Genetics
&
the Genotype-Phenotype relationship

Virginie Courtier-Orgogozo
Institut Jacques Monod, Paris

Evolution of Drosophila glue

soil

. grimshawi

rotten strawberry

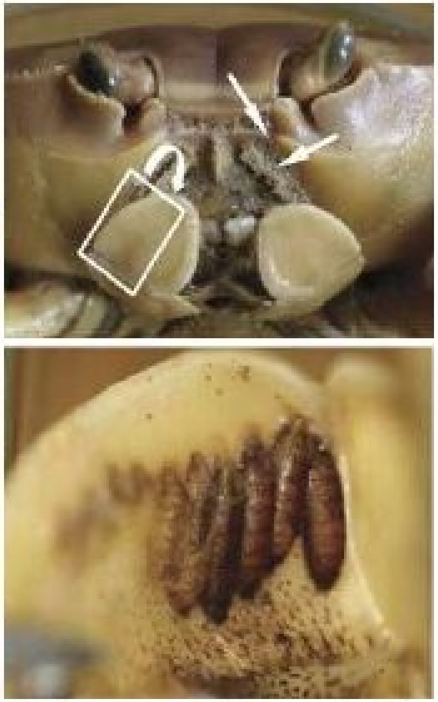
D. suzukii

grape

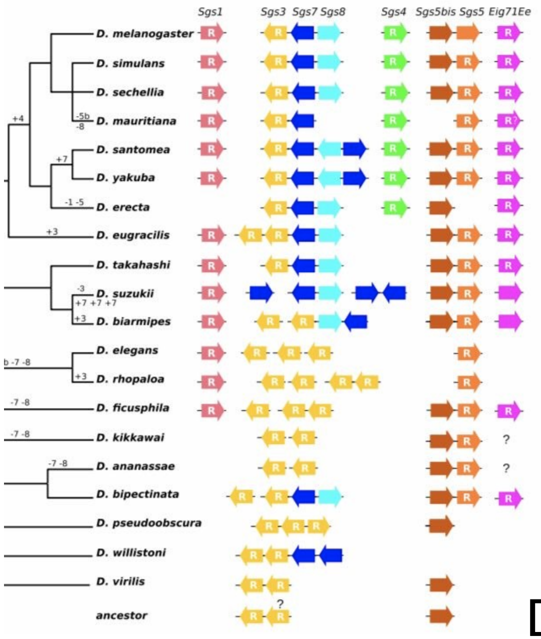
D. melanogaster

potato

D. repleta

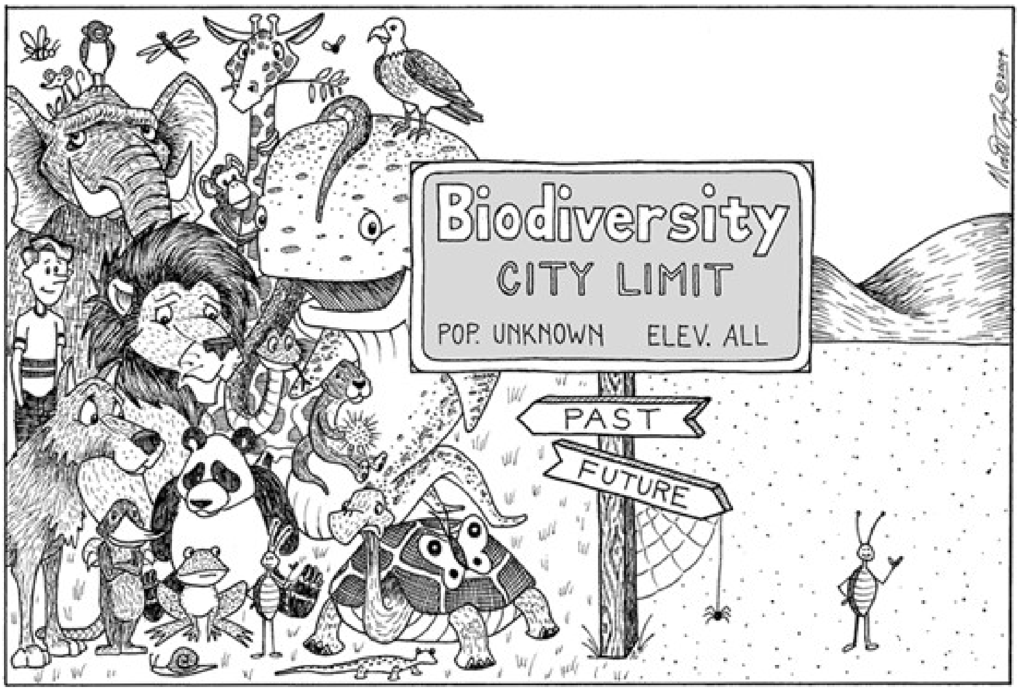


D. carcinophila

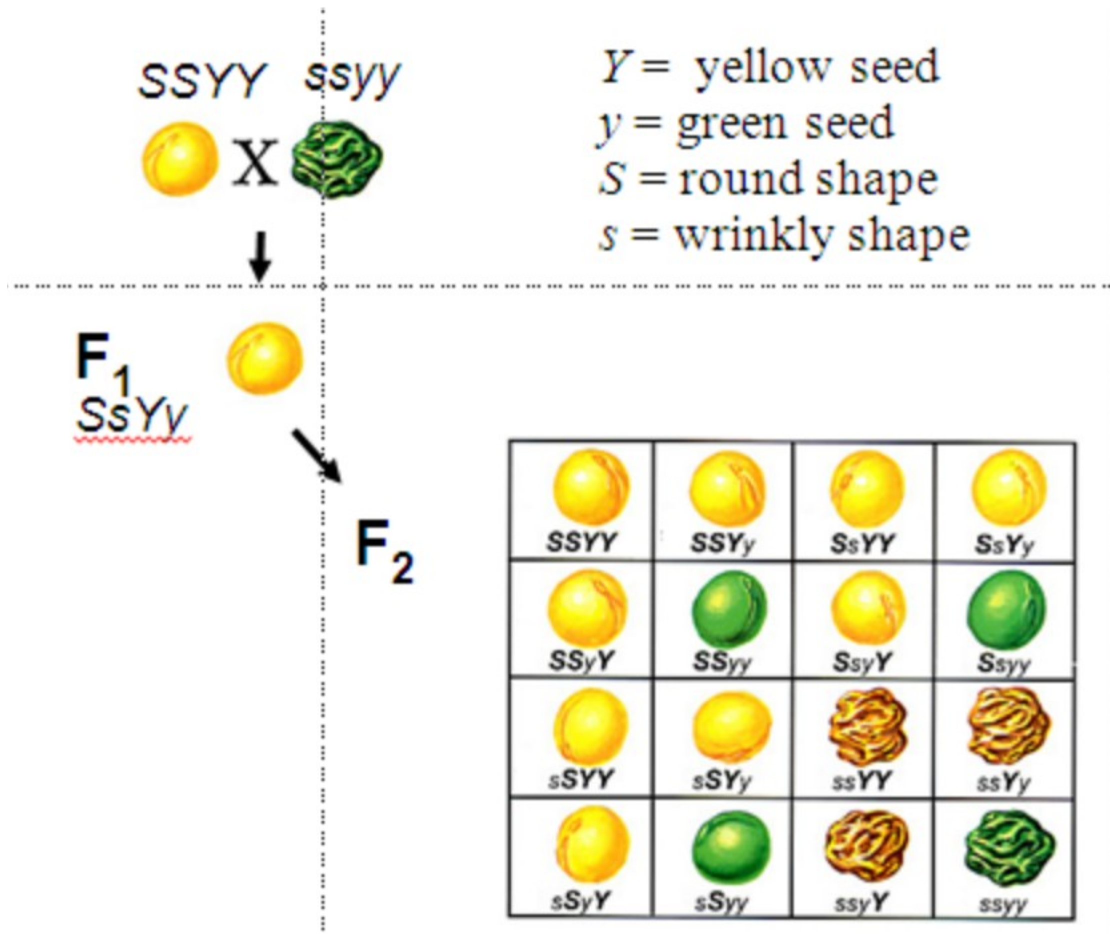


Da Lage et al. 2019 BMC Evol Biol

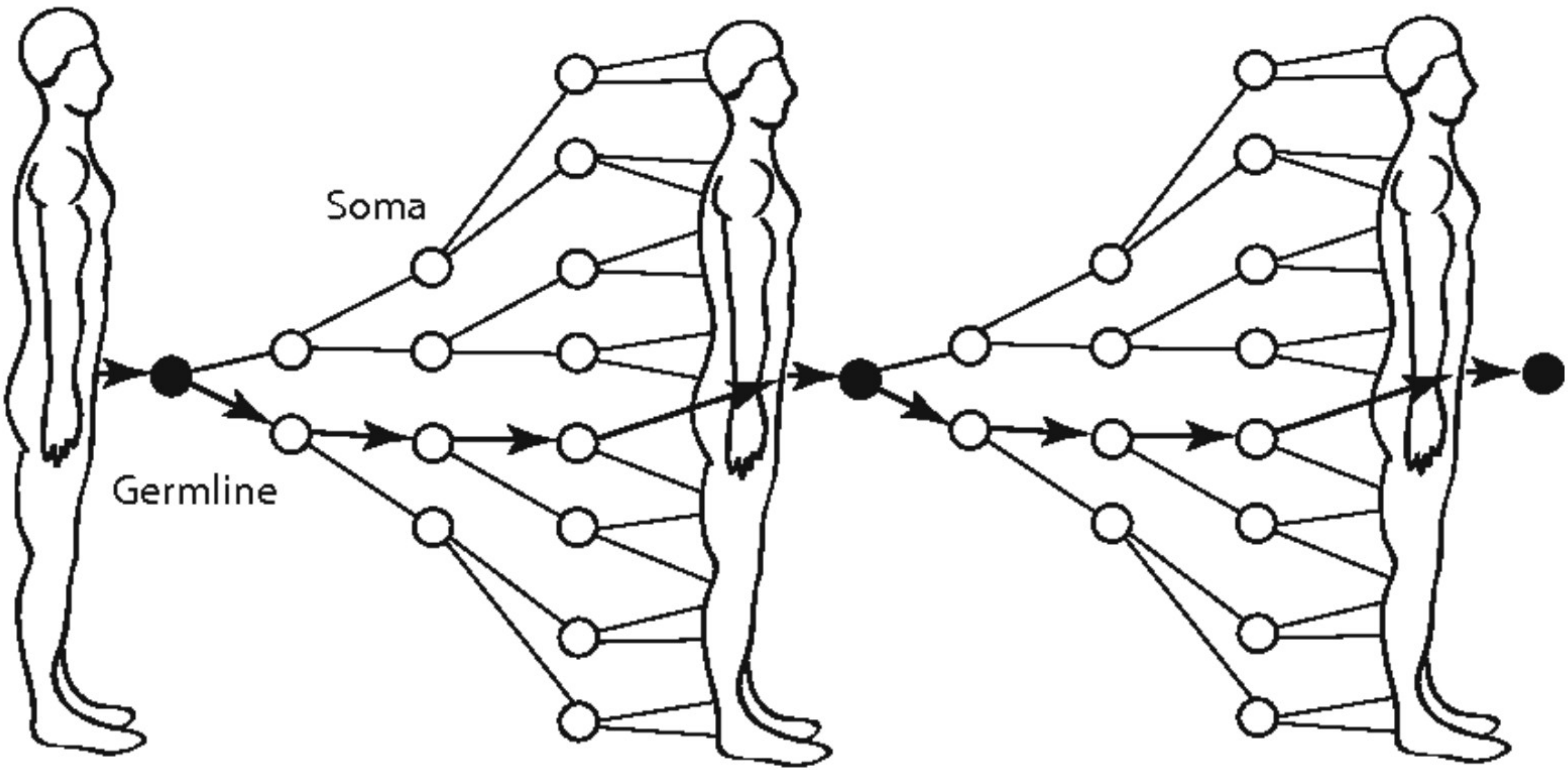
Goal of genetics: explain biological diversity and inheritance patterns



Mendel: half of the factors are transmitted (law of segregation, 1866)



August Weismann's germplast theory (1892)



Changes acquired during an organism's life cannot affect its offspring

The distinction between genotype and phenotype is the basis of genetics

“The view of natural inheritance as realized by an act of transmission, viz., the transmission of the parent's (or ancestor's) personal qualities to the progeny, is the most naive and oldest conception of heredity.”

“All "types" of organisms, distinguishable by direct inspection or only by finer methods of measuring or description, may be characterized as "**phenotypes**.”

“ A "**genotype**" is the sum of all the "genes" in a gamete or in a zygote.”

Johansen 1911

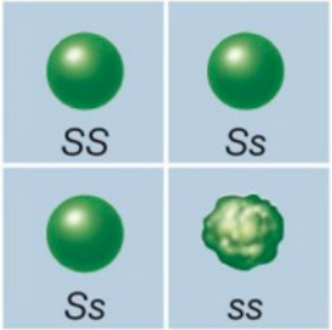
Phenotype = observable attributes of an individual

Genotype = inheritable genetic material
= DNA or RNA

How do genotypes map onto phenotypes ?

A very brief history of genetics

Transmission + *Phenotypic expression*



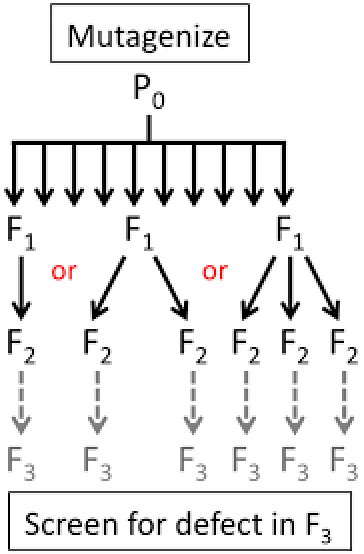
- Mendel 1860s
Controlled breeding, use of probabilities
Dominant/recessive/intermediate expression of traits
Random transmission of "factors"

- Cytology from 1880-90s Flemming, Boveri, Sutton
chromosomes as support of heredity, meiosis

- Classical genetics 1900-1950
distinction genotype-phenotype
segregation of characters & genes
genetic map, sex chromosomes
penetrance - expressivity

"Rediscovery" of Mendel's law
Extension to animals

- **Foundation of molecular biology 1940-1970**
  in part using bacteria and phage genetics
  DNA as the material support of heredity



- **Deciphering the role of the genes: 1970-**

Cellular, developmental, behavioral genetics: Screens
"High-throughput" versions 2000- (e.g. deletion libraries)
Reverse genetics

