

# Life cycle of organisms

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# **Escherichia coli**

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- *E.coli* is a gram negative, facultative anaerobic, rod-shaped, coliform bacterium that is commonly found in the lower intestine of warm-blooded organisms.
- *E.coli* is the most widely studied prokaryotic unicellular model organism.
- Most *E.coli* strains are harmless, but some can cause serious food poisoning. Shiga toxin-producing *E.coli* (STEC) is a bacterium that can cause severe foodborne disease.
- Can grow with or without oxygen.
- *E.coli* is about 2.0 micrometer long and 0.25-1.0 micrometer in diameter with a cell volume of 0.6-0.7 micro cubic meter.

## Taxonomy and classification

## Morphology

- Bacteria is arranged singly or in pairs.
- It is motile due to peritrichous flagella.
- Some strains are non-motile.
- Some strains may be fimbriated.
- The fimbriae are of type 1 (hemagglutinating and mannose-sensitive) and are present in both motile and non-motile strains.
- Some strains of *E. coli* isolated from extra-intestinal infections have polysaccharide capsule.
- They are non-sporing. They have thin cell wall with only 1 or 2 layers of peptidoglycan.
- They are facultative anaerobes.
- Growth occurs over a wide range of temperature from 15 – 45°C.

[Image of bacterium]

# Genetics

- Genome size :4.6Mb
- Chromosomes:Circular
- No.of genes:4000
- Percentage with human chromosomes:8%
- Average gene density:
- Gene sequenced:1997

# Reproduction

- *E.coli* reproduces by two means:
  - cell division
  - the transfer of genetic material through a sex pilus (conjugation).
- It takes about forty minutes to make one complete copy of the *E.coli* genome. Therefore each generation of *E.coli* should last just under one hour.
- Instead *E.coli* can divide (under ideal condition) in as little as 20 minutes.
- Prokaryotes reproduce asexually through binary fission.
- The cell divides leaving two copies of original bacterium called daughter cells

## Studies

- A German pediatrician named Odor Escheriehia in 1885 discovered *E.coli* in the faeces of healthy individual and named it Bacterium coli because of the fact that it is found in the colon.
- Early prokaryotic classification based on their motility and shape
- Afterwards E.haeckel's bacteria classification placed bacteria in the monera kingdom
- In 1985 Migula reclassified bacteria in the genus Escheria which was named so after its discover. This genus belongs to the bacterial group formally called 'coliforms' which are member of the enterics known as Enterubacterialeae family
- Because of its long history of laboratory culture and ease of manipulation *E.coli* plays an important role in modern biological engineering and industrial microbiology.

## Suitability as model organism

- Rapid reproduction and small size
- Under optimal condition, they can reproduce every 20 minutes, in a mere 7 hours, a single bacterial cells can give rise to more than 2million descendants.
- Thus enormous numbers of cells can be grown quickly, so that even very rare mutations will appear in a short period of time.
- Consequently, numerous mutations in *E.coli* everything from colony appearance to drug resistance, have been isolated and characterised.
- *E.coli* is easy to culture in the laboratory in liquid medium or on solid medium within petri plates.
- In liquid culture, *E.coli* cells will grow to a concentration of a billion cells per millilitre and trillion of bacterial cells can be easily grown on a single test tube.

- When *E.coli* cells are diluted and spread onto the solid medium of a petridish, individual bacteria reproduce asexually, giving rise to a concentrated clump of 10 million - 100 million genetically identical cells called a colony.
- This colony formation makes it easy to isolate genetically pure strains of the bacteria
- One of the first useful applications of recombinant DNA technology was the manipulation of *E.coli* to produce human insulin
- Modified *E.coli* has been used in vaccine development, bioremediation, production of biofuels, lightening and production of immobilised enzymes
- *E.coli* is a very versatile host for the production of heterogenous protein and protein expression systems have been developed which allow the production of recombinant proteins in *E.coli*.
- Researchers can introduce genes into the microbes using plasmids which permits high level expression of protein and such protein may be mass produced in industrial fermentation processes



# Escherichia coli

## Key organism for studying:

- Transcription, translation, replication, recombination
- Mutation
- Gene regulation
- Recombinant DNA technology

## Genetic "Vital Statistics"

Genome size:	4.6 Mb
Chromosomes:	1, circular
Number of genes:	4000
Percentage with human homologs:	8%
Average gene size:	1 kb, no introns
Transposons:	Strain specific, ~ 60 copies per genome
Genome sequenced in:	1997



The unicellular bacterium *Escherichia coli* is widely known as a disease-causing pathogen, a source of food poisoning and intestinal disease. However, this negative reputation is undeserved. Although some strains of *E. coli* are harmful, others are natural and essential residents of the human gut. As model organisms, strains of *E. coli* play an indispensable role in genetic analyses. In the 1940s, several groups began investigating the genetics of *E. coli*. The need was for a simple organism that could be cultured inexpensively to produce large numbers of individual bacteria to be able to find and analyze rare genetic events. Because *E. coli* can be obtained from the human gut and is small and easy to culture, it was a natural choice. Work on *E. coli* defined the beginning of "black box" reasoning in genetics: through the selection and analysis of mutants, the workings of cellular processes could be deduced even though an individual cell was too small to be seen.

***E. coli* genome.** Electron micrograph of the genome of the bacterium *E. coli*, released from the cell by osmotic shock.  
[G. Mumt/Science Source.]

## Special features

Much of *E. coli*'s success as a model organism can be attributed to two statistics: its 1-μm cell size and a 20-minute generation time. (Replication of the chromosome takes 40 minutes, but multiple replication forks allow the cell to divide in 20 minutes.) Consequently, this prokaryote can be grown in staggering numbers—a feature that allows geneticists to identify mutations and other rare genetic events such as intragenic recombinants. *E. coli* is also remarkably easy to culture. When cells are spread on plates of nutrient medium, each cell divides in situ and forms a visible colony. Alternatively, batches of cells can be grown in liquid shake culture. Phenotypes such as colony size, drug resistance, ability to obtain energy from particular carbon sources, and colored dye production take the place of the morphological phenotypes of eukaryotic genetics.



**Bacterial colonies.** [Biophoto Associates/Science Source.]

## Life Cycle

*Escherichia coli* reproduces asexually by simple cell fission; its haploid genome replicates and partitions with the dividing cell. In the 1940s, Joshua Lederberg and Edward Tatum discovered that *E. coli* also has a type of sexual cycle in which cells of genetically differentiated "sexes" fuse and exchange some or all of their genomes, sometimes leading to recombination (see Chapter 5). "Males" can convert "females" into males by the transmission of a particular plasmid. This circular extragenomic 100-kb DNA plasmid, called F, determines a type of "maleness." F<sup>+</sup> cells acting as male "donors" transmit a copy of the F plasmid to a recipient cell. The F plasmid can integrate into the chromosome to form an Hfr cell type, which transmits the chromosome linearly into F<sup>-</sup> recipients. Other plasmids are found in *E. coli* in nature. Some carry genes whose functions equip the cell for life in specific environments; R plasmids that carry drug-resistance genes are examples.

**Length of life cycle:** 20 minutes

Geneticists have also taken advantage of some unique genetic elements associated with *E. coli*. Bacterial plasmids and phages are used as vectors to clone the genes of other organisms within *E. coli*. Transposable elements from *E. coli* are harnessed to disrupt genes in cloned eukaryotic DNA. Such bacterial elements are key players in recombinant DNA technology.

#### Genetic analysis

Spontaneous *E. coli* mutants show a variety of DNA changes, ranging from simple base substitutions to the insertion of transposable elements. The study of rare spontaneous mutations in *E. coli* is feasible because large populations can be screened. However, mutagens also are used to increase mutation frequencies.

To obtain specific mutant phenotypes that might represent defects in a process under study, screens or selections must be designed. For example, nutritional mutations and mutations conferring resistance to drugs or phages can be obtained on plates supplemented with specific chemicals, drugs, or phages. Null mutations of any essential gene will result in no growth; these mutations can be selected by adding penicillin (an antibacterial drug isolated from a fungus), which kills dividing cells but not the nongrowing mutants. For conditional lethal mutations, replica plating can be used: mutated colonies on a master plate are transferred by a felt pad to other plates that are then subjected to some toxic environment. Mutations affecting the expression of a specific gene of interest can be screened by fusing it to a reporter gene such as the *lacZ* gene, whose protein product can make a blue dye, or the *GFP* gene, whose product fluoresces when exposed to light of a particular wavelength.

After a set of mutants affecting the process of interest have been obtained, the mutations are sorted into their genes by recombination and complementation. These genes are cloned and sequenced to obtain clues to function. Targeted mutagenesis can be used to tailor mutational changes at specific protein positions (see page 540).

In *E. coli*, crosses are used to map mutations and to produce specific cell genotypes (see Chapter 5). Recombinants are made by mixing Hfr cells (having an integrated F plasmid) and F<sup>-</sup> cells. Generally an Hfr donor transmits part of the bacterial genome, forming a temporary merozygote in which recombination takes place. Hfr crosses can be used to perform mapping by time-of-marker entry or by recombinant frequency. By transfer of F<sup>-</sup> derivatives carrying donor genes to F<sup>-</sup>, it is possible to make stable partial dipooids to study gene interaction or dominance.

