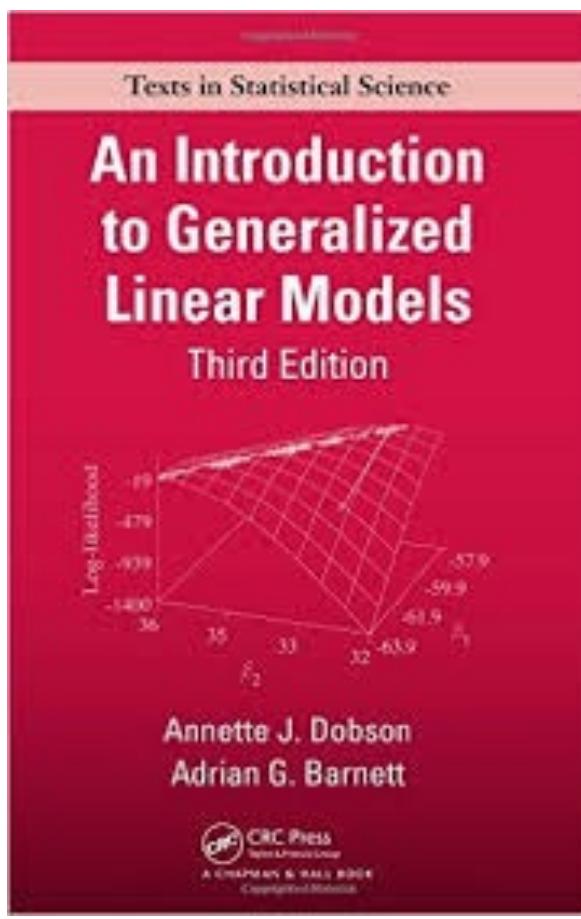
QTL mapping

- Lander ES, Botstein D 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185.

Introductory text book for probability



William Feller



Dobson+ Barnett

30 April: Mapping populations
 DNA sequencing

- Mapping population
- Introduction to DNA sequencing
- Next Generation Sequencing (NGS)
- Genome assembly
- Use of long read sequences