- **Association mapping on natural populations: 2000-**

Genetics of natural variation

- Evolutionary genetics   1900-

Biometricians versus Mendelians: continuous versus discrete
hereditary variation

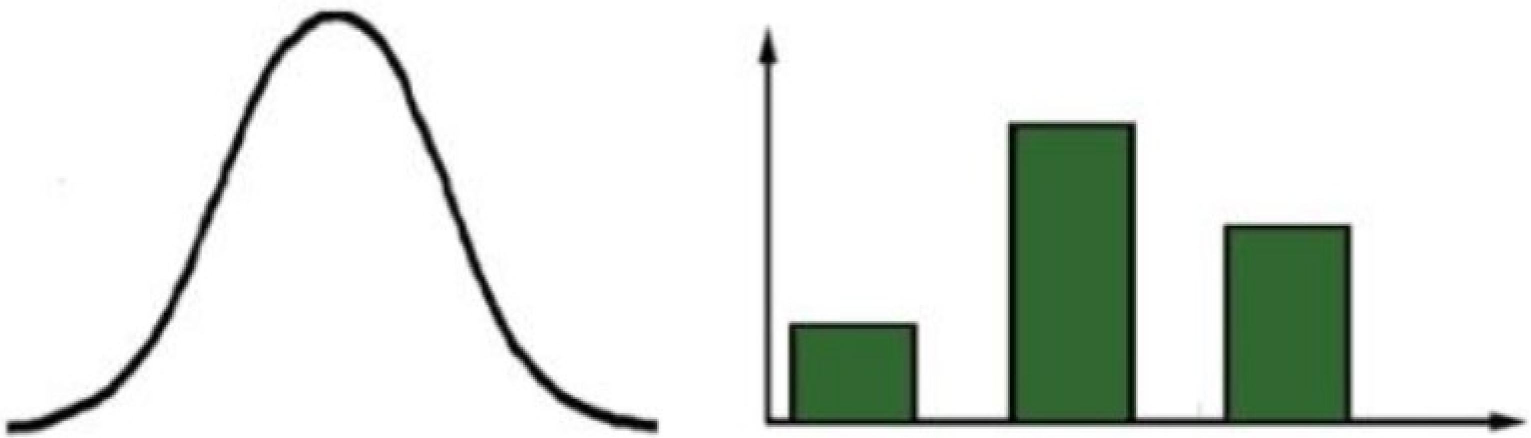
- Neo-darwinian synthesis 1930
mostly population genetics on allele frequencies, not phenotypes

- Human genetics

Now:
Bridging of "laboratory" and evolutionary genetics
Use of genomic data in model and "non-model" organisms
Role of the environment

Genetics of natural variation

Traits can be continuous or discontinuous
(also called discrete)

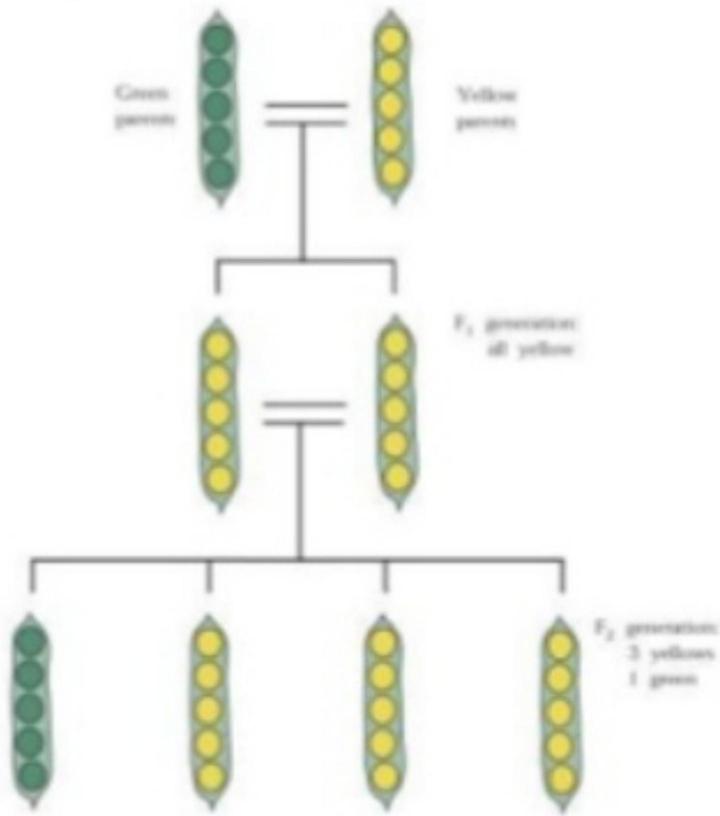


Continuous Variation
- No distinct catagories
- Tends to be quantitative
- Controlled by a lot of genes
- Strongly influenced by the environment

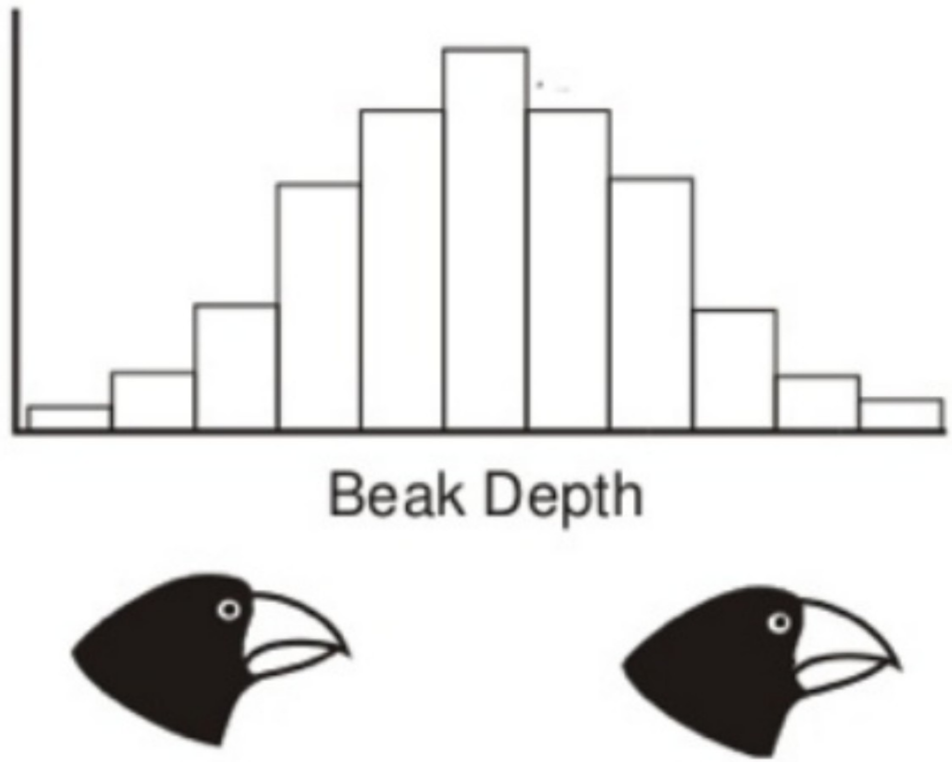
Discontinuous Variation
- Distinct catagories
- Tends to be qualitative
- Controlled by a few genes
- Unaffected by the environment

Mendel focused on variation of large effect while Darwin observed small variations that affect fitness

Mendel studied discontinuous (discrete) traits



Darwin observed continuous variation



Slide from Hasan Alhaddad

Is natural variation discrete or continuous? Mendelians against Biometricians

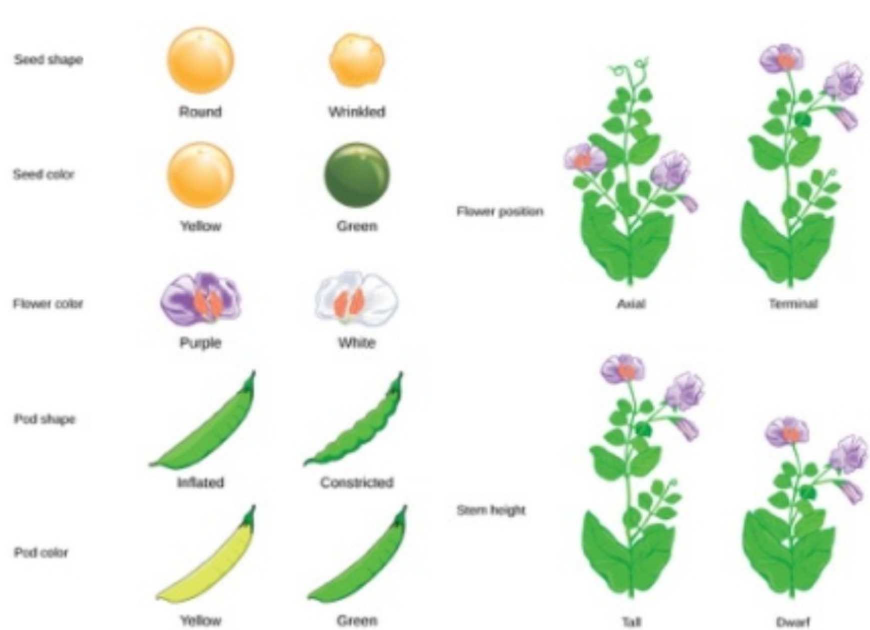
William Bateson
Hugo de Vries

- Discontinuous variation
with discrete heredity factors
- Mutation
- Evolutionary jumps

Karl Pearson
Walter Weldon

- Continuous variation
- Pre-existing variation
- Gradual change

Clear and distinct visual Traits/characters

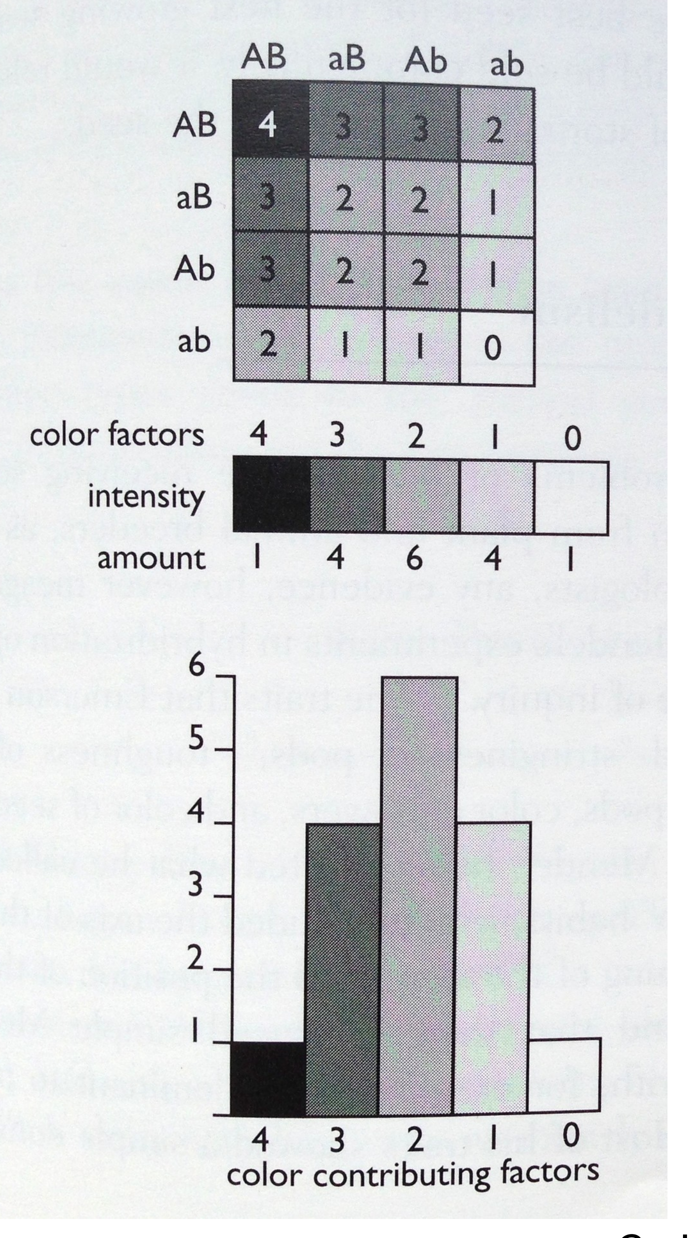


Human height

Reconciliation of Mendelian genetics and heredity of quantitative characters

Nilsson-Ehle (cereals)
East (corn)

example with only two factors with additive action:



Carlson 2004 book *Mendel's Legacy*

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Use of genomic data in model and "non-model" organisms
Role of the environment

Divergences and syntheses

Mendelian genetics
"Mendelians"

Statistical genetics
"Biometricians"

1900

agriculture

"Neodarwinian synthesis"

1930

Laboratory genetics

Population genetics
evolution
genotype

Quantitative genetics
phenotype

1950 Molecular biology

1975

Molecular genetics
cell biology,
development
physiology, etc.

Molecular evolution

Molecular markers

2000

Functional genomics

Evo-Devo

Evolutionary genomics

Quantitative genetics
molecular basis

Present syntheses

Basic principles of genetics

Genetic nomenclature

- Genetic nomenclature is different for each species

examples: *lacZ* in *Escherichia coli*
CDC28 in *Saccharomyces cerevisiae*,
cdc2 in *Schizosaccharomyces pombe*
cdk-1 in *Caenorhabditis elegans*
yellow (*y*) in *Drosophila melanogaster*
FUNNYNAME5 (*FNN5*) in *Arapidopsis thaliana*

Allele nomenclature...

- Wild-type allele "+" compared to mutation "*m*"

= for laboratory mutations
no reference wild-type allele in natural populations

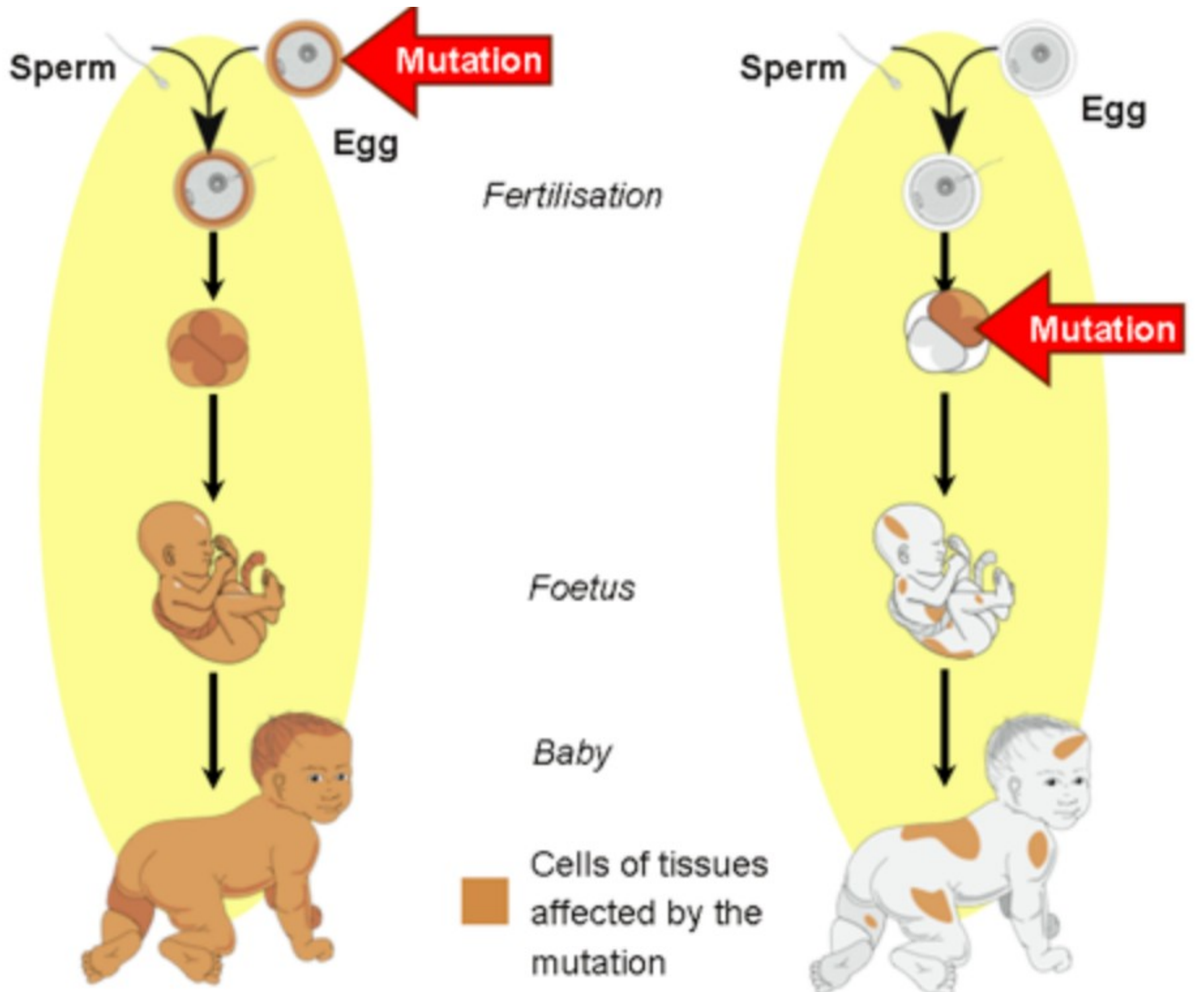
Aberration Types

Substitution

Aberration Types

Substitution
Insertion
Deletion
Indel
Inversion
Translocation
Complex change
(Epigenetic change)