#### Techniques of Genetic Modification

##### Standard mutagenesis:

Chemicals and radiation	Random somatic mutations
Transposons	Random somatic insertions

##### Transgenesis:

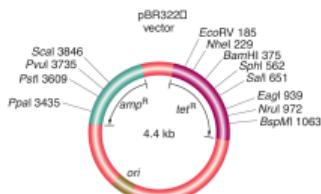
On plasmid vector	Free or integrated
On phage vector	Free or integrated
Transformation	Integrated

##### Targeted gene knockouts:

Null allele on vector	Gene replacement by recombination
Engineered allele on vector	Site-directed mutagenesis by gene replacement

#### Genetic engineering

**Transgenesis.** *E. coli* plays a key role in introducing transgenes to other organisms (see Chapter 10). It is the standard organism used for cloning genes of any organism. *E. coli* plasmids or bacteriophages are used as vectors, carrying the DNA sequence to be cloned. These vectors are introduced into a bacterial cell by transformation, if a plasmid, or by transduction, if a phage, where they replicate in the cytoplasm. Vectors are specially modified to include unique cloning sites that can be cut by a variety of restriction enzymes. Other “shuttle” vectors are designed to move DNA fragments from yeast (“the eukaryotic *E. coli*”) into *E. coli*, for its greater ease of genetic manipulation, and then back into yeast for phenotypic assessment.



**A plasmid designed as a vector for DNA cloning.** Successful insertion of a foreign gene into the plasmid is detected by inactivation of either drug-resistance gene (*tet*<sup>R</sup> or *amp*<sup>R</sup>). Restriction sites are identified.

**Targeted gene knockouts.** A complete set of gene knockouts is being accumulated. In one procedure, a kanamycin-resistance transposon is introduced into a cloned gene in vitro (by using a transposase). The construct is transformed in, and resistant colonies are knockouts produced by homologous recombination.

#### Main contributions

Pioneering studies for genetics as a whole were carried out in *E. coli*. Perhaps the greatest triumph was the elucidation of the universal 64-codon genetic code, but this achievement is far from alone on the list of accomplishments attributable to this organism. Other fundamentals of genetics that were first demonstrated in *E. coli* include the spontaneous nature of mutation (the fluctuation test, page 586), the various types of base changes that cause mutations, and the semiconservative replication of DNA (the Meselson and Stahl experiment, page 271). This bacterium helped open up whole new areas of genetics, such as gene regulation (the *lac* operon, page 401ff.) and DNA transposition (IS elements, page 553). Last but not least, recombinant DNA technology was invented in *E. coli*, and the organism still plays a central role in this technology today.

#### Other areas of contribution

- Cell metabolism
- Nonsense suppressors
- Colinearity of gene and polypeptide
- The operon
- Plasmid-based drug resistance
- Active transport

## ***Saccharomyces cerevisiae***

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# *Saccharomyces cerevisiae*

## Key organism for studying:

- Genomics
- Systems biology
- Genetic control of cell cycle
- Signal transduction
- Recombination
- Mating type
- Mitochondrial inheritance
- Gene interaction; two-hybrid



The ascomycete *S. cerevisiae*, alias "baker's yeast," "budding yeast," or simply "yeast," has been the basis of the baking and brewing industries since antiquity. In nature, it probably grows on the surfaces of plants, using exudates as nutrients, although its precise niche is still a mystery. Although laboratory strains are mostly haploid, cells in nature can be diploid or polyploid. In approximately 70 years of genetic research, yeast has become "the *E. coli* of the eukaryotes." Because it is haploid and unicellular, and forms compact colonies on plates, it can be treated in much the same way as a bacterium. However, it has eukaryotic meiosis, cell cycle, and mitochondria, and these features have been at the center of the yeast success story.

**Yeast cells, *Saccharomyces cerevisiae*.** [SciMAT/Science Source.]

## Special features

As a model organism, yeast combines the best of two worlds: it has much of the convenience of a bacterium, but with the key features of a eukaryote. Yeast cells are small (10  $\mu\text{m}$ ) and complete their cell cycle in just 90 minutes, allowing them to be produced in huge numbers in a short time. Like bacteria, yeast can be grown in large batches in a liquid medium that is continuously shaken. And, like bacteria, yeast produces visible colonies when plated on agar medium, can be screened for mutations, and can be replicated. In typical eukaryotic manner, yeast has a mitotic cell-division cycle, undergoes meiosis, and contains mitochondria housing a small unique genome. Yeast cells can respire anaerobically by using the fermentation cycle and hence can do without mitochondria, allowing mitochondrial mutants to be viable.

## Genetic analysis

Performing crosses in yeast is quite straightforward. Strains of opposite mating type are simply mixed on an appropriate medium. The resulting  $a/\alpha$  diploids are induced to undergo meiosis by using a special sporulation medium. Investigators can isolate ascospores from a single tetrad by using a machine called a micromanipulator. They also have the option of synthesizing  $a/a$  or  $\alpha/\alpha$  diploids for special purposes or creating partial diploids by using specially engineered plasmids.

Because a huge array of yeast mutants and DNA constructs are available within the research community, special-purpose strains for screens and selections can be built by crossing various yeast types. Additionally, new mutant alleles can be mapped by crossing with strains containing an array of phenotypic or DNA markers of known map position.

The availability of both haploid and diploid cells provides flexibility for mutational studies. Haploid cells are convenient for large-scale selections or screens because mutant phenotypes are expressed directly. Diploid cells are convenient for obtain-

## Genetic "Vital Statistics"

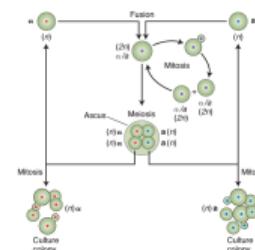
Genome size:	12 Mb
Chromosomes:	$n = 16$
Number of genes:	6000
Percentage with human homologs:	25%
Average gene size:	1.5 kb, 0.03 intron/gene
Transposons:	Small proportion of DNA
Genome sequenced in:	1996

ing dominant mutations, sheltering lethal mutations, performing complementation tests, and exploring gene interaction.

## Life Cycle

Yeast is a unicellular species with a very simple life cycle consisting of sexual and asexual phases. The asexual phase can be haploid or diploid. A cell divides asexually by budding: a mother cell throws off a bud into which is passed one of the nuclei that result from mitosis. For sexual reproduction, there are two mating types, determined by the alleles *MATr* and *MATa*. When haploid cells of different mating type unite, they form a diploid cell, which can divide mitotically or undergo meiotic division. The products of meiosis are a nonlinear tetrad of four ascospores.

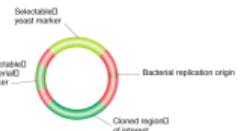
**Total length of life cycle:** 90 minutes to complete cell cycle



Techniques of Genetic Manipulation	
<b>Standard mutagenesis:</b>	
Chemicals and radiation	Random somatic mutations
Transposons	Random somatic insertions
<b>Transgenesis:</b>	
Integrative plasmid	Inserts by homologous recombination
Replicative plasmid	Can replicate autonomously ( $\lambda\mu$ or ARS origin of replication)
Yeast artificial chromosome	Replicates and segregates as a chromosome
Shuttle vector	Can replicate in yeast or <i>E. coli</i>
<b>Targeted gene knockouts:</b>	
Gene replacement	Homologous recombination replaces wild-type allele with null copy

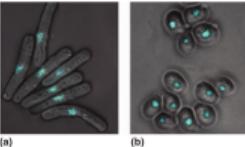
### Genetic engineering

**Transgenesis.** Budding yeast provides more opportunities for genetic manipulation than any other eukaryote (see Chapter 10). Exogenous DNA is taken up easily by cells whose cell walls have been partly removed by enzyme digestion or abrasion. Various types of vectors are available. For a plasmid to replicate free of the chromosomes, it must contain a normal yeast replication origin (ARS) or a replication origin from a  $2\text{-}\mu\text{m}$  plasmid found in certain yeast isolates. The most elaborate vector, the yeast artificial chromosome (YAC), consists of an ARS, a yeast centromere, and two telomeres. A YAC can carry large transgenic inserts, which are then inherited in the same way as Mendelian chromosomes. YACs have been important vectors in cloning and sequencing large genomes such as the human genome.



**A simple yeast vector.** This type of vector is called a yeast integrative plasmid (Yip).

**Targeted knockouts.** Transposon mutagenesis (transposon tagging) can be accomplished by introducing yeast DNA into *E. coli* on a shuttle vector; the bacterial transposons integrate into the yeast DNA, knocking out gene function. The shuttle vector is then transferred back into yeast, and the tagged mutants replace wild-type copies by homologous recombination. Gene knockouts can also be accomplished by replacing wild-type alleles with an engineered null copy through homologous recombination. By using these techniques, researchers have systematically constructed a complete set of yeast knockout strains (each carrying a different knockout) to assess null function of each gene at the phenotypic level.



**Cell-cycle mutants.** (a) Mutants that elongate without dividing. (b) Mutants that arrest without buckling. [Courtesy of Susan L. Forsburg, the Salk Institute. "The Art and Design of Genetic Screens: Yeast," *Nature Reviews Genetics* 2, 2001, 659–668.]