Mapping populations

1. Test cross population
2. F2 population
3. Recombinant Inbred Lines (RILs)
4. F1 population

Mapping populations

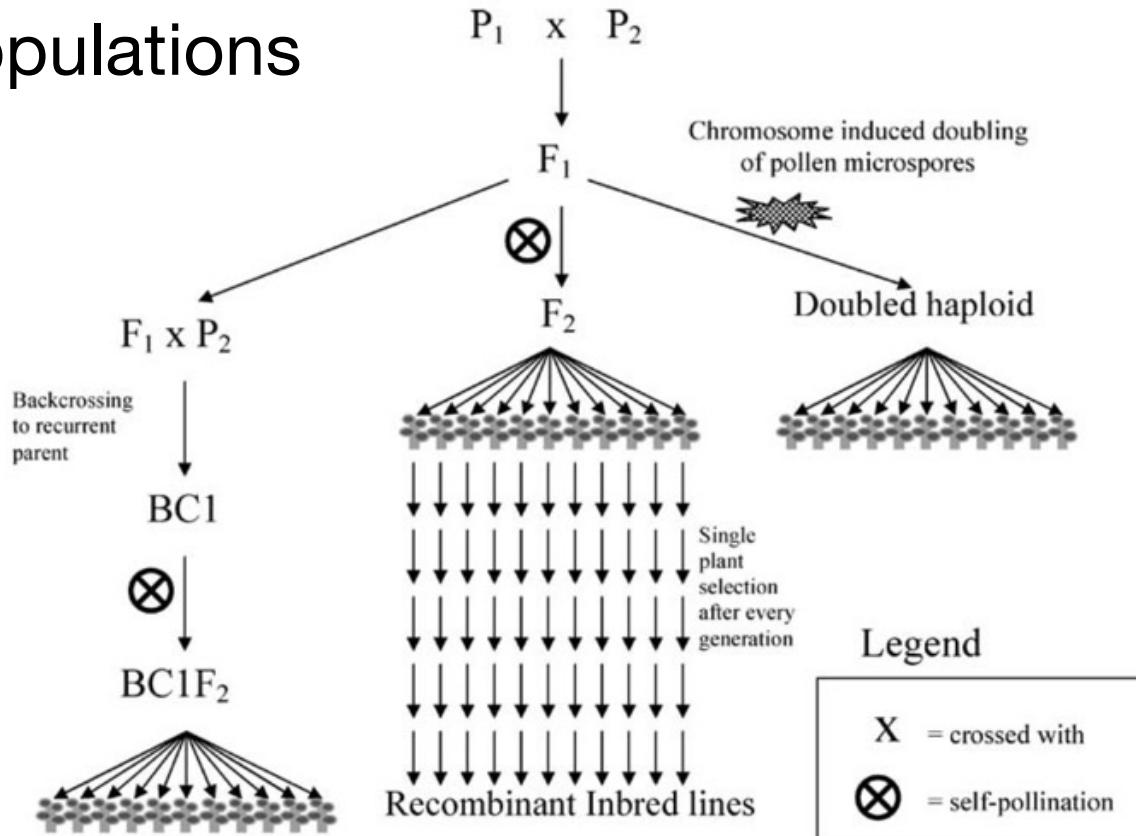
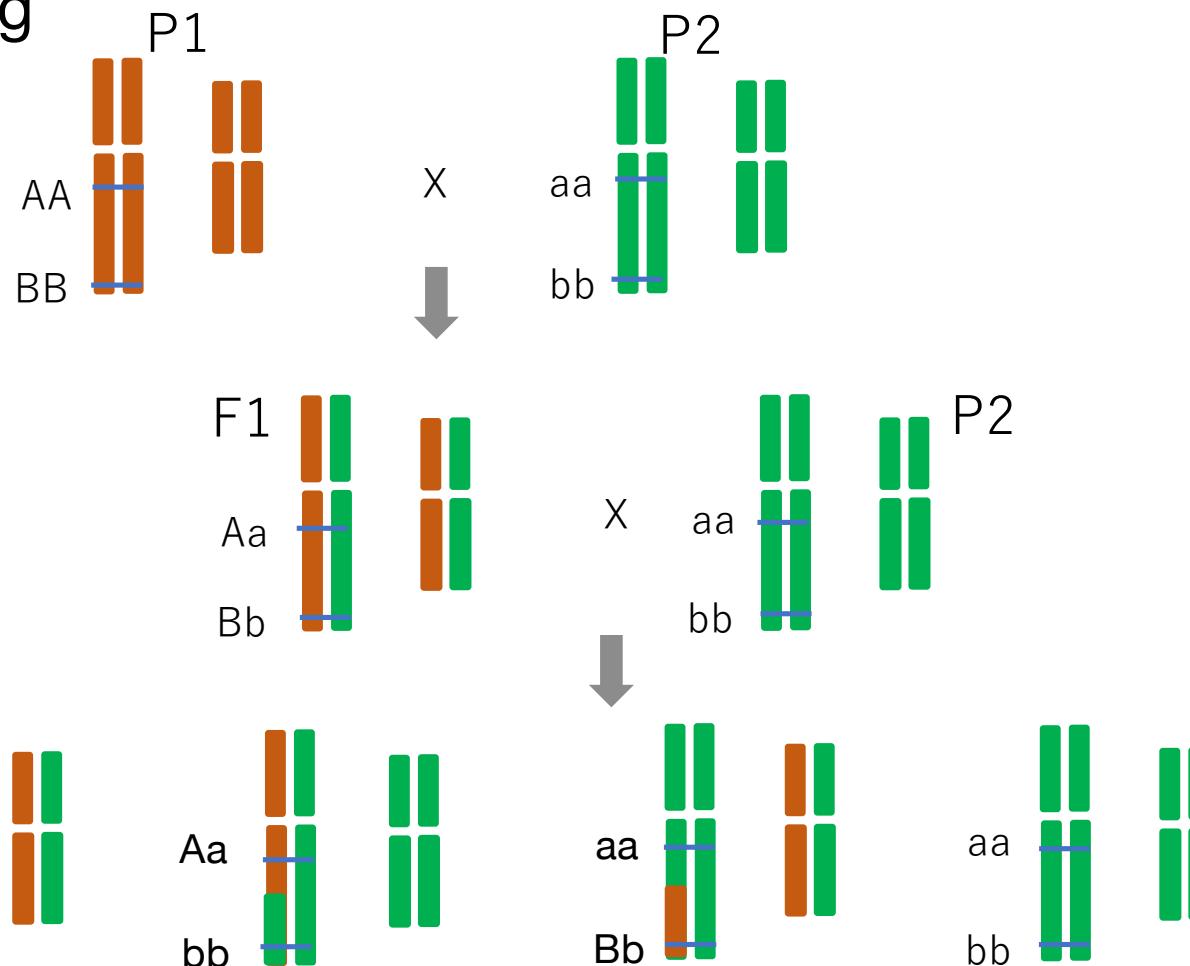