Estimation of mutation rates
Mutation accumulation lines, sequencing family trio, across a phylogeny

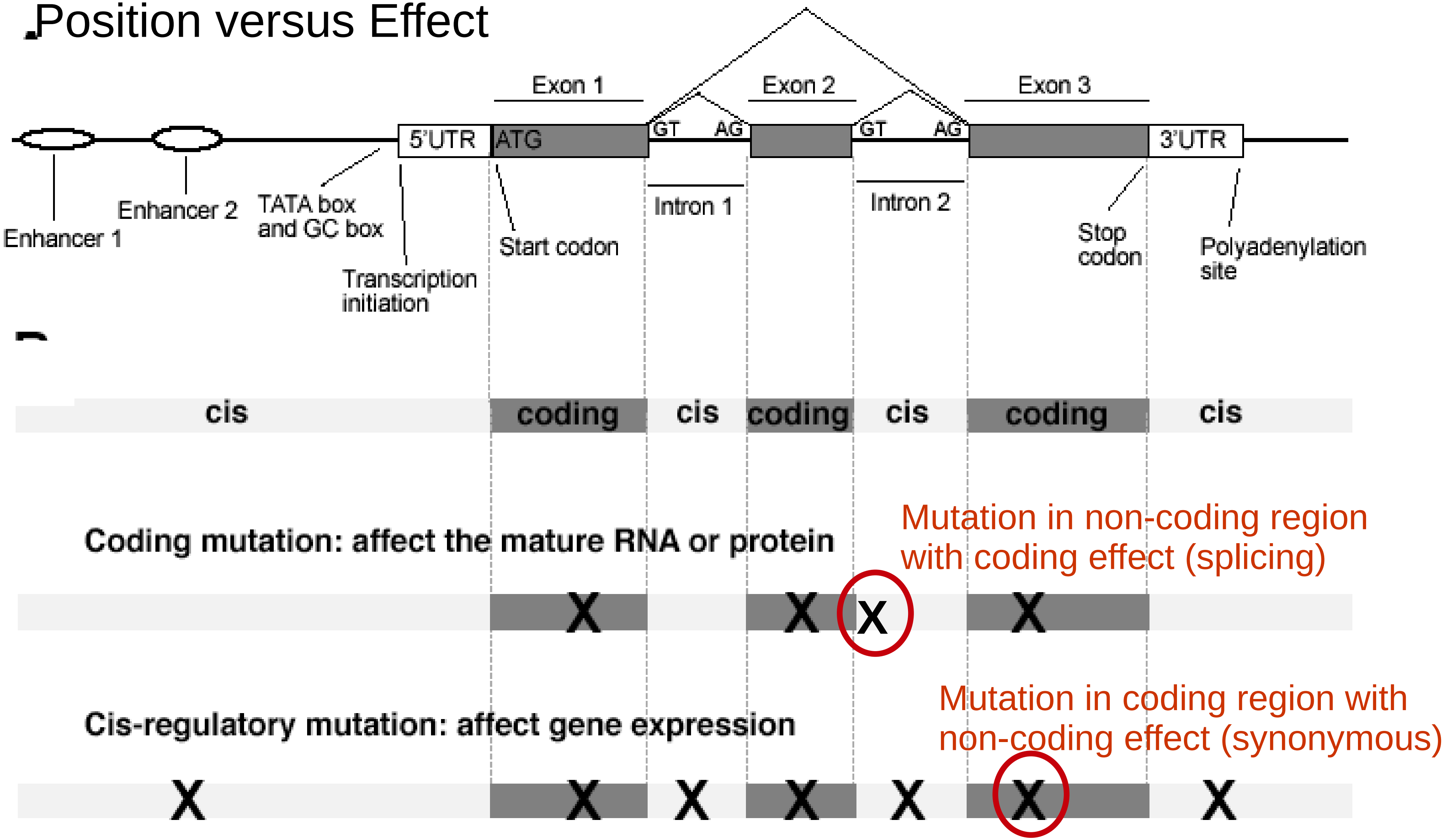


Gametic mutations are inherited and occur in the testes of males and the ovaries of females.

Somatic mutations occur in body cells. They are not inherited but may affect the person during their lifetime.

Coding versus cis-regulatory

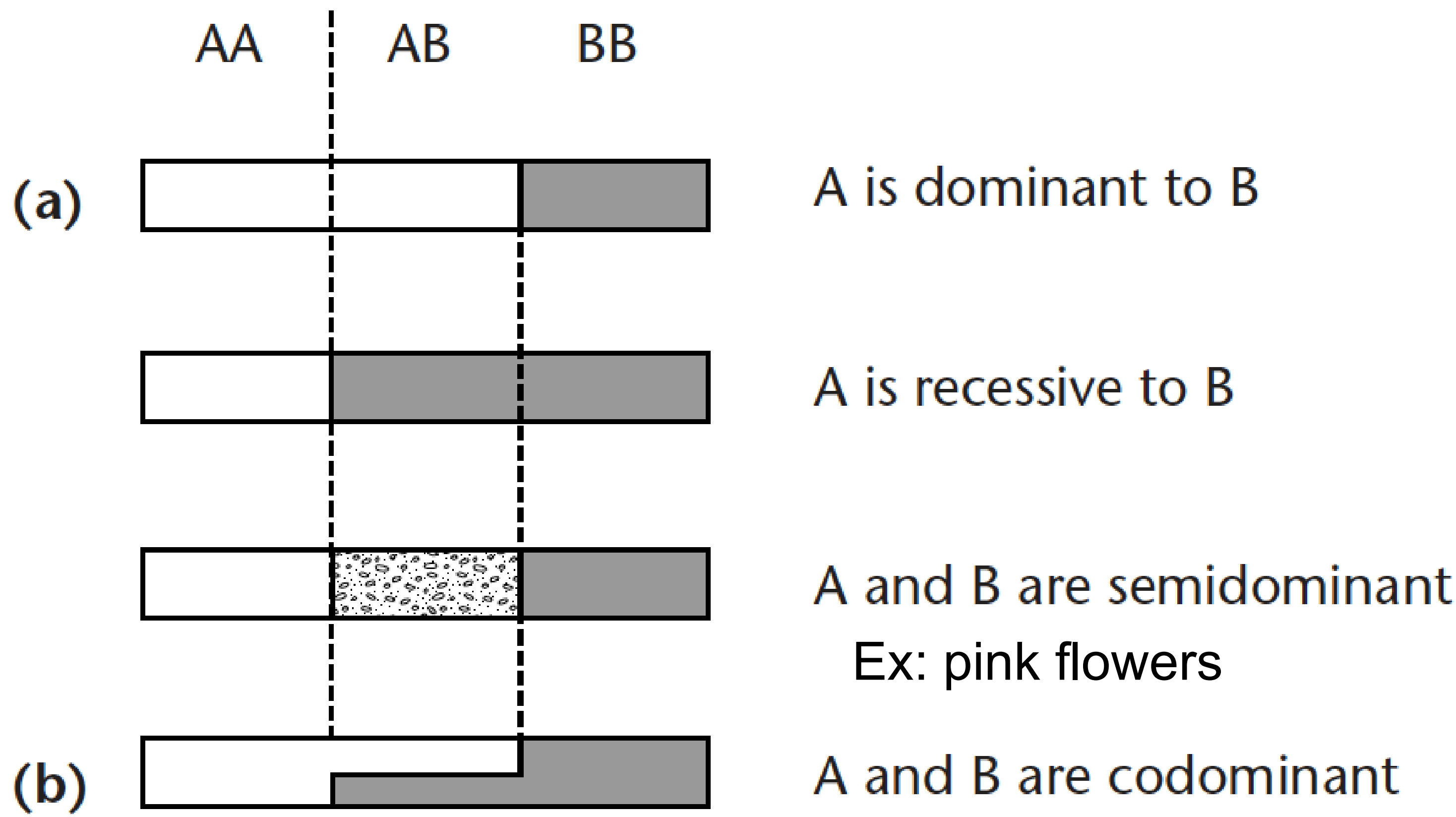
- Position versus Effect



Coding versus cis-regulatory

Coding
Cis-regulatory
Gene loss
Gene amplification
(Gene rearrangement)

Levels of dominance



Ex: AB blood groups, red and white flowers

Can be quantified as deviation from midpoint between parents

Levels of activity of the various alleles

- **Null**: no activity, equivalent to gene deletion
- **Hypomorph ou loss-of-function**: reduced activity
- **Silent**: no influence on the phenotype
- **Hypermorph or gain-of-function**: increased activity
- **Neomorph**: new activity, can be ectopic expression

Relationship with dominance

- Dominant (or semi-dominant) null allele:
  haplo-insufficiency – dose effect
  - Dominant-negative via a poison effect
  - Dominant gain-of-function/neomorph: common when gain-of-function, also with neomorph

Dominance is not an intrinsic to an allele

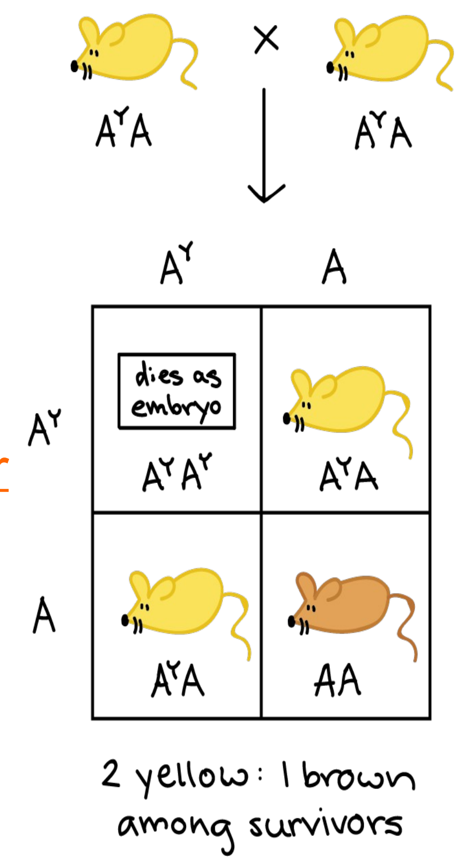
- It is <u>relative to another allele</u>, not to *all* other alleles

- It is a property of their effect <u>on a given phenotypic trait</u>

as in
"dominance of *a1* over *a2* for a particular trait"

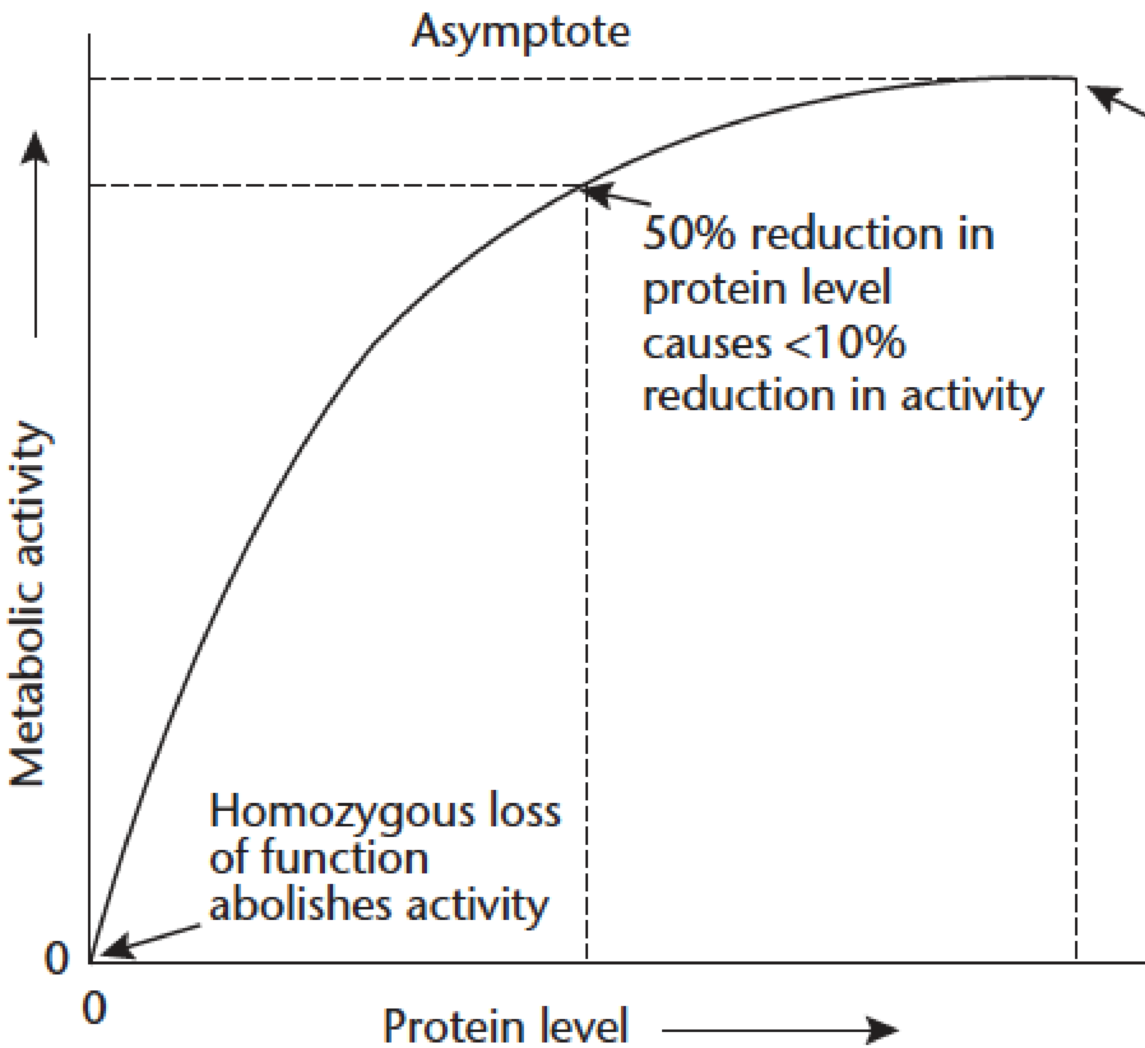
Example of the *agouti* locus in mouse

yellow allele is **dominant** over the + allele <u>for coat color</u>
yellow allele is **recessive** over the + allele <u>for lethality</u>