### Main contributions

Thanks to a combination of good genetics and good biochemistry, yeast studies have made substantial contributions to our understanding of the genetic control of cell processes.

**Cell cycle.** The identification of cell-division genes through their temperature-sensitive mutants (*cde* mutants) has led to a powerful model for the genetic control of cell division. The different *Cdc* phenotypes reveal the components of the machinery required to execute specific steps in the progression of the cell cycle. This work has been useful for understanding the abnormal cell-division controls that can lead to human cancer.

**Recombination.** Many of the key ideas for the current molecular models of crossing over (such as the double-strand-break model) are based on tetrad analysis of gene conversion in yeast (see page 155). Gene conversion (aberrant allele ratios such as 3:1) is quite common in yeast genes, providing an appropriately large data set for quantifying the key features of this process.

**Gene interactions.** Yeast has led the way in the study of gene interactions. The techniques of traditional genetics have been used to reveal patterns of epistasis and suppression, which suggest gene interactions (see Chapter 6). The two-hybrid plasmid system for finding protein interactions was developed in yeast and has generated complex interaction maps that represent the beginnings of systems biology (see page 537). Synthetic lethals—lethal double mutants created by intercrossing two viable single mutants—also are used to plot networks of interaction (see page 239).

**Mitochondrial genetics.** Mutants with defective mitochondria are recognizable as very small colonies called "petites." The availability of these petites and other mitochondrial mutants enabled the first detailed analysis of mitochondrial genome structure and function in any organism.

**Genetics of mating type.** Yeast *MAT* alleles were the first mating-type genes to be characterized at the molecular level. Interestingly, yeast undergoes spontaneous switching from one mating type to the other. A silent "spare" copy of the opposite *MAT* allele, residing elsewhere in the genome, enters into the mating-type locus, replacing the resident allele by homologous recombination. Yeast has provided one of the central models for signal transduction during detection and response to mating hormones from the opposite mating type.

### Other areas of contribution

- Genetics of switching between yeast-like and filamentous growth
- Genetics of senescence

## ***Neurospora crassa***

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# *Neurospora crassa*

## Key organism for studying:

- Genetics of metabolism and uptake
- Genetics of crossing over and meiosis
- Fungal cytogenetics
- Polar growth
- Circadian rhythms
- Interactions between nucleus and mitochondria



*Neurospora crassa* growing on sugarcane.  
[Courtesy of David Jacobson.]

## Special features

*Neurospora* holds the speed record for fungi because each hypha grows more than 10 cm per day. This rapid growth, combined with its haploid life cycle and ability to grow on defined medium, has made it an organism of choice for studying biochemical genetics of nutrition and nutrient uptake.

Another unique feature of *Neurospora* (and related fungi) allows geneticists to trace the steps of single meioses. The four haploid products of one meiosis stay together in a sac called an ascus. Each of the four products of meiosis undergoes a further mitotic division, resulting in a linear octad of eight ascospores (see page 103). This feature makes *Neurospora* an ideal system in which to study crossing over, gene conversion, chromosomal rearrangements, meiotic nondisjunction, and the genetic control of meiosis itself. Chromosomes, although small, are easily visible, and so meiotic processes can be studied at both the genetic and the chromosomal levels. Hence, in *Neurospora*, fundamental studies have been carried out on the mechanisms underlying these processes (see page 133).

## Genetic analysis

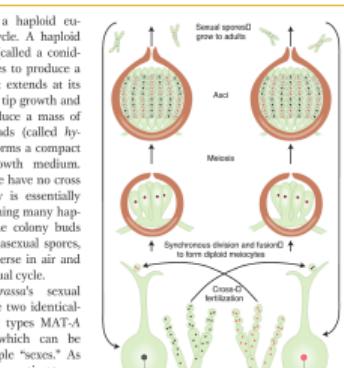
Genetic analysis is straightforward (see page 103). Stock centers provide a wide range of mutants affecting all aspects of the biology of the fungus. *Neurospora* genes can be mapped easily by crossing them with a bank of strains with known mutant loci or known RFLP alleles. Strains of opposite mating type are crossed simply by growing them together. A geneticist with a handheld needle can pick out a single ascospore for study. Hence, analyses in which

## Genetic "Vital Statistics"

Genome size:	43 Mb
Chromosomes:	7 autosomes ( $n = 7$ )
Number of genes:	10,000
Percentage with human homologs:	6%
Average gene size:	1.7 kb, 1.7 introns/gene
Transposons:	rare
Genome sequenced in:	2003

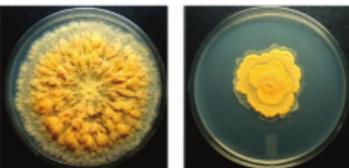
*Neurospora crassa*, the orange bread mold, was one of the first eukaryotic microbes to be adopted by geneticists as a model organism. Like yeast, it was originally chosen because of its haploidy, its simple and rapid life cycle, and the ease with which it can be cultured. Of particular significance was the fact that it will grow on a medium with a defined set of nutrients, making it possible to study the genetic control of cellular chemistry. In nature, it is found in many parts of the world growing on dead vegetation. Because fire activates its dormant ascospores, it is most easily collected after burns—for example, under the bark of burnt trees and in fields of crops such as sugar cane that are routinely burned before harvesting.

## Life Cycle



In *N. crassa*'s sexual cycle, there are two identical-looking mating types MAT-a and MAT-a, which can be viewed as simple "sexes." As in yeast, the two mating types are determined by two alleles of one gene. When colonies of different mating type come into contact, their cell walls and nuclei fuse. Many transient diploid nuclei arise, each of which undergoes meiosis, producing an octad of ascospores. The ascospores germinate and produce colonies exactly like those produced by asexual spores.

**Length of life cycle:** 4 weeks for sexual cycle



**Wild-type (left) and mutant (right) *Neurospora* grown in a petri dish.** [Courtesy of Anthony Griffith/Oliviera Cavalli.]

either complete ascii or random ascospores are used are rapid and straightforward.

Because *Neurospora* is haploid, newly obtained mutant phenotypes are easily detected with the use of various types of screens and selections. A favorite system for study of the mechanism of mutation is the *adl-3* gene, because *adl-3* mutants are pure and easily detected.

Although vegetative diploids of *Neurospora* are not readily obtainable, geneticists are able to create a "mimic diploid," useful for complementation tests and other analyses requiring the presence of two copies of a gene (see page 229). Namely, the fusion of two different strains produces a heterokaryon, an individual containing two different nuclear types in a common cytoplasm. Heterokaryons also enable the use of a version of the specific-locus test, a way to recover mutations in a specific recessive allele. (Cells from a *+/+* heterokaryon are plated and *m/m* colonies are sought.)

#### Techniques of Genetic Manipulation

##### Standard mutagenesis:

Chemicals and radiation	Random somatic mutations
Transposon mutagenesis	Not available

##### Transgenesis:

Plasmid-mediated transformation	Random insertion
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##### Targeted gene knockouts:

RIP	GC → AT mutations in transgenic duplicate segments before a cross
Quelling	Somatic posttranscriptional inactivation of transgenes

#### Genetic engineering

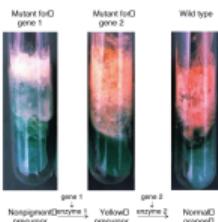
**Transgenesis.** The first eukaryotic transformation was accomplished in *Neurospora*. Today, *Neurospora* is easily transformed with the use of bacterial plasmids carrying the desired transgene, plus a selectable marker such as hygromycin resistance to show that the plasmid has entered. No plasmids replicate in *Neurospora*, and so a transgene is inherited only if it integrates into a chromosome.

**Targeted knockouts.** In special strains of *Neurospora*, transgenes frequently integrate by homologous recombination. Hence, a transgenic strain normally has the resident gene plus the homologous

transgene, inserted at a random ectopic location. Because of this duplication of material, if the strain is crossed, it is subject to RIP, a genetic process that is unique to *Neurospora*. RIP is a premeiotic mechanism that introduces many GC-to-AT transitions into both duplicate copies, effectively disrupting the gene. RIP can therefore be harnessed as a convenient way of deliberately knocking out a specific gene.

#### Main contributions

George Beadle and Edward Tatum used *Neurospora* as the model organism in their pioneering studies on gene–enzyme relations, in which they were able to determine the enzymatic steps in the synthesis of arginine (see page 224). Their work with *Neurospora* established the beginning of molecular genetics. Many comparable studies on the genetics of cell metabolism with the use of *Neurospora* followed.



#### Pathway synthesizing orange carotenoid pigment in *Neurospora*.

[Courtesy of Anthony Griffith.]

Pioneering work has been done on the genetics of meiotic processes, such as crossing over and disjunction, and on co-ordination rhythms. Continuously growing cultures show a daily rhythm of conidiospore formation. The results of pioneering studies using mutations that alter this rhythm have contributed to a general model for the genetics of circadian rhythms.

*Neurospora* serves as a model for the multitude of pathogenic filamentous fungi affecting crops and humans because these fungi are often difficult to culture and manipulate genetically. It is even used as a simple eukaryotic test system for mutagenic and carcinogenic chemicals in the human environment.

Because crosses can be made by using one parent as female, the cycle is convenient for the study of mitochondrial genetics and nucleus–mitochondria interaction. A wide range of linear and circular mitochondrial plasmids have been discovered in natural isolates. Some of them are retroelements that are thought to be intermediates in the evolution of viruses.