Figure 4. Diagram of main types of mapping populations for self-pollinating species.

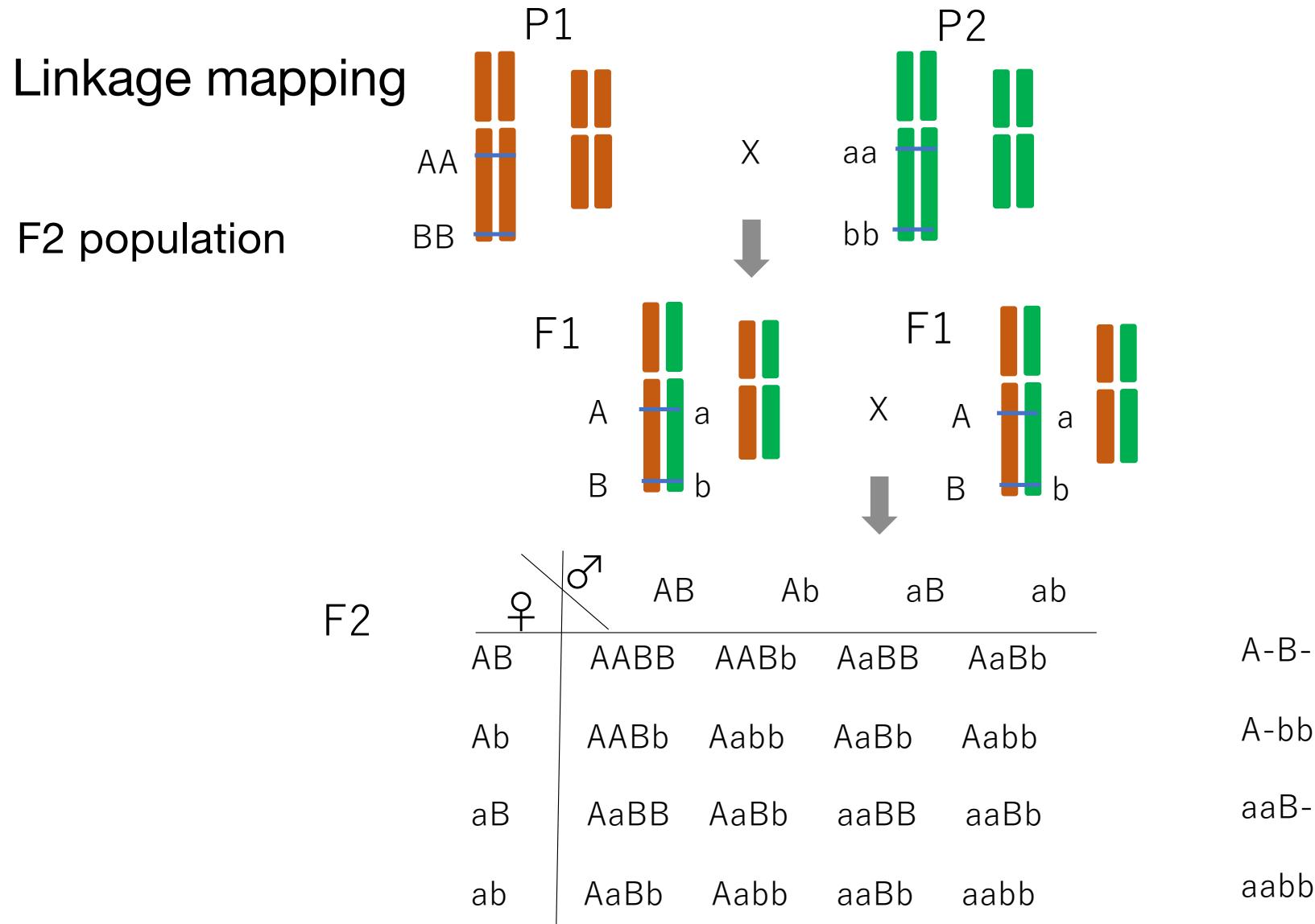
Collard et al. 2005 Euphytica 142:169

Linkage mapping

Test cross
(Back cross)



$$AaBb : Aabb : aaBb : aabb \rightarrow 1 : <1 : <1 : 1$$



Linkage mapping

Recombinant Inbred Lines
(RILs)

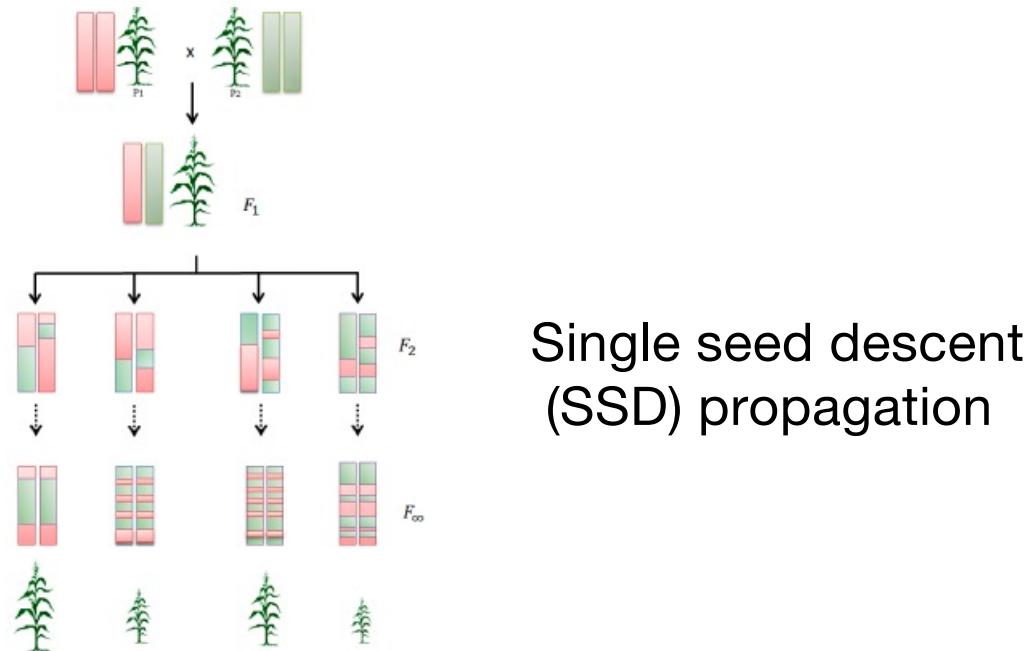


Figure 1: The production of recombinant inbred lines (RILs) by repeated selfing.

Source:

Detecting Epistatic Selection with Partially Observed Genotype Data Using Copula Graphical Models