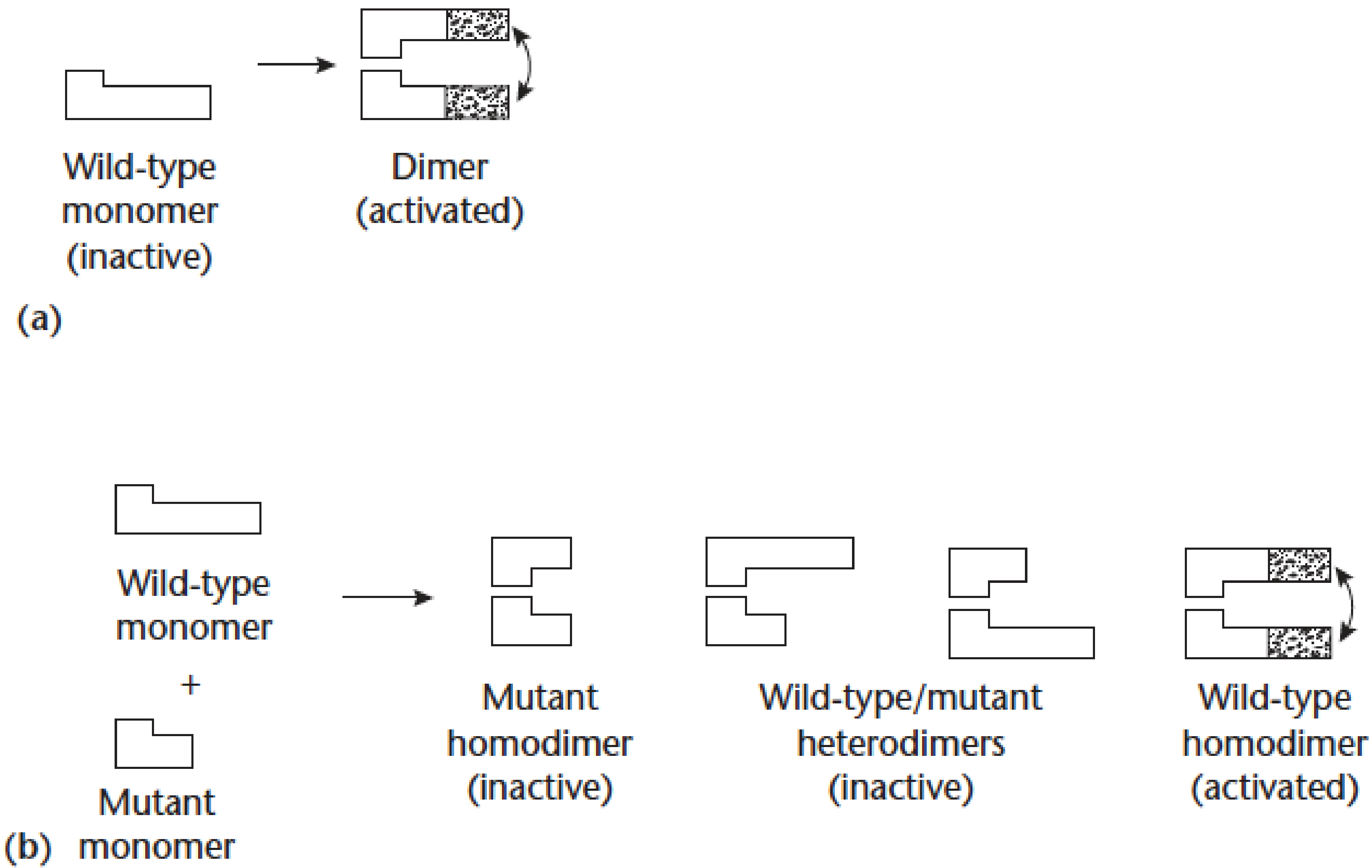
Why are most wild alleles dominant?

Robustness to half-dose:



Wilkie 2005

One type of dominant-negative mutation sequestration of wild-type in dimer



Wilkie 2005

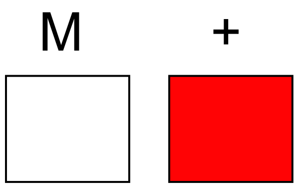
Mechanisms of dominance

Category of mutation	Mechanism	Affected gene/protein
Loss-of-function		
Haploinsufficiency	Metabolic rate determining step	LDL receptor
	Developmental regulator	Transcription factors
Dominant-negative		
Substrate sequestration	Binding by inactive monomer	Ligands, transcription factors
Dimer sequestration	Formation of inactive dimers	Receptors
Disruption of structure	Missense substitution	Collagens
Dominant-positive		
Increased gene dosage	Duplication	*PMP22*
	Amplification	Oncogene products
Altered mRNA expression	Increased gene expression	γ Hemoglobin
	Alternative splicing	*WT1*
	Toxic RNA inclusions	*DMPK*
Altered protein activity	Constitutive activity	Ion channels, receptors
	Increased binding affinity	Hemoglobin
	Formation of toxic proteins	Diverse
Novel protein activity	Altered substrate specificity	α1 Antitrypsin
	Chimeric protein (translocation)	Transcription factors

Wilkie 2005

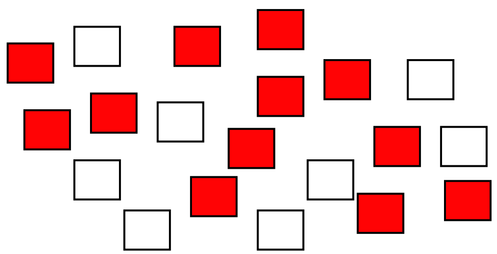
Penetrance

Discrete binary phenotype



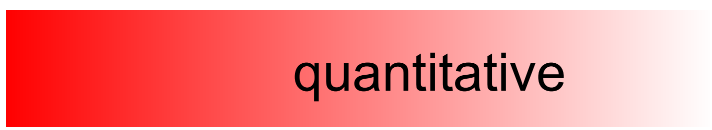
% of individuals showing the phenotype

ex: 40% of individuals have a white color
Partial penetrance

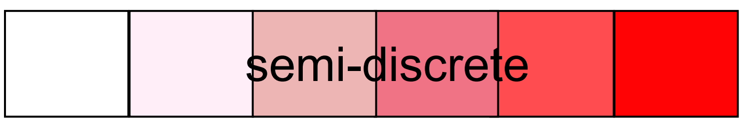


Expressivity

Phenotype with different degrees of severity



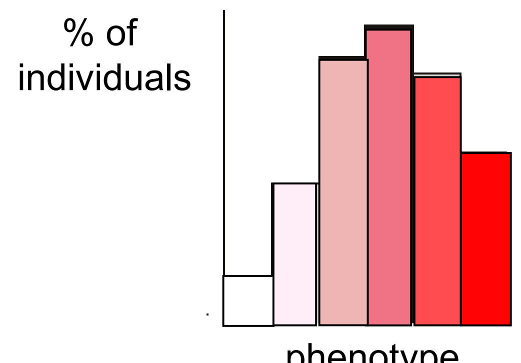
or



Degree of severity of the phenotype

ex: - number of affected ommatidia
- light pink color

often shown as a distribution of phenotypic values of individuals:



Maternal (or paternal) effect

The genotype of the parent matters,
not that of the individual itself.

Frequent for mutations affecting early embryonic development

m/m ♀ x *+/+* ♂

phenotype ? phenotype ?

↓

F1 *m/+* x *+/+* ♂ or *m/m* or *m/+*

phenotype **M**

↓

F2 *m/+* or *m/m or +/+*

phenotype **+**

+/+ ♀ x *m/m* ♂

phenotype ? phenotype ?

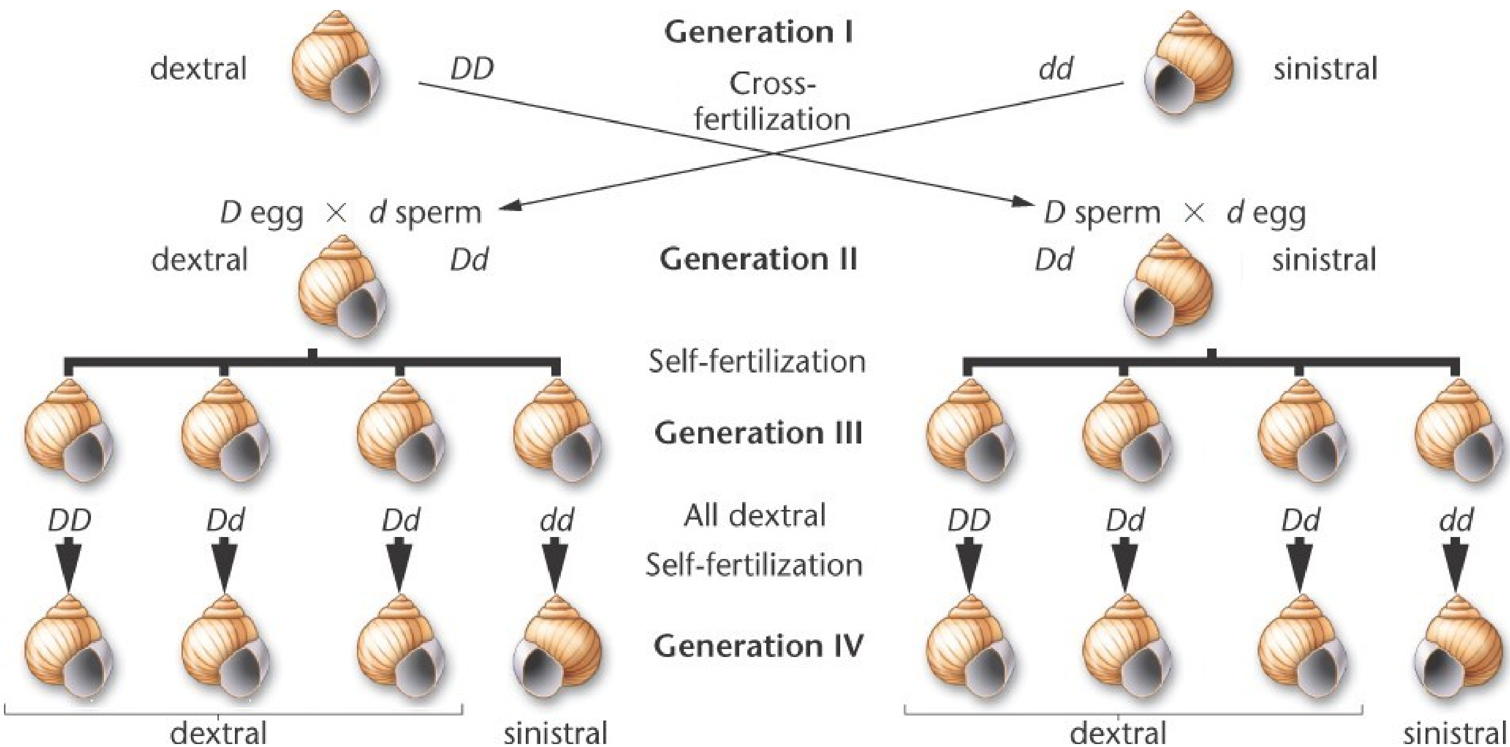
↓

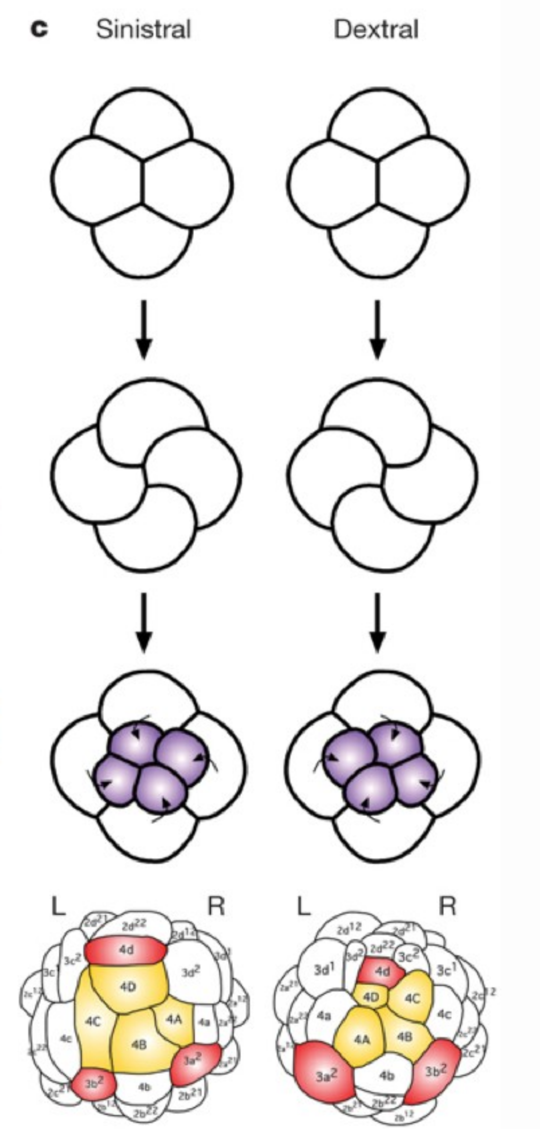
F1 *m/+*

phenotype **+**

Historical example of maternal effect

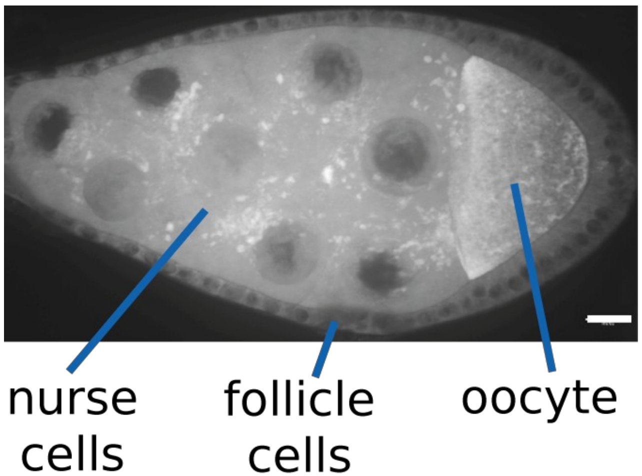
Alfred Sturtevant: "delayed inheritance"

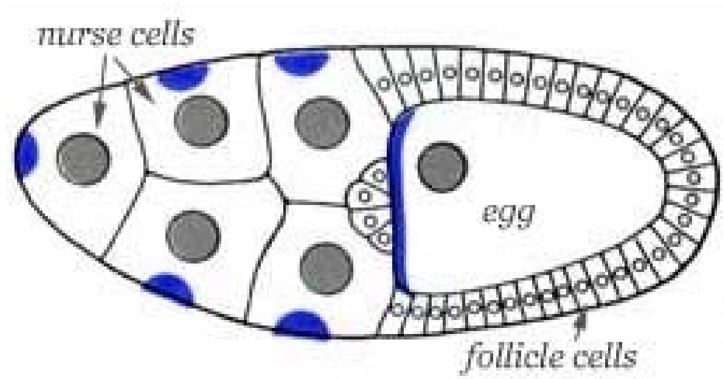


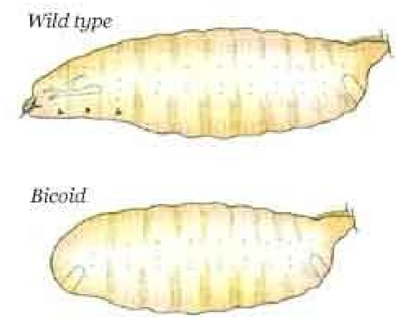


An example of maternal effect

deposition of mRNA or protein by maternal accessory cells into oocyte







Heredity with sex-linked transmission

Example: mutation on the X chromosome
in a species reproducing with XX ♀ x XY ♂

m/m ♀ x *+/Y* ♂

phenotype M phenotype +

↓

F1 *m/+* ♀ + *m/Y* ♂

females of phenotype + males of phenotype M

Heredity with sex-linked expression

Example: mutation that affects the phenotype only in females

Alleles

***m1* and *m2* are allelic if they are in the same gene**

in practice
- if the two mutations do not complement (at the phenotype level)
- or defined using sequence variations