#### Other areas of contribution

- Fungal diversity and adaptation
- Cytogenetics (chromosomal basis of genetics)
- Mating-type genes
- Heterokaryon-compatibility genes (a model for the genetics of self and nonself recognition)

## ***Arabidopsis thaliana***

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## Arabidopsis thaliana

### Key organism for studying:

- Development
- Gene expression and regulation
- Plant genomics

### Genetic "Vital Statistics"

Genome size:	125 Mb
Chromosomes:	diploid, 5 autosomes ( $2n = 10$ )
Number of genes:	25,000
Percentage with human homologs:	18%
Average gene size:	2 kb, 4 introns/gene
Transposons:	10% of the genome
Genome sequenced in:	2000



*Arabidopsis thaliana*, a member of the Brassicaceae (cabbage) family of plants, is a relatively late arrival as a genetic model organism. Most work has been done in the past 20 years. It has no economic significance: it grows prolifically as a weed in many temperate parts of the world. However, because of its small size, short life cycle, and small genome, it has overtaken the more traditional genetic plant models such as corn and wheat and has become the dominant model for plant molecular genetics.

*Arabidopsis thaliana* growing in the wild. The versions grown in the laboratory are smaller. [iStock Images/Alamy.]

### Special features

In comparison with other plants, *Arabidopsis* is small in regard to both its physical size and its genome size—features that are advantageous for a model organism. *Arabidopsis* grows to a height of less than 10 cm under appropriate conditions; hence, it can be grown in large numbers, permitting large-scale mutant screens and progeny analyses. Its total genome size of 125 Mb made the genome relatively easy to sequence compared with other plant model organism genomes, such as the maize genome (2500 Mb) and the wheat genome (16,000 Mb).

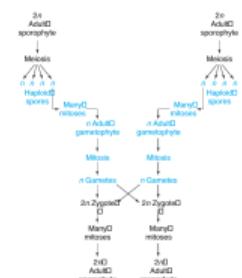
### Genetic analysis

The analysis of *Arabidopsis* mutations through crossing relies on tried and true methods—essentially those used by Mendel. Plant stocks carrying useful mutations relevant to the experiment in hand are obtained from public stock centers. Lines can be manually crossed with each other or self-fertilized. Although the flowers are small, cross-pollination is easily accomplished by removing undehisced anthers (which are sometimes eaten by the experimenter as a convenient means of disposal). Each pollinated flower then produces a long pod containing a large number of seeds. This abundant production of offspring (thousands of seeds per plant) is a boon to geneticists searching for rare mutants or other rare events. If a plant carries a new recessive mutation in the germ line, selfing allows progeny homozygous for the recessive mutation to be recovered in the plant's immediate descendants.

### Life Cycle

*Arabidopsis* has the familiar plant life cycle, with a dominant diploid stage. A plant bears several flowers, each of which produces many seeds. Like many annual weeds, its life cycle is rapid: it takes only about 6 weeks for a planted seed to produce a new crop of seeds.

Total length of life cycle: 6 weeks





**Arabidopsis mutants.** (Left) Wild-type flower of *Arabidopsis*. (Middle) The *agamous* mutation (*ag*), which results in flowers with only petals and sepals (no reproductive structures). (Right) A double-mutant *ap1*, *cal*, which makes a flower that looks like a cauliflower. (Similar mutations in cabbage are probably the cause of real cauliflower.) [George Haughn.]

#### Techniques of Genetic Modification

##### Standard mutagenesis:

Chemicals and radiation

Random germ-line or somatic mutations

##### T-DNA itself or transposons

Random tagged insertions

##### Transgenesis:

T-DNA carries the transgene

Random insertion

##### Targeted gene knockouts:

T-DNA or transposon-mediated mutagenesis

Random insertion; mutagenesis knockouts selected with PCR

##### RNAi

Mimics targeted knockout

#### Genetic engineering

**Transgenesis.** *Agrobacterium* T-DNA is a convenient vector for introducing transgenes (see Chapter 10). The vector-transgene construct inserts randomly throughout the genome. Transgenesis offers an effective way to study gene regulation. The transgene is spliced to a reporter gene such as GUS, which produces a blue dye at whatever positions in the plant the gene is active.

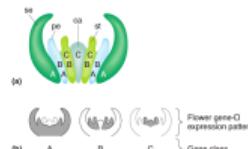
**Targeted knockouts.** Because homologous recombination is rare in *Arabidopsis*, specific genes cannot be easily knocked out by homologous replacement with a transgene. Hence, in *Arabidopsis*, genes are knocked out by the random insertion of a T-DNA vector or transposon (maize transposons such as *Ac-Ds* are used), and then specific gene knockouts are selected by applying PCR analysis to DNA from large pools of plants. The PCR uses a sequence in the T-DNA or in the transposon as one primer and a sequence in the gene of interest as the other primer. Thus, PCR amplifies only copies of the gene of interest that carry an insertion. Subdividing the pool and repeating the process lead to the specific plant carrying the knockout. Alternatively, RNAi may be used to inactivate a specific gene.

Large collections of T-DNA insertion mutants are available; they have the flanking plant sequences listed in public databases; so, if you are interested in a specific gene, you can see if the collection contains a plant that has an insertion in that gene. A convenient feature of knockout populations in plants is that they can be easily and inexpensively maintained as collections of seeds for

many years, perhaps even decades. This feature is not possible for most populations of animal models. The worm *Ceaeorhabditis elegans* can be preserved as a frozen animal, but fruit flies (*Drosophila melanogaster*) cannot be frozen and revived. Thus, lines of fruitfly mutants must be maintained as living organisms.

#### Main contributions

As the first plant genome to be sequenced, *Arabidopsis* has provided an important model for plant genome architecture and evolution. In addition, studies of *Arabidopsis* have made key contributions to our understanding of the genetic control of plant development. Geneticists have isolated homeotic mutations affecting flower development, for example. In such mutants, one type of floral part is replaced by another. Integration of the action of these mutants has led to an elegant model of flower-whorl determination based on overlapping patterns of regulatory-gene expression in the flower meristem. *Arabidopsis* has also contributed broadly to the genetic basis of plant physiology, gene regulation, and the interaction of plants and the environment (including the genetics of disease resistance). Because *Arabidopsis* is a natural plant of worldwide distribution, it has great potential for the study of evolutionary diversification and adaptation.



**The establishment of whorl fate.** (a) Patterns of gene expression corresponding to the different whorl fates. From outermost to innermost, the fates are sepal (se), petal (pe), stamen (st), and carpel (ca). (b) The shaded regions of the cross-sectional diagrams of the developing flower indicate the gene-expression patterns for the genes of the A, B, and C classes.

#### Other areas of contribution

- Environmental-stress response
- Hormone control systems

## ***Caenorhabditis elegans***

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## Introduction

- *Caenorhabditis elegans* is a free-living, transparent nematode, about 1 mm in length, that lives in temperate soil environments.
- In 1955, Dougherty raised *Caenorhabditis* to the status of genus.
- *C. elegans* is an unsegmented pseudocoelomate and lacks respiratory or circulatory systems.
- Most of these nematodes are **hermaphrodites** and a few are males.
- Males have specialised tails for mating that include spicules.
- In 1963, Sydney Brenner proposed research into *C. elegans* primarily in the area of neuronal development. In 1974, he began research into the molecular and developmental biology of *C. elegans*, which has since been extensively used as a model organism.
- It was the first multicellular organism to have its whole genome sequenced, and as of 2012, is the only organism to have its connectome (neuronal “wiring diagram”) completed.

## Taxonomy and classification

## Morpho-anatomy of adult and lifecycle of worm

- The wild-type nematode *Caenorhabditis elegans* has an elongated and spindle-shaped body, whereas the dumpy mutant has a shorter body with approximately the same diameter.
- A temperature-sensitive morphological mutant which develops into an adult with dumpy phenotype, at 15°C, but with roller phenotype at 25°C, in addition to dumpy phenotype, was isolated.
- The heterozygous offspring (F1), produced by crossing the mutant hermaphrodite with wild-type males, were rollers in both hermaphrodites and males.

[Image of adult]

- It has a cuticle (a tough outer covering, as an exoskeleton), four main epidermal cords, and a fluid-filled pseudocoelom (body cavity).
- It also has some of the same organ systems as larger animals. About one in a thousand individuals is male and the rest are hermaphrodites.
- The basic anatomy of *C. elegans* includes a mouth, pharynx, intestine, gonad, and collagenous cuticle.