October 2017

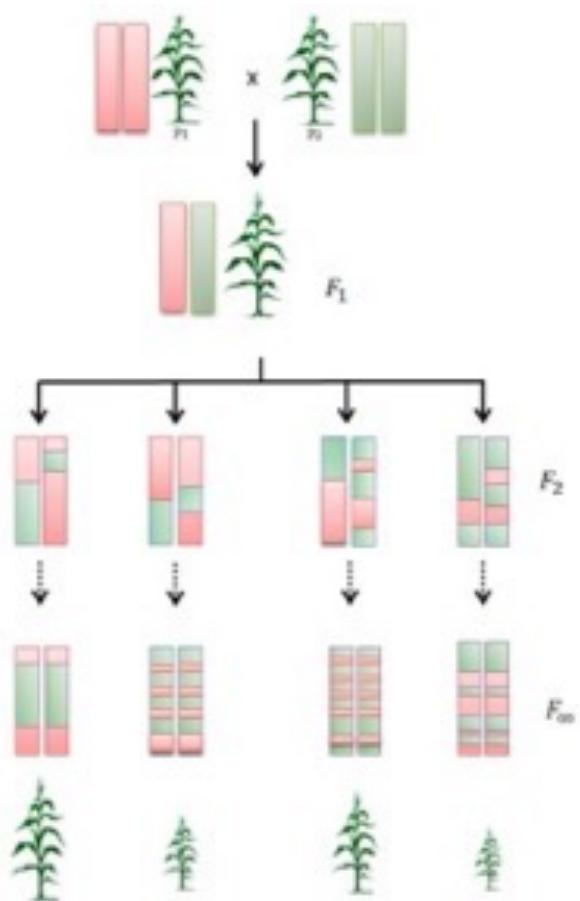
[Journal of the Royal Statistical Society Series C Applied Statistics](#) 68(1)

DOI: [10.1111/rssc.12287](https://doi.org/10.1111/rssc.12287)

License [CC BY-NC 4.0](#)

Linkage mapping

Recombinant Inbred Lines (RILs)