Complementation test

if *m1/m1* and *m2/m2* show the phenotype M
and act recessively

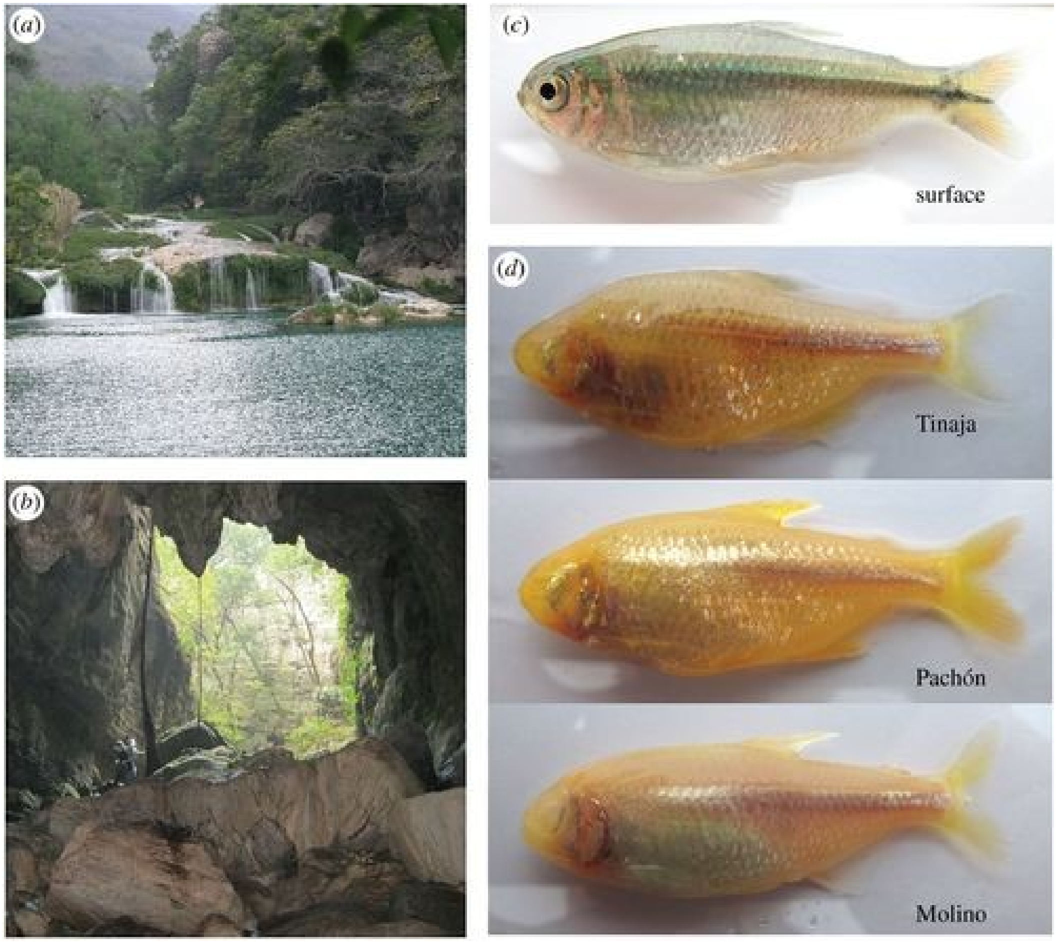
m1/m1 x *m2/m2*

↓

Interpretation

F1

- if Phenotype M **non-complementation** *m1/m2* same gene (alleles)
- if Phenotype + **complementation** *m1/+; +/m2* two genes (non allelic)


(a)
(b)
(c)
surface
(d)
Tinaja
Pachón
Molino


Molino × Pachón

The importance of DNA

Why is DNA an important molecule in biology?

Newsweek, May 23, 2005

Slide from
S. Gilbert

DNA still sells cars in the USA

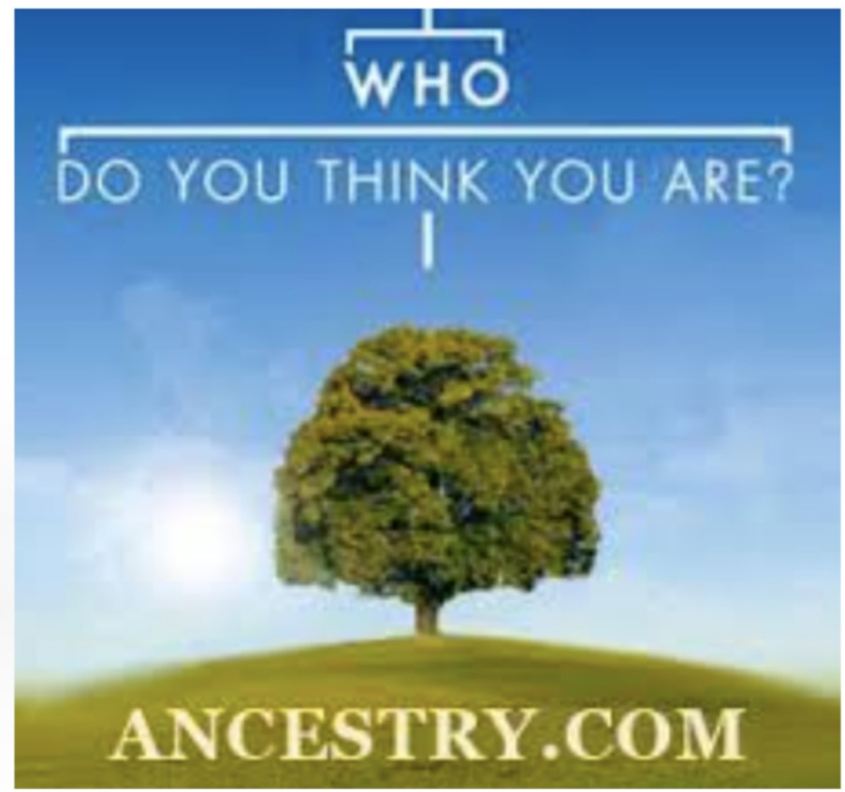
Subaru: “Genetic superstar”
Toyota: “Has a great set of genes

Slide from S. Gilbert

GENETIC INDIVIDUALITY:

Slide from S. Gilbert

Each of us is a genetically unique individual, and the genes determine who we are.



"…revealing what it is that makes you, you."

-American television ad for ancestry.com 2015

The importance of DNA in biology

Major basis of heritable variation (genotype-phenotype)

Transmitted (can help reconstruct history)

Present in all living entities (DNA/RNA)

Stable molecule (ancient DNA – oldest = horse in permafrost = 500 000 years, forensic)

String of letters, can be easily analyzed with computers (compared to anatomical traits for taxonomy)

Genetic Individuality

Slide from S. Gilbert

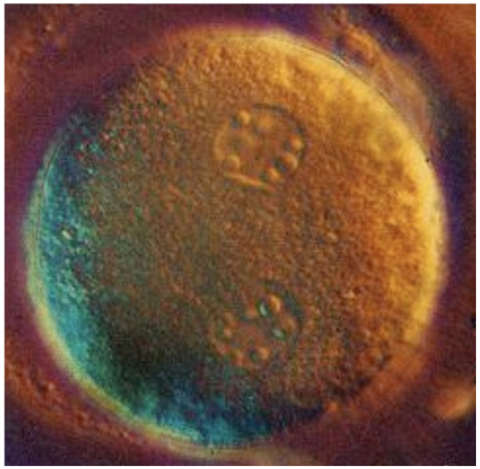
Genes determine who you are, and they act the same in each person.

LIFE Magazine, *First Days of Creation,* 1990:

"The result of fertilization is a single nucleus that contains an entire biological blueprint for a new individual, genetic information governing everything from the length of the nose to the diseases that will be inherited."

Standupgirl.com (anti-Choice website):

"And even more amazingly, intelligence and personality—the way you look and feel—were already in place in your genetic code. At the moment of conception you were essentially and uniquely you."



Disclaimer:
DNA is not the cause of everything

Monozygotic twins are not identical

Cardiovascular disease associates better with lifestyle than with DNA sequence (Mozaffarian 2008)

Lung cancer associated with smoking habits

Drug metabolism is mostly due to the microbiome

Several genes associated with autism, depression, etc. were “lost” in larger studies

Distilbene: anti-miscarriage drug, increases cancer risks in daughters and malformations in grand-daughters

What the HGP Taught us with the first genome sequenced: Genes act differently and non-additively in different people

Cockayne syndrome: **Mutation in the DNA repair enzyme ERCC6 at position 5q12.1.** Homozygous recessive persons are characterized by growth failure, impaired neural development, premature aging, sensitivity to sunlight.

Usher Syndrome: **Mutation in the retinal and cochlear basement membrane myosin MYO7A at 11q13.** Homozygous recessive persons are characterized by congenital deafness and gradual loss of vision.

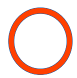
James Watson, presently 90 years old; not deaf, blind, nor stunted

Slide from S. Gilbert

First "synthetic" cell developed by scientists

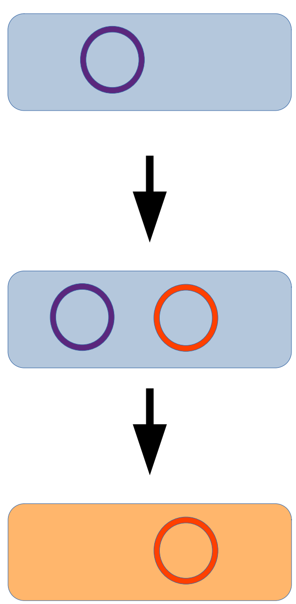
M. mycoides JCVI-syn1.0
1.08 million base pairs and 901 genes

single yeast artificial chromosome

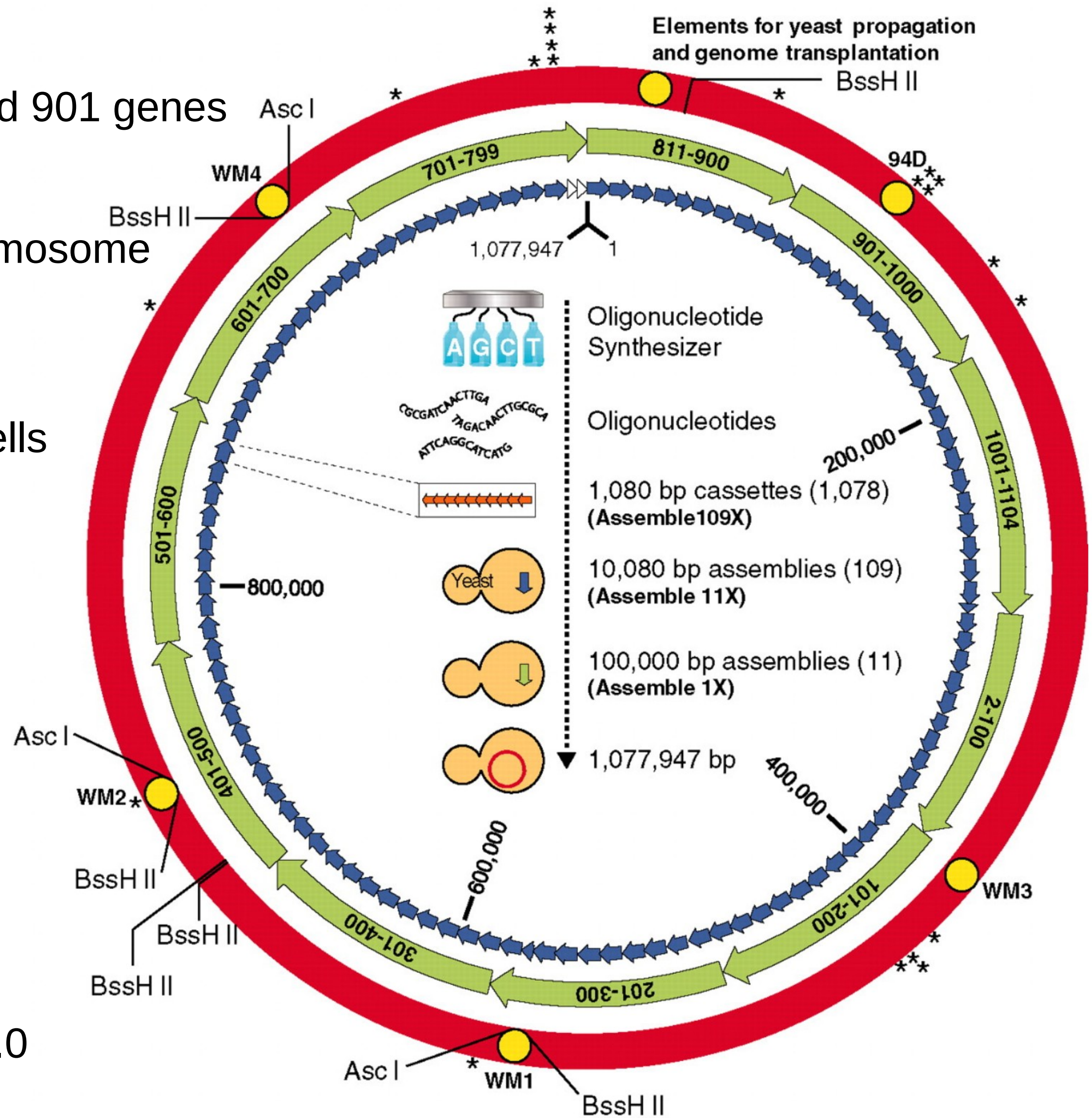


+

M. capricolum recipient cells



M. mycoides JCVI-syn1.0



Gibson et al. 2016

First “synthetic” cell developed by scientists

M. mycoides JCVI-syn1.0 contains strings of bases that, in code, spell out:

a web address to send emails to if you can successfully crack the new code,

the names of 46 authors and other key contributors,

three famous quotations. One of which by James Joyce, perfectly encapsulates the ups and downs of a the 15 year project—“To live, to err, to fall, to triumph, to recreate life out of life.”