## Genetics

- The genome sequence was published in 1998, although some small gaps were present; the last gap was finished by October 2002.
- Neurons of humans and *C. elegans* are almost identical. Both human and *C. elegans* neurons contain a dendrite which extends from the cell to receive neurotransmitters, and extends to the nerve ring or brain for a synaptic connection between neurons.
- The biggest difference is that *C. elegans* has motor excitatory and inhibitory neurons.
- The *C. elegans* genome is about 100 million base pairs long and consists of six chromosomes and a mitochondrial genome.
- Its gene density is about one gene per five kilo-base pairs. Introns make up 26% and intergenic regions 47% of the genome.
- *C. elegans* and other nematodes are among the few eukaryotes currently known to have operons; these include trypanosomes, flatworms (notably the trematode *Schistosoma mansoni*), and a primitive chordate tunicate .
- The genome contains an estimated 20,470 protein-coding genes. About 35% of *C.*

## Reproduction

- The hermaphroditic worm is considered to be a specialized form of self-fertile female, as its soma is female.
- The hermaphroditic germline produces male gametes first, and lays eggs through its uterus after internal fertilization.
- Hermaphrodites produce all their sperm in the L4 stage (150 sperm cells per gonadal arm) and then produce only oocytes.
- The hermaphroditic gonad acts as an ovotestis with sperm cells being stored in the same area of the gonad as the oocytes until the first oocyte pushes the sperm into the spermatheca (a chamber wherein the oocytes become fertilized by the sperm).
- Once a male recognizes hermaphrodite worm, it begins tracing the hermaphrodite with his tail until it reaches the vulval region. The male then probes the region with its spicules to locate the vulva, inserts them, and releases sperm.

## Studies

- First studied in the laboratory by Victor Nigon and Ellsworth Dougherty in the 1940s.
- Adopted by Sydney Brenner in 1963 as a model organism for the study of developmental biology using genetics.
- In the 1980s, John Sulston and co-workers identified the lineage of all 959 cells in the adult hermaphrodite, the first genes were cloned, and the physical map began to be constructed.
- Notable research using *C. elegans* includes the discoveries of caspases, RNA interference, and microRNAs. Six scientists have won the Nobel prize for their work on *C. elegans*.
- *C. elegans* made news when specimens were discovered to have survived the Space Shuttle Columbia disaster in February 2003.



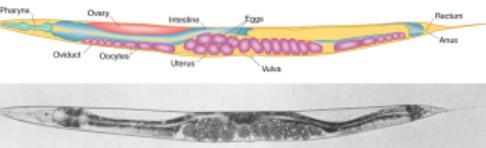
# *Caenorhabditis elegans*

- Key organism for studying:
- Development
  - Behavior
  - Nerves and muscles
  - Aging

## Genetic "Vital Statistics"

Genome size:	97 Mb
Chromosomes:	5 autosomes ( $2n = 10$ ), X chromosome
Number of genes:	19,000
Percentage with human homologs:	25%
Average gene size:	5 kb, 5 exons/gene
Transposons:	Several types, active in some strains
Genome sequenced in:	1998

*Caenorhabditis elegans* may not look like much under a microscope, and, indeed, this 1-mm-long soil-dwelling roundworm (a nematode) is relatively simple as animals go. But that simplicity is part of what makes *C. elegans* a good model organism. Its small size, rapid growth, ability to self, transparency, and low number of body cells have made it an ideal choice for the study of the genetics of eukaryotic development.



Photomicrograph and drawing of an adult *Caenorhabditis elegans*.

[From J. E. Sulston and H. R. Horvitz, *Developmental Biology*, 56, 1977, 111.]

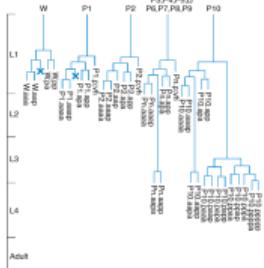
## Special features

Geneticists can see right through *C. elegans*. Unlike other multicellular model organisms, such as fruit flies or *Arabidopsis*, this tiny worm is transparent, making it efficient to screen large populations for interesting mutations affecting virtually any aspect of anatomy or behavior. Transparency also lends itself well to studies of development: researchers can directly observe all stages of development

simply by watching the worms under a light microscope. The results of such studies have shown that *C. elegans*'s development is tightly programmed and that each worm has a surprisingly small and consistent number of cells (959 in hermaphrodites and 1031 in males). In fact, biologists have tracked the fates of specific cells as the worm develops and have determined the exact pattern of cell division leading to each adult organ. This effort has yielded a lineage pedigree for every adult cell (see page 497).

## Genetic analysis

Because the worms are small and reproduce quickly and prolifically (selfing produces about 300 progeny and crossing yields



A symbolic representation of the lineages of 11 cells. A cell that undergoes programmed cell death is indicated by a blue X at the end of a branch of a lineage.

## Life Cycle

*C. elegans* is unique among the major model animals in that one of the two sexes is hermaphrodite (XX). The other is male (XO). The two sexes can be distinguished by the greater size of the hermaphrodites and by differences in their sex organs. Hermaphrodites produce both eggs and sperm, and so they can be selfed. The progeny of a selfed hermaphrodite also are hermaphrodites, except when a rare nondisjunction leads to an XO male. If hermaphrodites and males are mixed, the sexes copulate, and many of the resulting zygotes will have been fertilized by the males' amoeboid sperm. Fertilization and embryo production take place within the hermaphrodite, which then lays the eggs. The eggs finish their development externally.

Total length of life cycle:  $3\frac{1}{2}$  days

about 1000), they produce large populations of progeny that can be screened for rare genetic events. Moreover, because hermaphrodites in *C. elegans* make selfing possible, individual worms with homozygous recessive mutations can be recovered quickly by selfing the progeny of treated individual worms. In contrast, other animal models, such as fruit flies or mice, require matings between siblings and take more generations to recover recessive mutations.

#### Techniques of Genetic Modification

##### Standard mutagenesis:

Chemical (EMS) and radiation	Random germ-line mutations
Transposons	Random germ-line insertions

##### Transgenesis:

Transgene injection of gonad	Unintegrated transgene array; occasional integration
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##### Targeted gene knockouts:

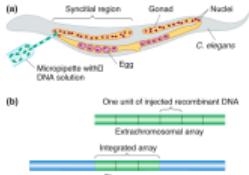
Transposon-mediated mutagenesis	Knockouts selected with PCR
RNAi	Mimics targeted knockout
Laser ablation	Knockout of one cell

#### Genetic engineering

**Transgenesis.** The introduction of transgenes into the germ line is made possible by a special property of *C. elegans* gonads. The nuclei of the worm are syncytial, meaning that there are many nuclei in a common cytoplasm. The nuclei do not become incorporated into cells until meiosis, when formation of the individual egg or sperm begins. Thus, a solution of DNA containing the transgene injected into the gonad of a hermaphrodite exposes more than 100 germ-cell precursor nuclei to the transgene. By chance, a few of these nuclei will incorporate the DNA (see Chapter 10).

Transgenes recombine to form multicopy tandem arrays. In an egg, the arrays do not integrate into a chromosome, but transgenes from the arrays are still expressed. Hence, the gene carried on a wild-type DNA clone can be identified by introducing it into a specific recessive recipient strain (functional complementation). In some but not all cases, the transgenic arrays are passed on to progeny. To increase the chance of inheritance, worms are exposed to ionizing radiation, which can induce the integration of an array into an ectopic chromosomal position, and, in this site, the array is reliably transmitted to progeny.

**Targeted knockouts.** In strains with active transposons, the transposons themselves become agents of mutation by inserting into random locations in the genome, knocking out the interrupted genes. If we can identify organisms with insertions into a specific gene of interest, we can isolate a targeted gene knockout. Inserts into specific genes can be detected by using PCR if one PCR primer is based on the transposon sequence and another one is based on the sequence of the gene of interest. Alternatively, RNAi can be used to nullify the function of specific genes. As an alternative to mutation, individual cells can be killed by a laser beam to observe the effect on worm function or development (laser ablation).

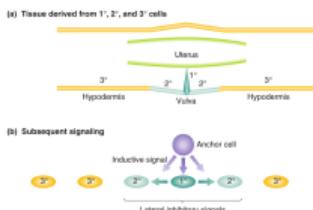


**Creation of *C. elegans* transgenes.** (a) Method of injection. (b) Extrachromosomal and integrated arrays.

#### Main contributions

*C. elegans* has become a favorite model organism for the study of various aspects of development because of its small and invariant number of cells. One example is programmed cell death, a crucial aspect of normal development. Some cells are genetically programmed to die in the course of development (a process called apoptosis). The results of studies of *C. elegans* have contributed a useful general model for apoptosis, which is also known to be a feature of human development.

Another model system is the development of the vulva, the opening to the outside of the reproductive tract. Hermaphrodites with defective vulvas still produce progeny, which in screens are easily visible clustered within the body. The results of studies of hermaphrodites with no vulva or with too many have revealed how cells that start off completely equivalent can become differentiated into different cell types (see page 497).



**Production of the *C. elegans* vulva.** (a) The final differentiated tissue. (b) Method of differentiation. The cells begin completely equivalent. An anchor cell behind the equivalent cells sends a signal to the nearest cells, which become the vulva. The primary vulva cell then sends a lateral signal to its neighbors, preventing them from becoming primary cells, even though they, too, have received the signal from the anchor cell.

Behavior also has been the subject of genetic dissection. *C. elegans* offers an advantage in that worms with defective behavior can often still live and reproduce. The worm's nerve and muscle systems have been genetically dissected, allowing behavior to be linked to specific genes.

#### Other area of contribution

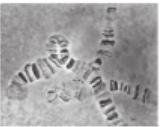
- Cell-to-cell signaling

## **Drosophila melanogaster**

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# Drosophila melanogaster

- Key organism for studying:
- Transmission genetics
  - Cytogenetics
  - Development
  - Population genetics
  - Evolution



Polytene chromosomes. [William M. Gelbart, Harvard University.]