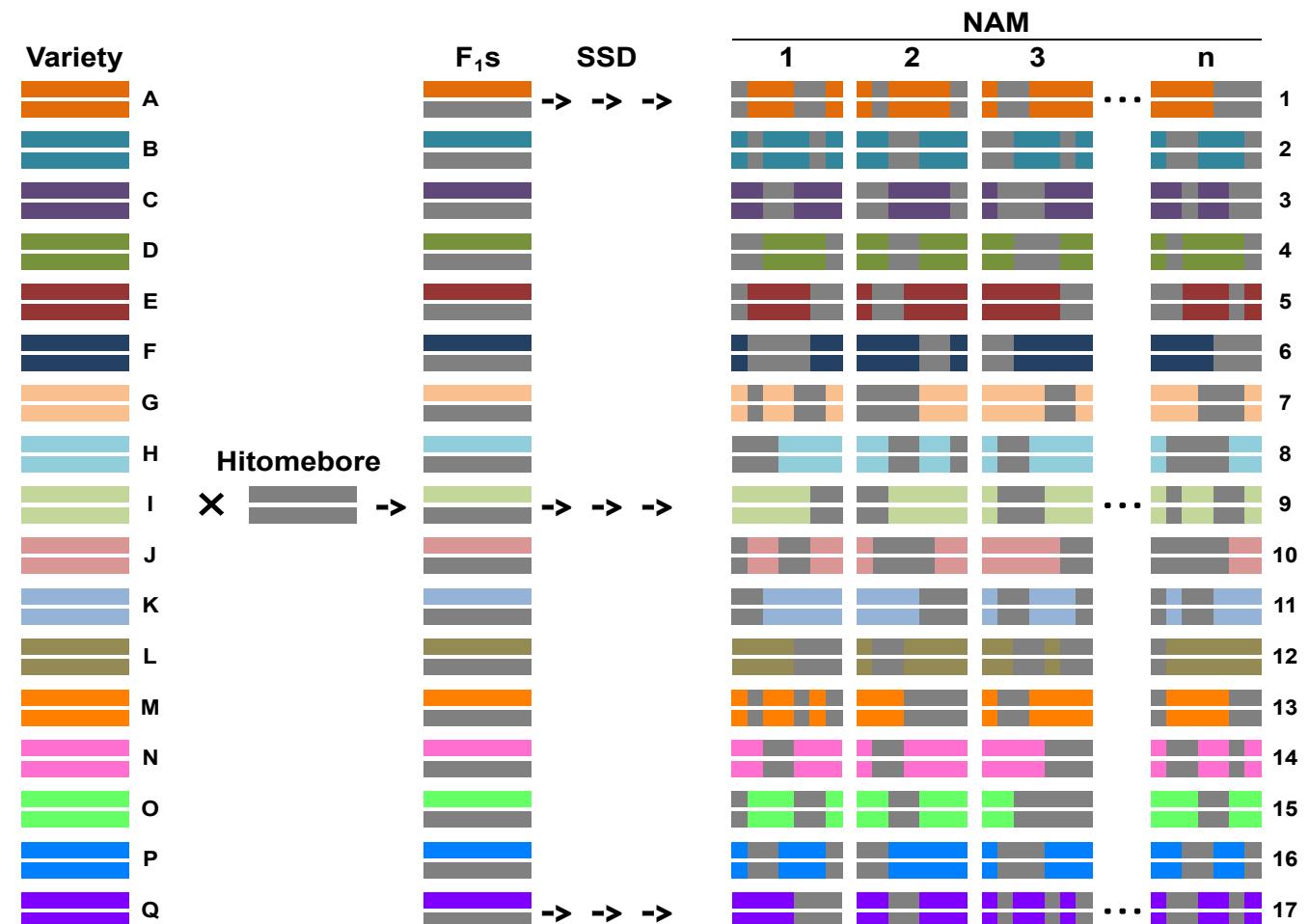
Advantages of use of RILS

- Pure lines with mosaic genomes
- Multiple individuals per genotype
- No effect of heterozygosity (heterosis)
- Genotyping cost-effective
- Doubled-haploid (DH)

Linkage mapping

Yu et al. 2008. Genetics 107: 539-551.

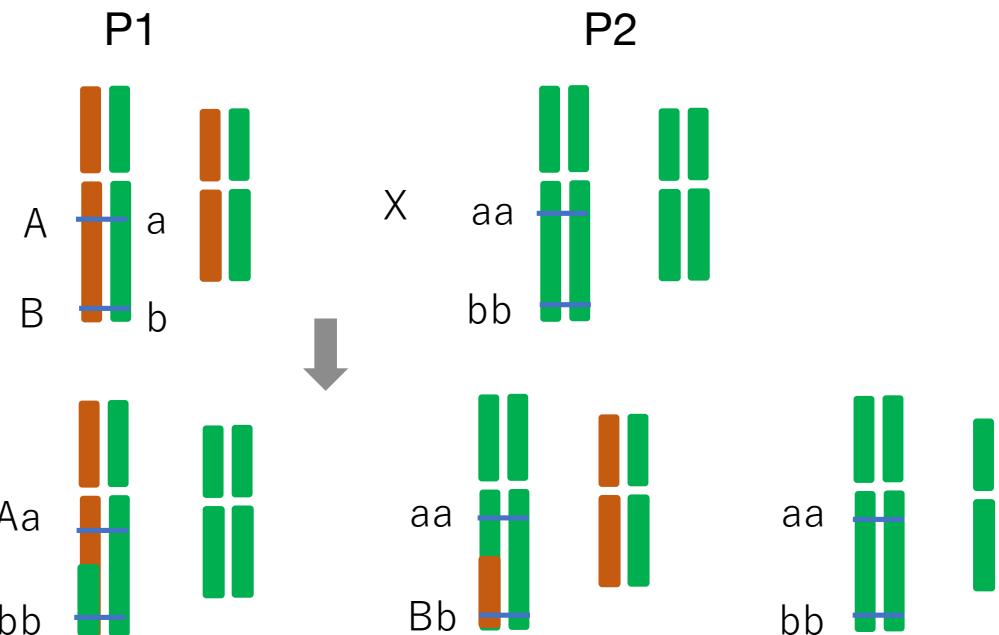
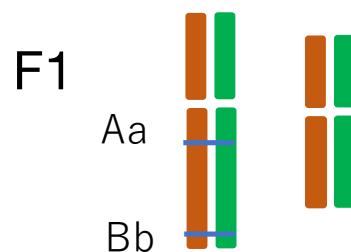
Nested Association Mapping
(NAM) population



Linkage mapping

F1 population

Pseudo testcross
mapping



Explain later an example of *Dioscorea* linkage mapping

Grattapaglia, D., and Sederoff, R. (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics*, 137.4:1121-1137.