Gibson et al. 2016

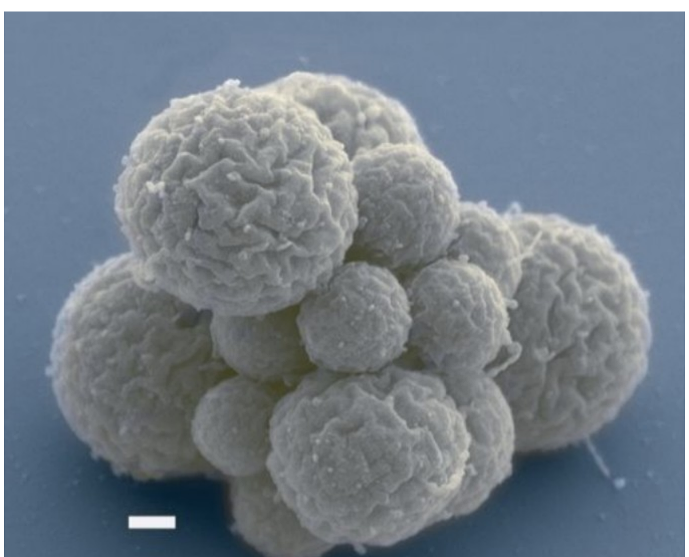
Smallest “synthetic” cell

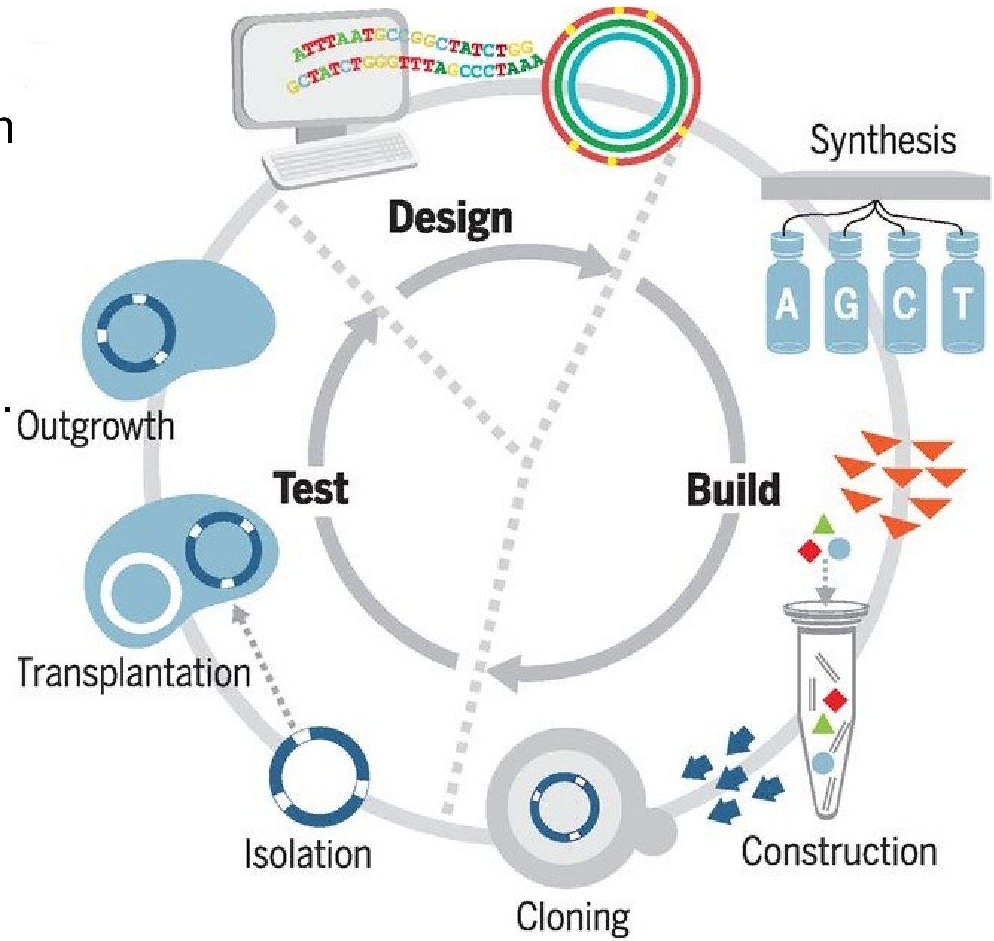
M. mycoides JCVI-syn3.0
531,560 base pairs and 473 genes

retains genes involved in key processes such as transcription and translation, but also contains 149 genes of unknown function

genome smaller than that of any autonomously replicating cell found in nature.

doubling time of ~180 min
colonies morphologically similar to those of JCVI-syn1.0





Hutchison III et al. 2016

Theodore Schwann, 1839

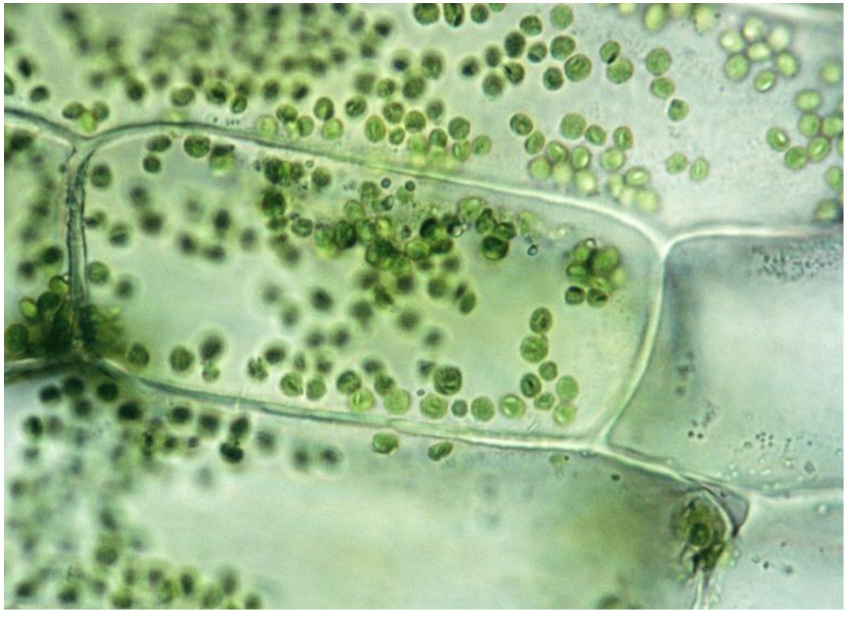
"*All living things are composed of cells and cell products*"

1) The cell is the unit of structure, physiology, and organization in living things.

2) The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.

Rudolf Virchow, 1857

Every cell arises from another cell

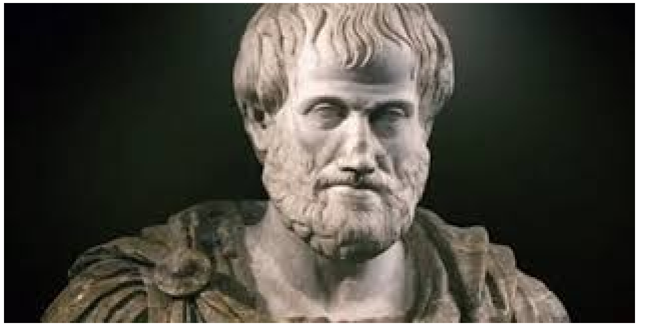


The genotype-phenotype relationship

Different kinds of phenotypes

Morphology

- **Color**
- **Size and shape**
- **Presence/ absence**
- **Position**



Aristote, Historia animalium, book I, 2, 300BC

Physiology

Behavior

Genotype & Phenotype

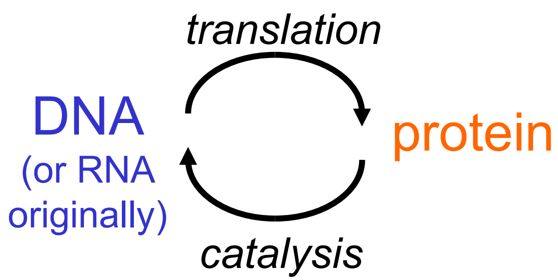
= what engenders = what is apparent

DNA/RNA

- **Regulation of gene expression**
- **Biochemical reactions**
- **Subcellular architecture**
- **Assembly of cells**
- **Organism morphology and behavior**

etc.

distinction appeared at the origin of life:



Francis Crick Central Dogma
A reductionist view of the GP relationship

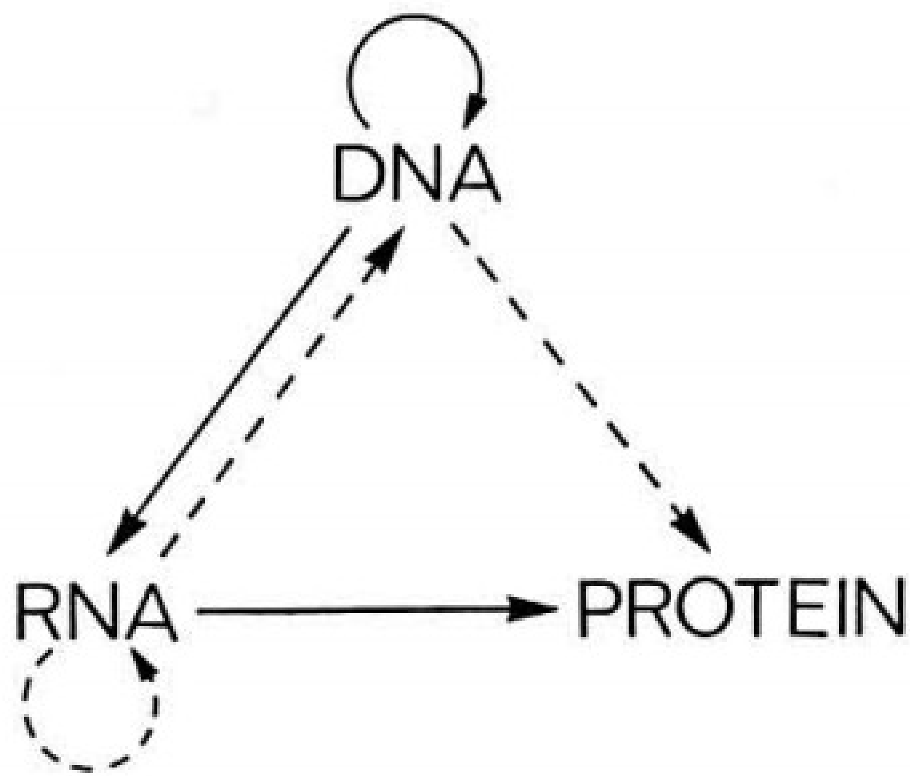


Fig. 3. A tentative classification for the present day. Solid arrows show general transfers; dotted arrows show special transfers. Again, the absent arrows are the undetected transfers specified by the central dogma.

Crick 1958
Crick 1970

How do genotypes map onto phenotypes ?

DEVELOPMENTAL BIOLOGY

EVOLUTIONARY GENETICS

Both are direct descendants of Morgan's school. Emphasis on genes.

How does an organism form from a single cell?

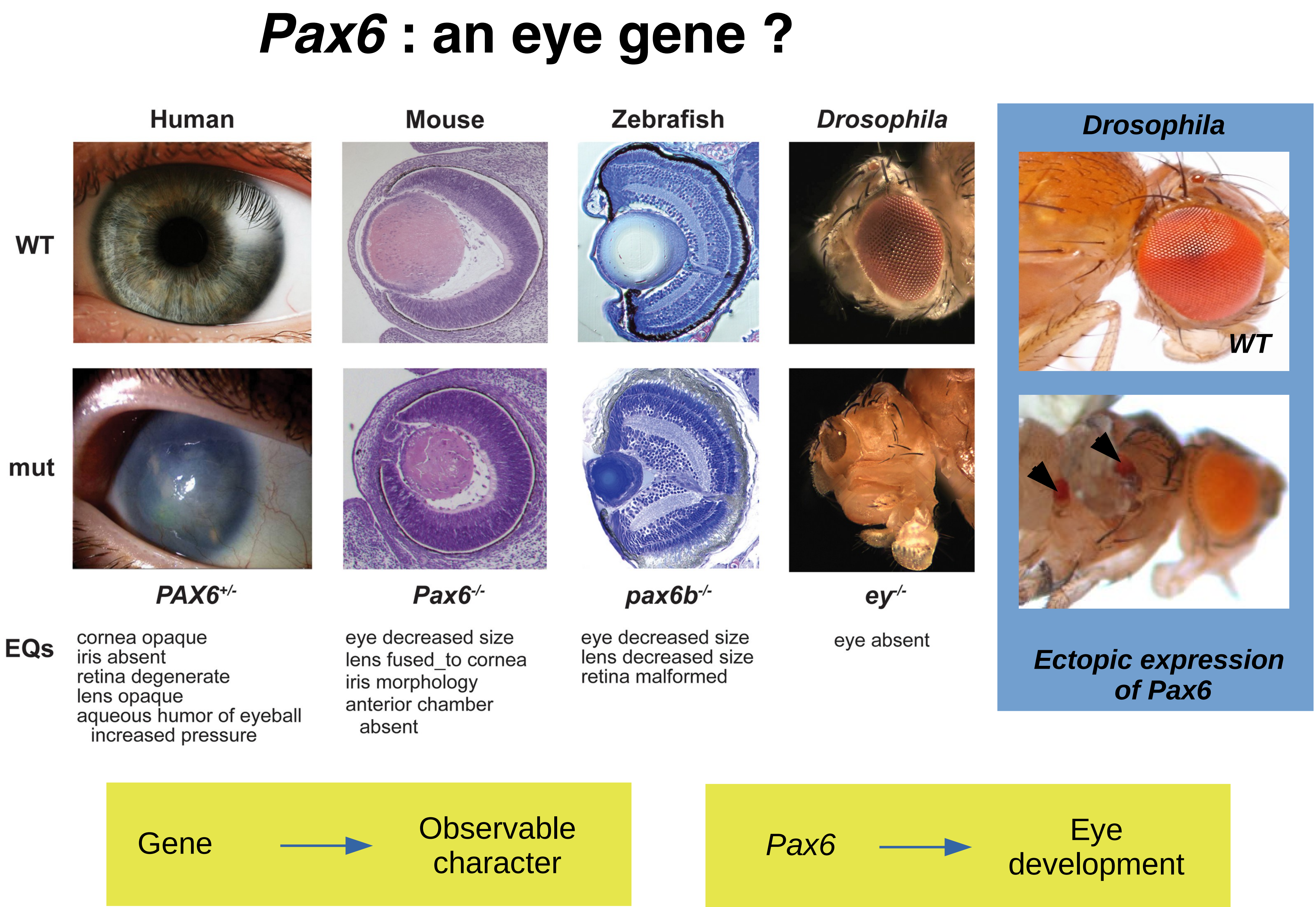
What makes one organism different from another one?

One of the central problems of biology is that of differentiation - how does an egg develop into a complex many-celled organism? That is, of course, the traditional major problem of embryology; but it also appears in genetics in the form of the question,
"How do genes produce their effects?

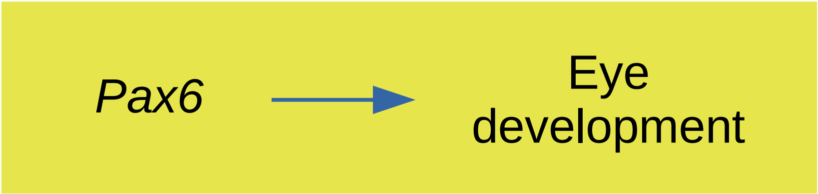
Sturtevant, 1932

How do genes produce observable traits?