## Genetic "Vital Statistics"

Genome size:	180 Mb
Chromosomes:	Diploid, 3 autosomes, X and Y ( $2n = 8$ )
Number of genes:	13,000
Percentage of human homologs:	~ 50%
Average gene size:	3 kb, 4 exons/gene
Transposons:	<i>P</i> elements, among others
Genome sequenced in:	2000

The fruit fly *Drosophila melanogaster* (loosely translated as “dusky syrup-lover”) was one of the first model organisms to be used in genetics. It was chosen in part because it is readily available from ripe fruit, has a short life cycle of the diploid type, and is simple to culture and cross in jars or vials containing a layer of food. Early genetic analysis showed that its inheritance mechanisms have strong similarities to those of other eukaryotes, underlining its role as a model organism. Its popularity as a model organism went into decline during the years when *E. coli*, yeast, and other microorganisms were being developed as molecular tools. However, *Drosophila* has experienced a renaissance because it lends itself so well to the study of the genetic basis of development, one of the central questions of biology. *Drosophila*'s importance as a model for human genetics is demonstrated by the discovery that approximately 60 percent of known disease-causing genes in humans, as well as 70 percent of cancer genes, have counterparts in *Drosophila*.

## Special features

*Drosophila* came into vogue as an experimental organism in the early twentieth century because of features common to most model organisms. It is small (3 mm long), simple to raise (originally, in milk bottles), quick to reproduce (only 12 days from egg to adult), and easy to obtain (just leave out some rotting fruit). It proved easy to amass a large range of interesting mutant alleles that were used to lay the ground rules of transmission genetics. Early researchers also took advantage of a feature unique to the fruit fly: polytene chromosomes (see page 638). In salivary glands and certain other tissues, these “giant chromosomes” are produced by multiple rounds of DNA replication without chromosomal segregation. Each polytene chromosome displays a unique banding pattern, providing geneticists with landmarks that could be used to correlate recombination-based maps with actual chromosomes. The momentum provided by these early advances, along with the large amount of accumulated knowledge about the organism, made *Drosophila* an attractive genetic model.

## Genetic analysis

Crosses in *Drosophila* can be performed quite easily. The parents may be wild or mutant stocks obtained from stock centers or as new mutant lines.

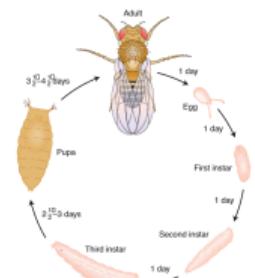


Two morphological mutants of *Drosophila*, with the wild type for comparison.

## Life Cycle

*Drosophila* has a short diploid life cycle that lends itself well to genetic analysis. After hatching from an egg, the fly develops through several larval stages and a pupal stage before emerging as an adult, which soon becomes sexually mature. Sex is determined by X and Y sex chromosomes (XX is female, XY is male), although, in contrast with humans, the number of X's in relation to the number of autosomes determines sex (see page 54).

**Total length of life cycle:** 12 days from egg to adult



To perform a cross, males and females are placed together in a jar, and the females lay eggs in semisolid food covering the jar's bottom. After emergence from the pupae, offspring can be anesthetized to permit counting members of phenotypic classes and to distinguish males and females (by their different abdominal stripe patterns). However, because female progeny stay virgin for only a few hours after emergence from the pupae, they must immediately be isolated if they are to be used to make controlled crosses. Crosses designed to build specific gene combinations must be carefully planned, because crossing over does not take place in *Drosophila* males. Hence, in the male, linked alleles will not recombine to help create new combinations.

For obtaining new recessive mutations, special breeding programs (of which the prototype is Muller's CIB test) provide convenient screening systems. In these tests, mutagenized flies are crossed with a stock having a balancer chromosome (see page 645). Recessive mutations are eventually brought to homozygosity by inbreeding for one or two generations, starting with single F<sub>1</sub> flies.

#### Techniques of Genetic Modification

##### Standard mutagenesis:

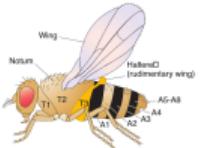
Chemical (EMS) and radiation      Random germ-line and somatic mutations

##### Transgenesis:

P element mediated      Random insertion

##### Targeted gene knockouts:

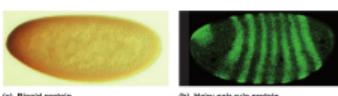
Induced replacement      Null ectopic allele exists and recombinates with wild-type allele  
RNAi      Mimics targeted knockout



The normal thoracic and abdominal segments of *Drosophila*.

Their discoveries opened the door to other pioneering studies:

- Early studies on the kinetics of mutation induction and the measurement of mutation rates were performed with the use of *Drosophila*. Muller's CIB test and similar tests provided convenient screening methods for recessive mutations.
- Chromosomal rearrangements that move genes adjacent to heterochromatin were used to discover and study position-effect variegation.
- In the last part of the twentieth century, after the identification of certain key mutational classes such as homeotic and maternal-effect mutations, *Drosophila* assumed a central role in the genetics of development, a role that continues today (see Chapter 13). Maternal-effect mutations that affect the development of embryos, for example, have been crucial in the elucidation of the genetic determination of the *Drosophila* body plan; these mutations are identified by screening for abnormal developmental phenotypes in the embryos from a specific female. Techniques such as enhancer trap screens have enabled the discovery of new regulatory regions in the genome that affect development. Through these methods and others, *Drosophila* biologists have made important advances in understanding the determination of segmentation and of the body axes. Some of the key genes discovered, such as the homeotic genes, have widespread relevance in animals generally.



Photomicrographs showing gradients of body plan determinants.

(a) mRNA for the gene *bcd* is shown localized to the anterior (left-hand) tip of the embryo. (b) mRNA of the *nos* gene is localized to the posterior (right-hand tip of the embryo). The distribution of the proteins encoded by these genes and other genes determines the body axis.  
[(a) Courtesy of Ruth Lehmann; (b) Courtesy of James Langford.]

#### Genetic engineering

**Transgenesis.** Building transgenic flies requires the help of a *Drosophila* transposon called the *P* element. Geneticists construct a vector that carries a transgene flanked by *P*-element repeats. The transgene vector is then injected into a fertilized egg along with a helper plasmid containing a transposase. The transposase allows the transgene to jump randomly into the genome in germinal cells of the embryo (see Chapter 15).

**Targeted knockouts.** Targeted gene knockouts can be accomplished by, first, introducing a null allele transgenically into an ectopic position and, second, inducing special enzymes that cause excision of the null allele. The excised fragment (which is linear) then finds and replaces the endogenous copy by homologous crossing over. However, functional knockouts can be produced more efficiently by RNAi.

#### Main contributions

Much of the early development of the chromosome theory of heredity was based on the results of *Drosophila* studies. Geneticists working with *Drosophila* made key advances in developing techniques for gene mapping, in understanding the origin and nature of gene mutation, and in documenting the nature and behavior of chromosomal rearrangements (see pages 130 and 135).

#### Other areas of contribution

- Population genetics
- Evolutionary genetics
- Behavioral genetics

## **Mus musculus**

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- *Mus musculus* is a small mammal of the order Rodentia, characteristically having a pointed snout, large rounded ears, and a long and hairy tail.
- Mouses dates long back on the genetic research as a model organism
- Out of the order rodentia mouse is a organism with short breeding period.
- They are widely used to study human genetic disorders (downs syndrome, cystic fibrosis, epilepsy, heart diseases, cancers, tumours and many more).

## Classification and taxonomy

- The complete mouse genome was discovered in 2002 AD.
- Chromosome number ( $2n$ ): 40;  $n=20$
- The haploid genome is about 3 picograms, similar to humans.
- Unlike the mostly metacentric chromosomes of humans, all mouse chromosomes are acrocentric.
- Haploid chromosome contains 3.1 billion base pairs long
- Genome length (3000 mb distributed over 19 autosomes plus one and two sex chromosome).
- The primary coding genes are 23,139 out of which 20210 protein coding genes are found.

# Chromosomes

[Images]

## Morphology

- A mouse is a small with pointed nose, furry round body, large ears and a long often hairless tail.
- There are hundred types of mice divided into subfamilies of either old World or new world species.
- Common varieties includes deermouse, housemouse, wood mouse, spingmose and zebramouse.
- Colour of mouse ranges from white,brown to grey
- Length: 1 to 7 inches
- Weight: 0.23kg to 0.28kg
- Smallest mouse: African pygmy measure (1.2-3.1 inches).
- Some mice have tail that are as long as their body.

## Reproduction

- *Mus musculus* is characterized by tremendous reproductive potential.
- Breeding occurs throughout the year. A single female is capable of producing up to eight litters per year. Female can reproduce up to ten times per year.
- Females go into heat for four to five days. Once pregnant, rodent gives birth after three weeks.
- At about 21 days, the young are weaned from their mothers and may begin to take short trips away from nest to explore their surroundings.
- Most mice reach sexual maturity at about 35 days age and begin start mating when they are six weeks old.

## Suitability

- A model organism is a species that has been widely studied, usually because it is easy to maintain and breed in a laboratory setting and has particular experimental advantages.
- *Mus musculus* represents humans and a broad range of mammals as they are closely related in terms of anatomy, physiology and genetics.
- They are widely used as disease models for humans.
- They can breed large number of off-spring in a short period of time.
- The similar sized gene/genome of mouse and humans gave preference to mouse in genetic studies.
- They are easy to look after and care and are cost effective.
- The time between a mouse being born and giving birth is short, usually around 10 weeks. Thus several generations can be observed at once.
- Immunodeficient mice can also be used as hosts to grow both normal and diseased normal human tissue. This has been a useful tools in cancer and AIDS research.

## Studies

- William Harvey studied blood circulation and reproduction in 16th century.
- Gregor Mendel carried out his early investigations of inheritance on mouse coat color but was asked by his superior to stop breeding in his cell “smelly creatures that, in addition, copulated and had sex”
- Frederick Griffith in 1928 conducted an experiment on mouse and found that bacteria are capable of transferring genetic information through a process known as transformation.
- In 1902 Lucien Cuer not published the results of his experiments using mice which showed that Mendel's laws of inheritance were also valid for animals.
- Mouse has been used as a biological model from early 20th century as the three inbred lines DBA (dilute, brown and non-agouti colored) were discovered.
- In recent studies, researchers have generated blastocyst like structure from stem cell; blastocyst like structure resemble very closely to real blastocysts.
- Methods have been developed for successfully generating hair growth in nude mice, this has a potential hair loss treatment in human.
- Study have related good health in mice to plenty of *Clostridia* in its gut, at the

# Mus musculus

- Key organism for studying:
- Human disease
  - Mutation
  - Development
  - Coat color
  - Immunology

## Genetic "Vital Statistics"

Genome size:	2600 Mb
Chromosomes:	19 autosomes, X and Y ( $2n = 40$ )
Number of genes:	30,000
Percentage with human homologs:	99%
Average gene size:	40 kb, 8.3 exons/gene
Transposons:	Source of 38% of genome
Genome sequenced in:	2002



Because humans and most domesticated animals are mammals, the genetics of mammals is of great interest. However, mammals are not ideal for genetics: they are relatively large in size compared with other model organisms, thereby taking up large and expensive facilities, their life cycles are long, and their genomes are large and complex. Compared with other mammals, however, mice (*Mus musculus*) are relatively small, have short life cycles, and are easily obtained, making them an excellent choice for a mammal model. In addition, mice had a head start in genetics because mouse "fanciers" had already developed many different interesting lines of mice that provided a source of variants for genetic analysis. Research on the Mendelian genetics of mice began early in the twentieth century.

An adult mouse and its litter. [Anthony Griffiths.]

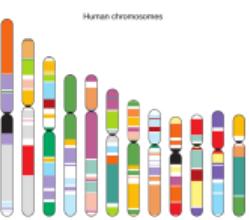
## Special features

Mice are not exactly small, furry humans, but their genetic makeup is remarkably similar to ours. Among model organisms, the mouse is the one whose genome most closely resembles the human genome. The mouse genome is about 14 percent smaller than that of humans (the human genome is 3000 Mb), but it has approximately the same number of genes (current estimates are just under 30,000). A surprising 99 percent of mouse genes seem to have homologs in humans. Furthermore, a large proportion of the genome is synteny with that of humans; that is, there are large blocks containing the same genes in the same relative positions (see page 531). Such genetic similarities are the key to

the mouse's success as a model organism; these similarities allow mice to be treated as "stand-ins" for their human counterparts in many ways. Potential mutagens and carcinogens that we suspect of causing damage to humans, for example, are tested on mice, and mouse models are essential in studying a wide array of human genetic diseases.

## Genetic analysis

Mutant and "wild type" (though not actually from the wild) mice are easy to come by: they can be ordered from large stock centers that provide mice suitable for crosses and various other types of experiments. Many of these lines are derived from mice bred in past centuries by mouse fanciers. Controlled crosses can be performed simply by pairing a male with a nonpregnant female. In most cases, the parental genotypes can be provided by male or female.

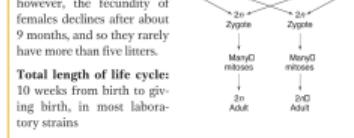
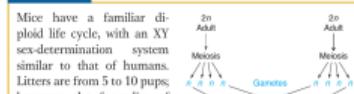


A mouse-human synteny map of 12 chromosomes from the human genome. Color coding is used to depict the regional matches of each block of the human genome to the corresponding sections of the mouse genome. Each color represents a different mouse chromosome.

## Life Cycle

Mice have a familiar diploid life cycle, with an XY sex-determination system similar to that of humans. Litters are from 5 to 10 pups; however, the fecundity of females declines after about 9 months, and so they rarely have more than five litters.

**Total length of life cycle:**  
10 weeks from birth to giving birth, in most laboratory strains



Most of the standard estimates of mammalian mutation rates (including those of humans) are based on measurements in mice. Indeed, mice provide the final test of agents suspected of causing mutations in humans. Mutation rates in the germ line are measured with the use of the specific-locus test: mutagenize  $+/+$  gonads, cross to  $m/m$  (m is a known recessive mutation at the locus under study), and look for  $m^+/m$  progeny ( $m^+$  is a new mutation). The procedure is repeated for seven sample loci. The measurement of somatic mutation rates uses a similar setup, but the mutagen is injected into the fetus. Mice have been used extensively to study the type of somatic mutation that gives rise to cancer.

#### Techniques of Genetic Modification

##### Standard mutagenesis:

Chemicals and radiation Germ-line and somatic mutations

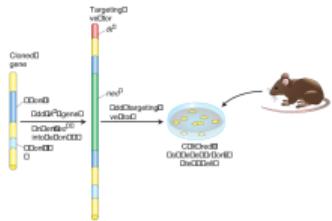
##### Transgenesis:

Transgene injection into zygote Random and homologous insertion  
Transgene uptake by stem cells Random and homologous insertion

##### Targeted gene knockouts:

Null transgene uptake by stem cells Targeted knockout stem cells selected

#### Production of ES cells with a gene knockout



**Producing a gene knockout.** A drug-resistance gene ( $neo^R$ ) is inserted into the transgene, both to serve as a marker and to disrupt the gene, producing a knockout. (The  $tk$  gene is a second marker.) The transgene construct is then injected into mouse embryo cells.

containing a defective allele and two drug-resistance markers into a wild-type embryonic stem cell (see Chapter 10). The markers are used to select those specific transformant cells in which the defective allele has replaced the homologous wild-type allele. The transgenic cells are then introduced into mouse embryos. A similar method can be used to replace wild-type alleles with a functional transgene (gene therapy).

#### Main contributions

Early in the mouse's career as a model organism, geneticists used mice to elucidate the genes that control coat color and pattern, providing a model for all fur-bearing mammals, including cats, dogs, horses, and cattle (see page 221). More recently, studies of mouse genetics have made an array of contributions with direct bearing on human health:

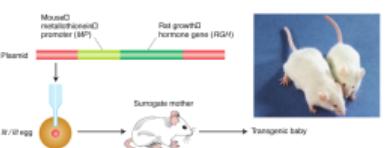
- A large proportion of human genetic diseases have a mouse counterpart—called a “mouse model”—useful for experimental study.
- Mice serve as models for the mechanisms of mammalian mutation.
- Studies on the genetic mechanisms of cancer are performed on mice.
- Many potential carcinogens are tested on mice.
- Mice have been important models for the study of mammalian developmental genetics (see page 480). For example, they provide a model system for the study of genes affecting cleft lip and cleft palate, a common human developmental disorder.
- Cell lines that are fusion hybrids of mouse and human genomes played an important role in the assignment of human genes to specific human chromosomes. There is a tendency for human chromosomes to be lost from such hybrids, and so loss of specific chromosomes can be correlated with loss of specific human alleles.

#### Other areas of contribution

- Behavioral genetics
- Quantitative genetics
- The genes of the immune system

#### Genetic engineering

**Transgenesis.** The creation of transgenic mice is straightforward but requires the careful manipulation of a fertilized egg (see Chapter 10). First, mouse genomic DNA is cloned in *E. coli* with the use of bacterial or phage vectors. The DNA is then injected into a fertilized egg, where it integrates at ectopic (random) locations in the genome or, less commonly, at the normal locus. The activity of the transgene's protein can be monitored by fusing the transgene with a reporter gene such as *GFP* before the gene is injected. With the use of a similar method, the somatic cells of mice also can be modified by transgene insertion: specific fragments of DNA are inserted into individual somatic cells and these cells are, in turn, inserted into mouse embryos.



**Producing a transgenic mouse.** The transgene, a rat growth-hormone gene joined to a mouse promoter, is injected into a mouse egg homozygous for dwarfism ( $b/b$ ). (Photo: R. L. Brunster, School of Veterinary Medicine, University of Pennsylvania.)

**Targeted knockouts.** Knockouts of specific genes for genetic dissection can be accomplished by introducing a transgene

# Corn

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**Figure 1:** Ancestor of modern day maize.