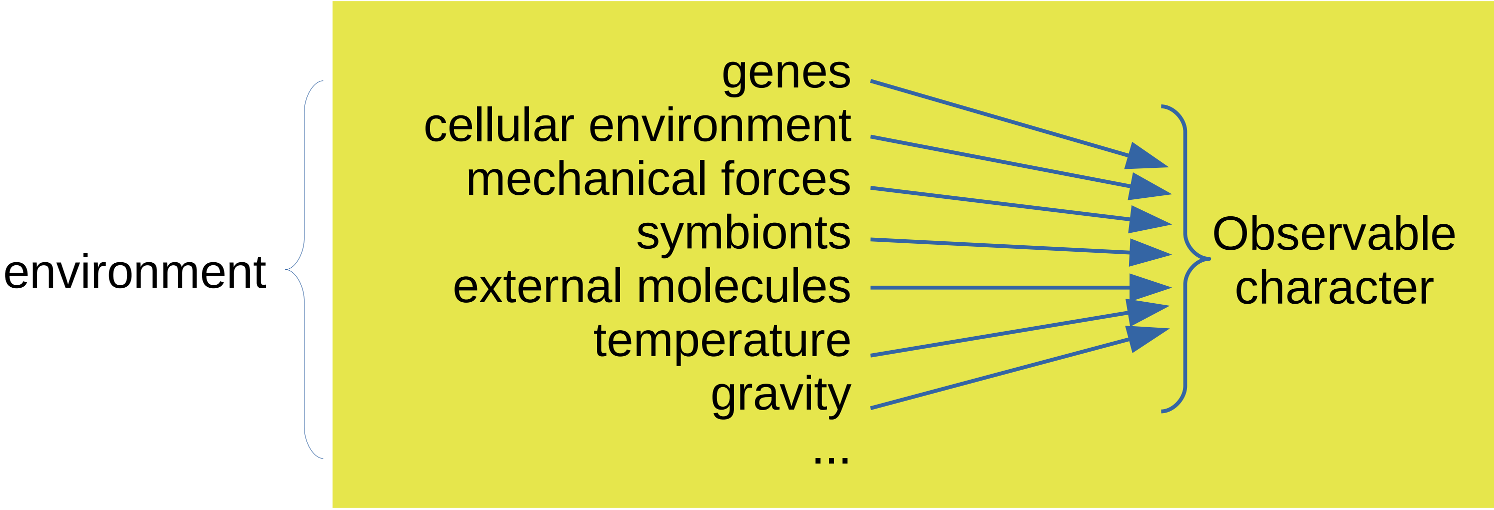
Gene → Observable character



Pax6 : an eye gene ?
Human
Mouse
Zebrafish
Drosophila
WT
mut
PAX6+/-
Pax6-/-
pax6b-/-
ey-/-
EQs
cornea opaque
iris absent
retina degenerate
lens opaque
aqueous humor of eyeball increased pressure
eye decreased size
lens fused_to cornea
iris morphology
anterior chamber absent
eye decreased size
lens decreased size
retina malformed
eye absent
Drosophila
WT
Ectopic expression of Pax6
Gene
Observable character
Pax6
Eye development

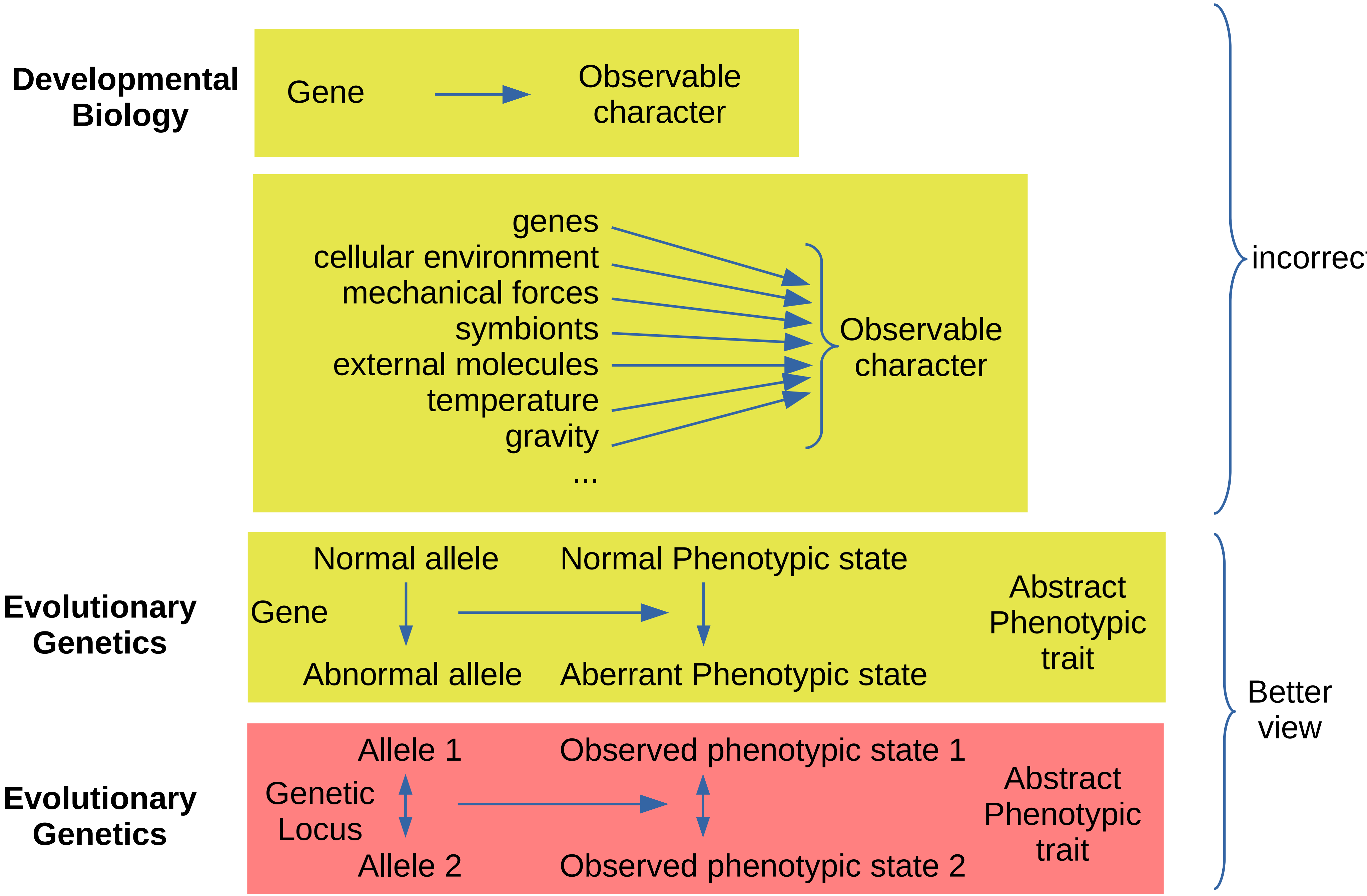


Too simplistic

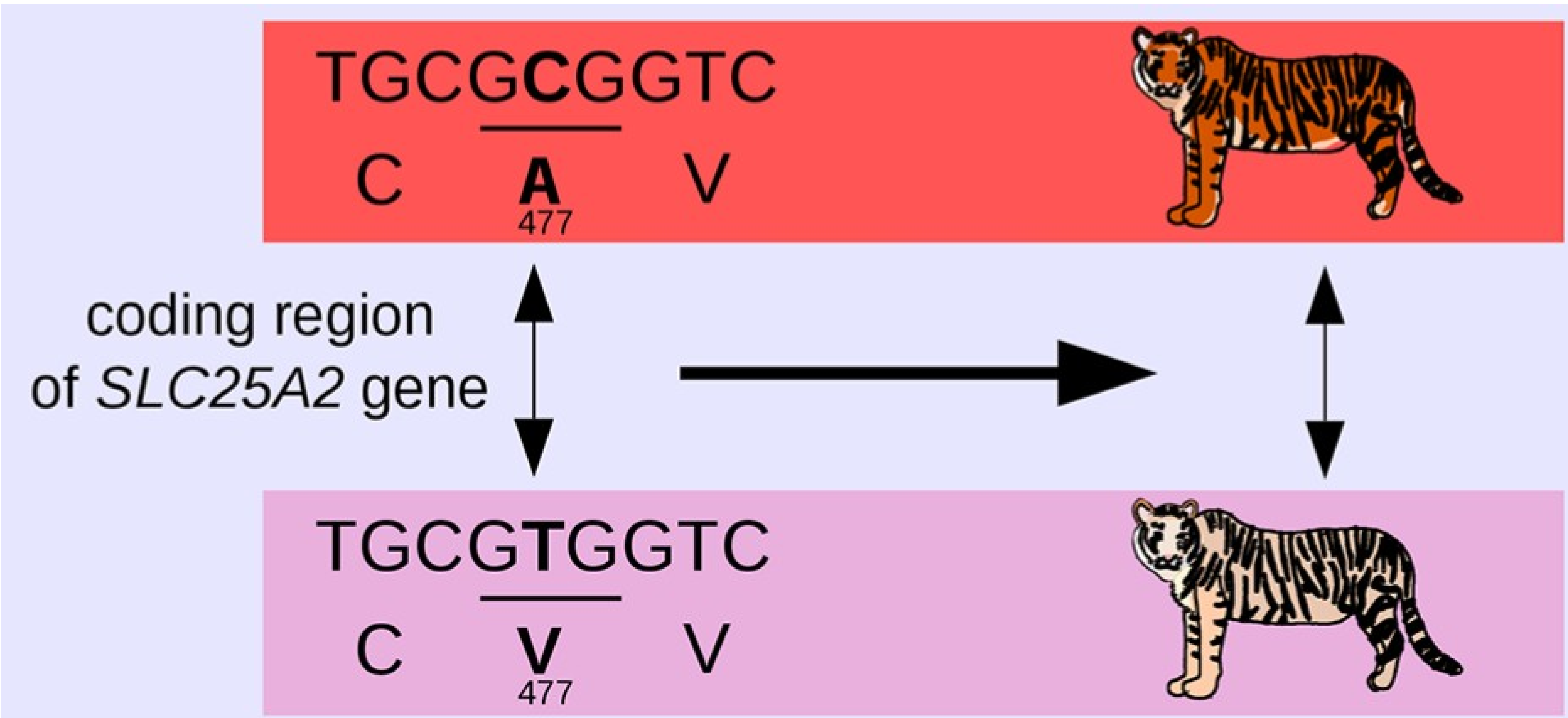


Better, but difficult to disentangle the effects

The wrong and the right perspectives



The genotype-phenotype connection



Xu et al 2013 Current Biology

Orgogozo et al 2015 Frontiers Genetics

Genotype = "the genetic makeup of an organism that determines a specific phenotype (trait), from one generation to the next, and potentially throughout the population".

Report of the National Academy of Sciences on gene drive, 2016

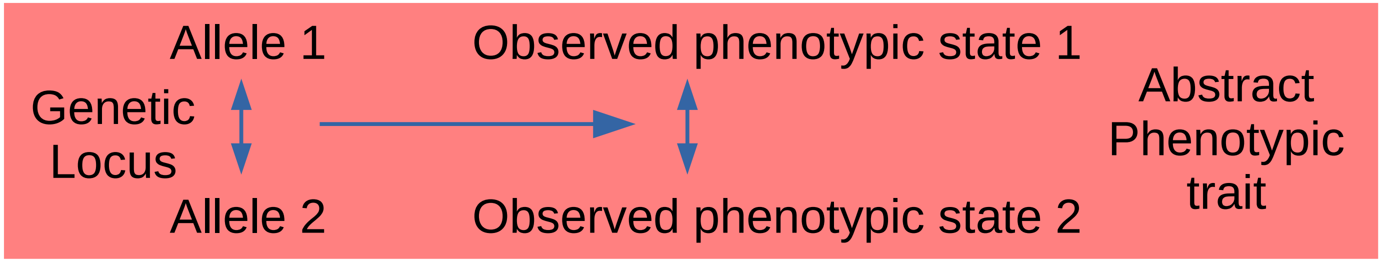
NO!

The genotype-phenotype connection is about differences

Example: the causes of a difference in hair color

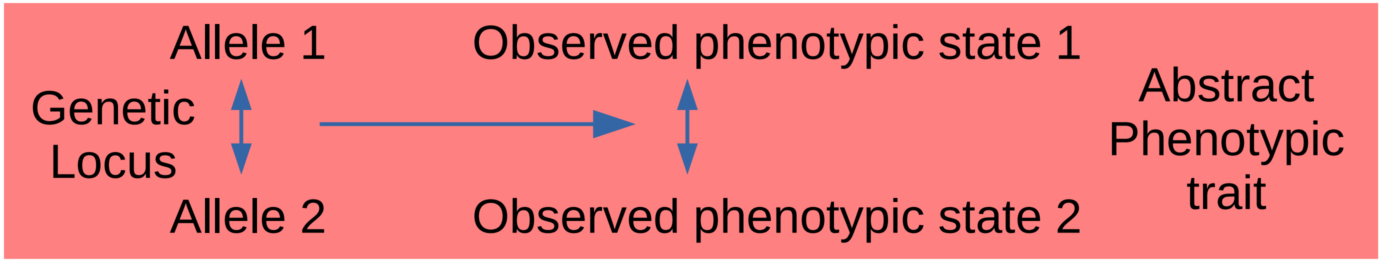
Genes coding for pigment synthesizing enzymes
Amount of received sun light
Hair dyeing
Age
...

Gephe: a relationship between two levels of variation



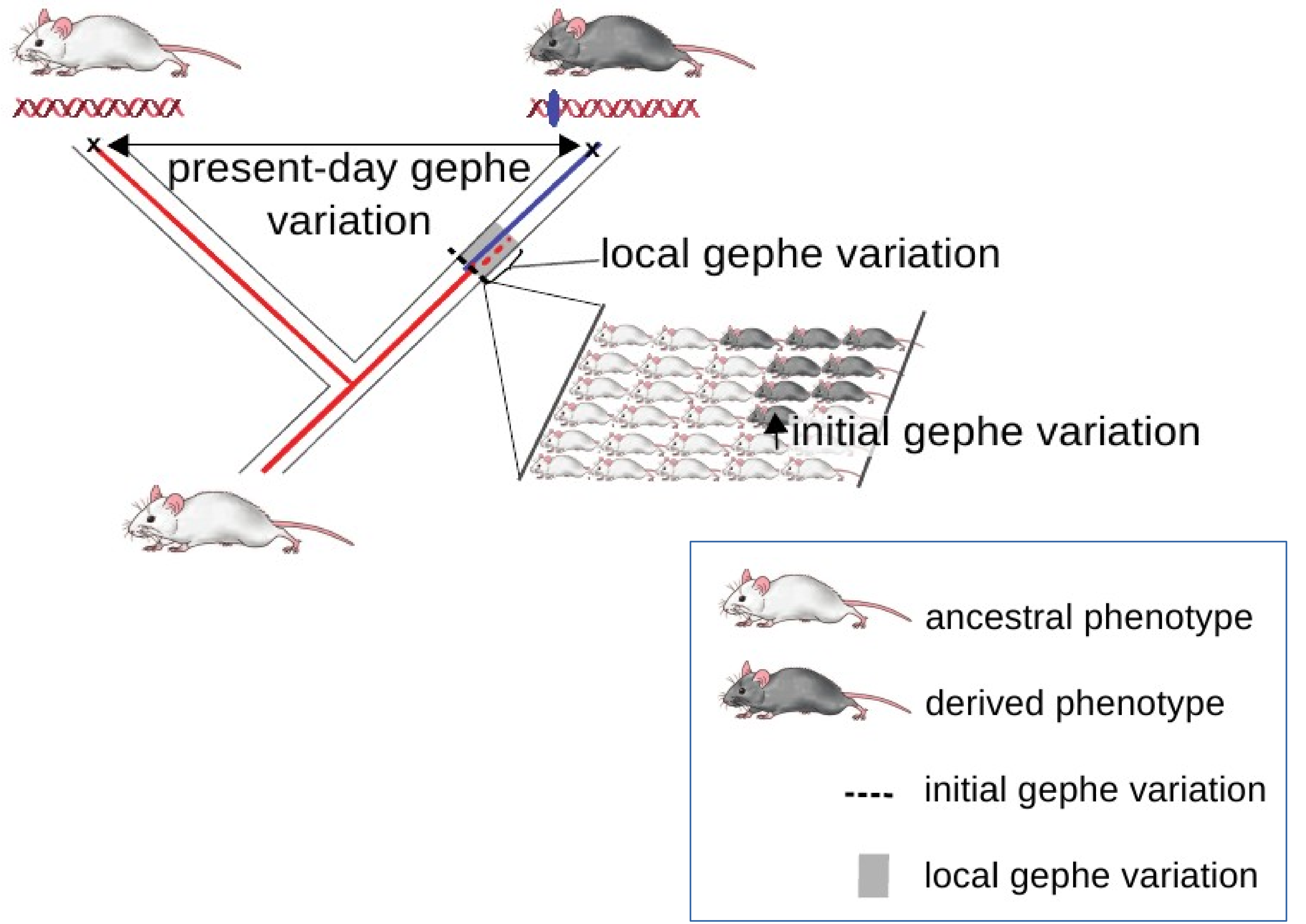
Genetic locus	**Phenotypic trait**
One particular coding site in the *Nav1.4* gene	Resistance to tetrodotoxin or saxotoxin
Various coding sites in *opsin* genes	Color vision
SLC45A2 coding region	Pigmentation of eye, hair and skin
Mc1R coding region	Pigmentation of hair and skin, but not eye
Cis-regulatory element in the *lactase* gene	Ability to digest milk
Cis-regulatory element in the *pitx1* gene	Pelvis morphology
Cis-regulatory elements in the *optix* gene	Red color pattern on butterfly wings
Cis-regulatory and coding regions of the *FRIGIDA* gene	Flowering time