**Figure 2:** Male inflorescence of a maize plant



**Figure 3:** Maize crop (ear)

- Lower plants have haploid condition for the major part of the life cycle, with different haploid mating types producing the gametes that combine to form a diploid zygote.
- Propagation of diploid chromosome constitution of a zygote to a later stage of life cycle is called the sporophytic generation because some diploid cells (sporocytes) undergo meiosis to form haploid spores (sporogenesis).
- These spores, although not gametes, multiply mitotically to yield the gametophytes (haploid male and female stages) which, in turn, form gametes.
- In maize (or angiosperms), as the plants reach in this progression, the haploid gametophytes are reduced in size as to be microscopic.

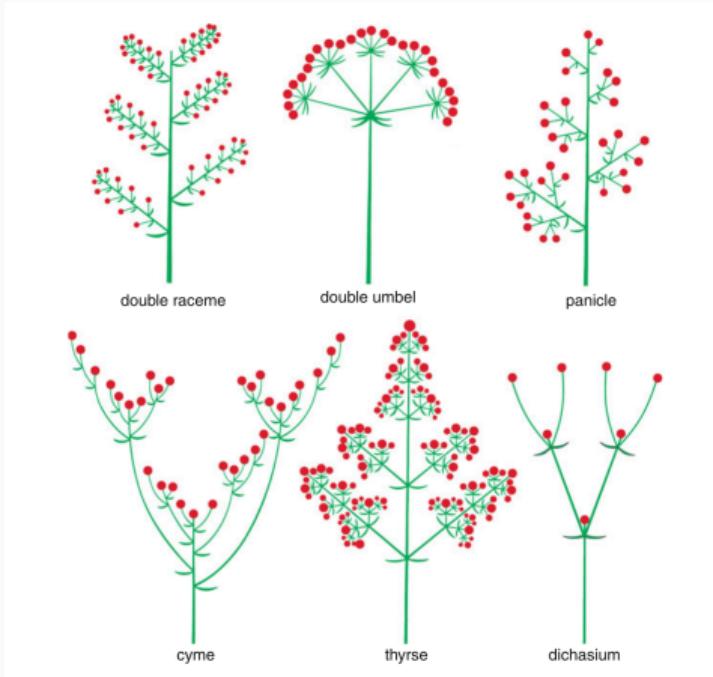
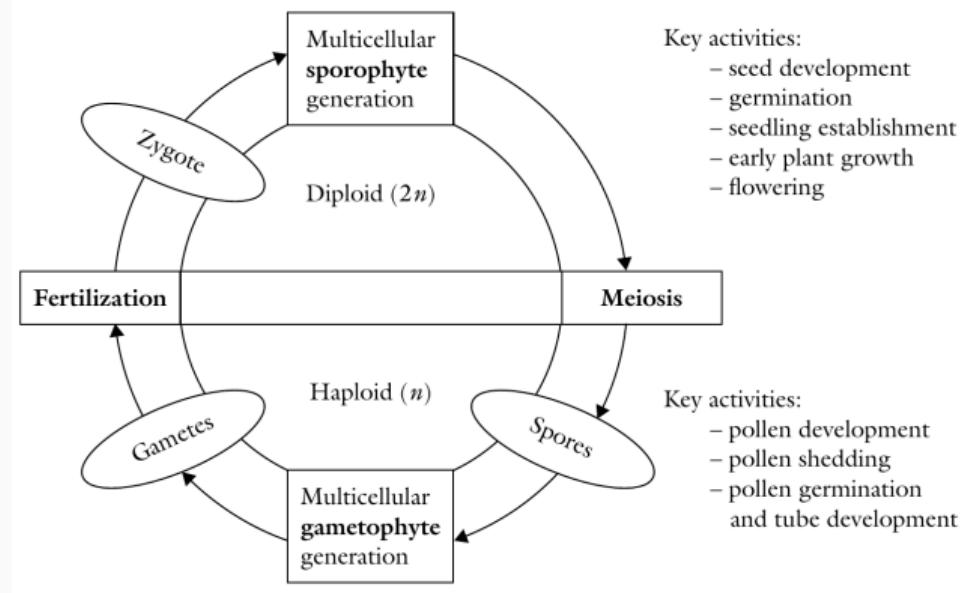


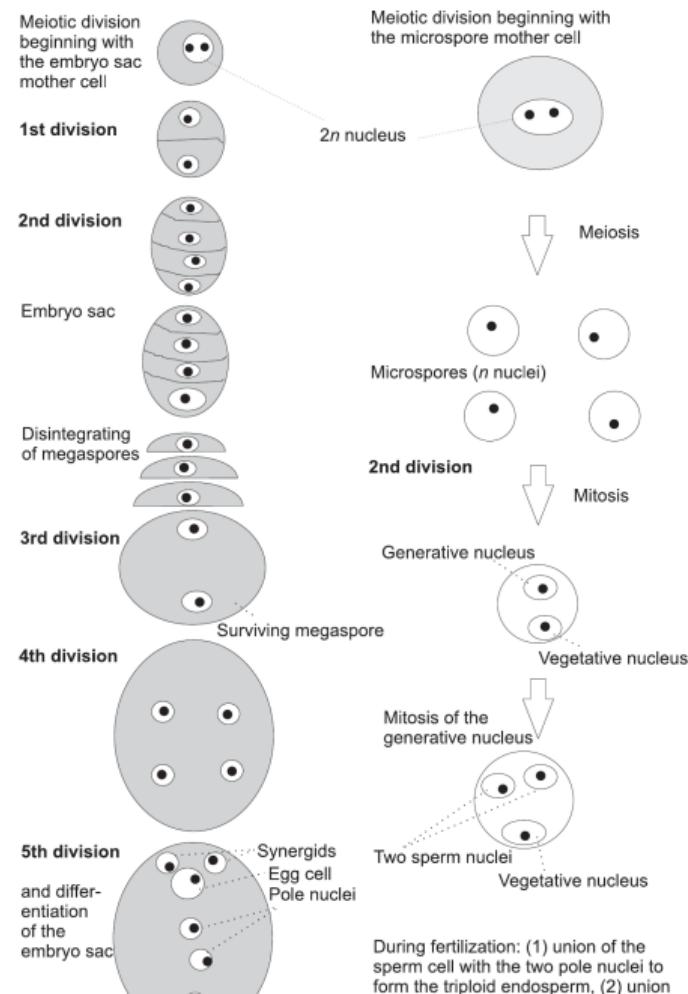
Figure 4: Types of compound inflorescences

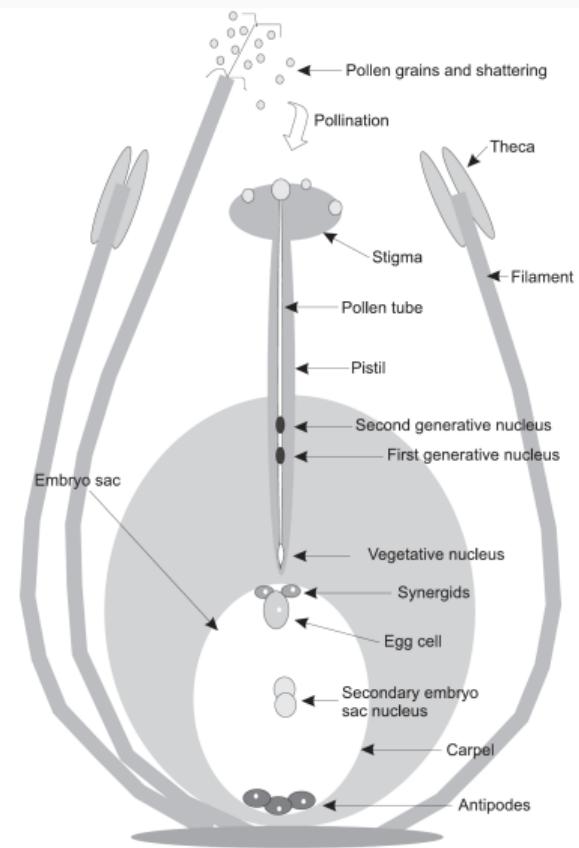
- In corn two types of gametophytes are represented by small **microspores** in the stamens (found in tassels), and by large **megaspores** in the pistils (found in developing ears).
- In stamen single diploid microspore mother cell cell (**PMC**) divide meiotically to yield four haploid microspores, each becoming encapsulated as a pollen grain (the **male gametophyte**), the haploid pollen then divides mitotically to form a **tube nucleus** and a **generative nucleus**.
- In pistils similar event occur in each megasporangium mother cell (**MMC**), except that only one of four haploid macrospore nuclei becomes functional occupant of the **embryo sac** (the **female gametophyte**). The nuclei of this cell divides mitotically into two daughter nuclei, which divide twice more, forming a total of eight haploid nuclei, four at each end of the embryo sac.

# Alternation of generation in flowering plants



- A single nucleus from each end group of the four then unites at the center to form the diploid endosperm nucleus.
- Of the remaining six nuclei in the embryo sac, the group of three farthest away from the pollen tube point of entry (**micropyle**) are called the **antipodal** cells, while the other group differentiates into a single **female gametic nucleus** and two **synergids**.
- In the process of fertilization, the pollen grain makes contact with the stigma (silk) of the pistil and then germinates into a long **pollen tube** carrying the two male sperm nuclei to the embryo sac.
- One male gamete fertilizes the female gametic nucleus to form the diploid zygote, and the other male gamete combines with the diploid endosperm to form triploid tissue that will nourish the embryo. This constitutes the **double fertilization**.





**Figure 6: Fertilization in self pollinated flower**