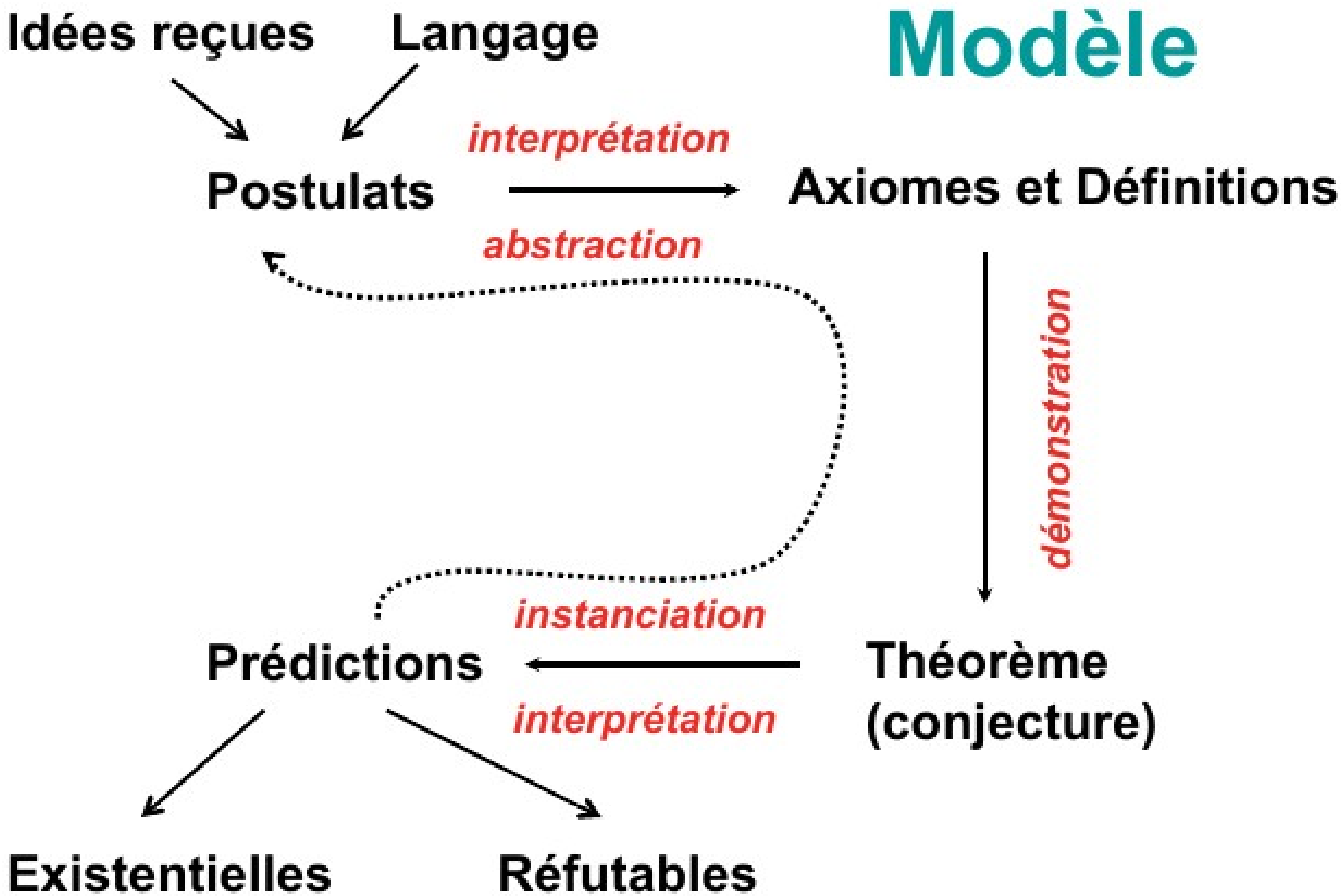
Gephe: a relationship between two levels of variation



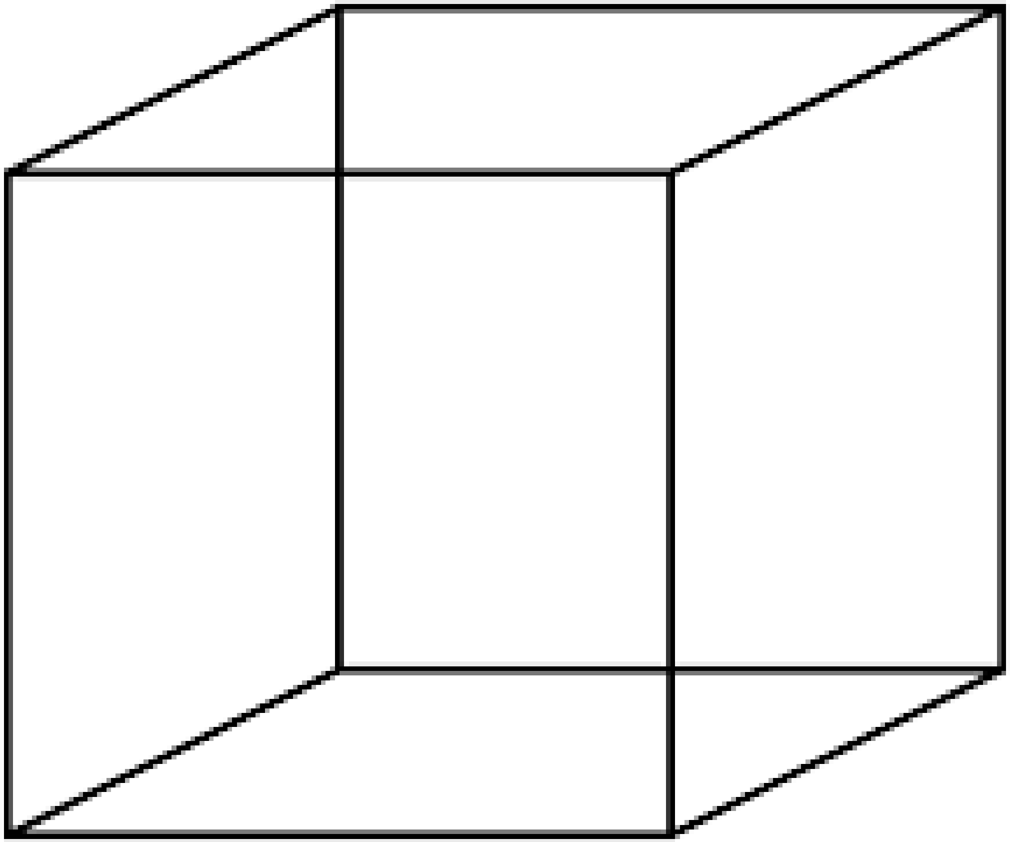
The genetic locus in the gephe concept is **not necessarily a gene.**
it can span a particular base-pair, a coding region, a cis-regulatory region, or extend to an entire gene or even a gene cluster.
This view rejoins previous reflections by Scott Gilbert (2000), David Stern (2000) and Lenny Moss (2003) on the distinct concepts of gene in developmental biology and in evolutionary biology.

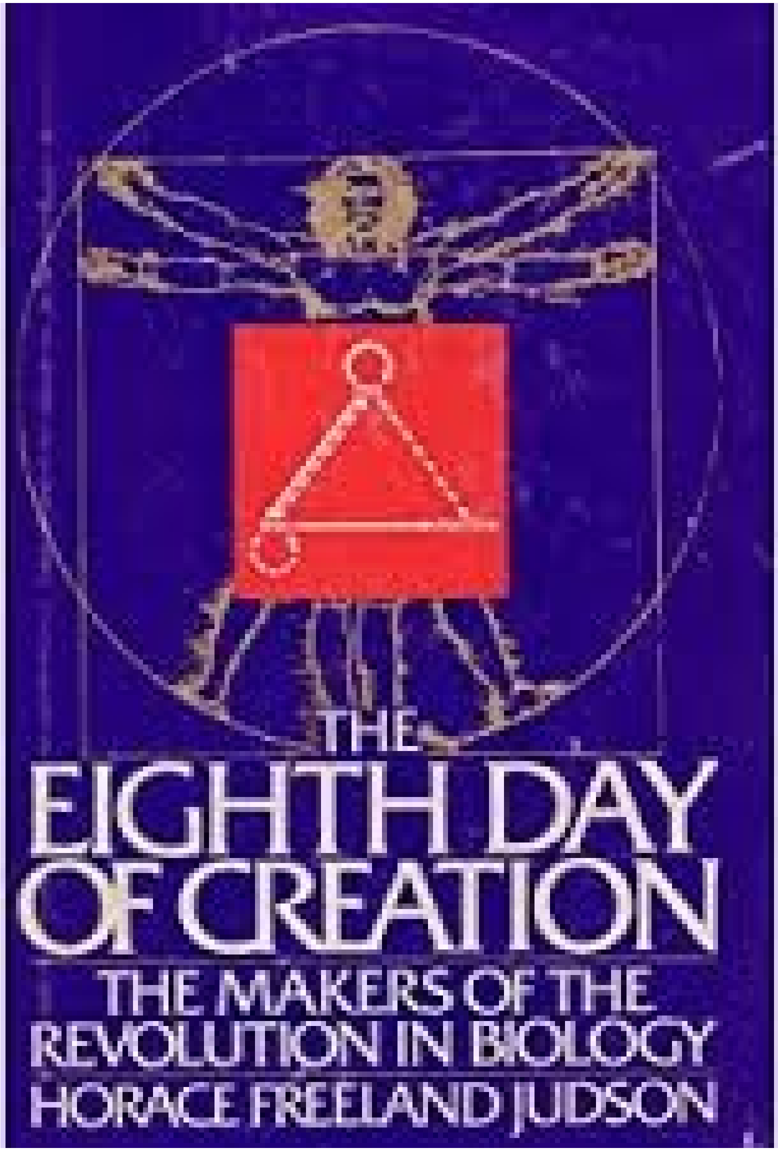
Gephe: a difference at various levels

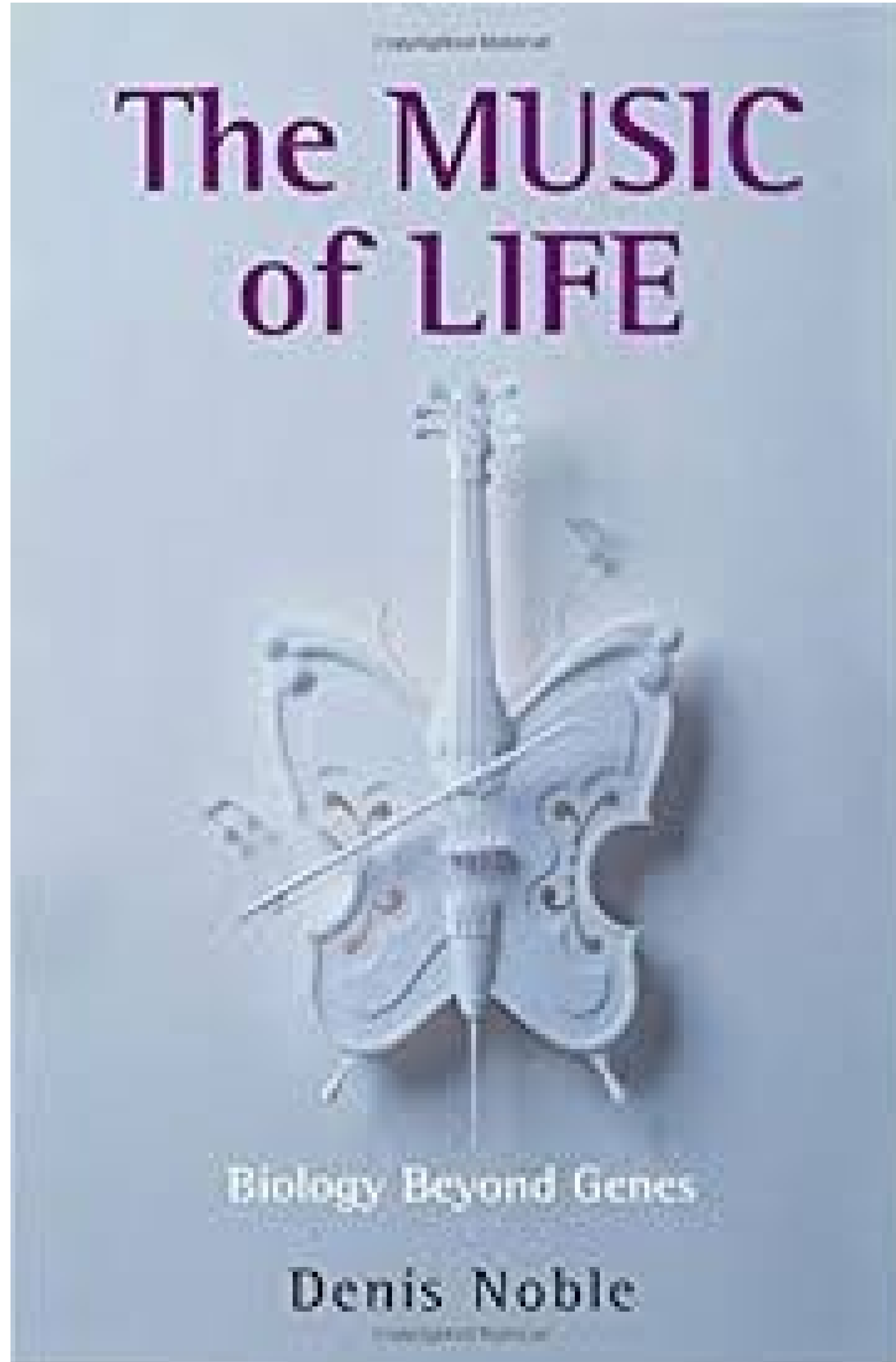




We sometimes seem to have forgotten that the original question in genetics was not what makes a protein but rather 'what makes a dog a dog, a man a man'.
(D. Noble – The Music of Life)




THE
EIGHTH DAY
OF CREATION
THE MAKERS OF THE
REVOLUTION IN BIOLOGY
HORACE FREELAND JUDSON


The MUSIC
of LIFE
Biology Beyond Genes
Denis Noble