Discipline Courses-I
Semester-II
Paper III: Mycology and Phytopathology
Unit-IV
Lesson: Morphology and life cycles of Alternaria and Neurospora
Lesson Developer: K.R. Sharma, Bhawna Saxena
College/Department: Hindu College/Department of Botany, University of Delhi

Table of Contents

Chapter: Morphology and life cycles of *Alternaria* and *Neurospora*

Alternaria species

- **Introduction**
- **Systematic Position of *Alternaria***
- **Occurrence and Distribution**
- **Symptoms**
- **Thallus Structure and perpetuation**
- **Anamorphic(Asexual) stage of Alternaria**
- **Teleomorphic(Sexual) stage of Alternaria**
- **Disease Control**
- **Economic Importance**

Neurospora species

- **Introduction**
- **Systematic Position of *Neurospora***
- **Occurrence and Distribution**
- **Thallus organization and cell Structure**
- **Anamorphic or Asexual Reproduction**
- **Teleomorphic or Sexual Reproduction**
- **Economic Importance**
- **Scientific Achievements by using *Neurospora* as a "Model Organism"**

- **Summary**
- **Exercise/ Questions for Practice**
- **Glossary**
- **References/ Bibliography/ Further Reading**

Alternaria species

Introduction

The most common species of *Alternaria* are generally **anamorphic** (asexual) imperfect filamentous fungi belonging to the Class-Deuteromycetes (=The Fungi Imperfecti). Some perfect or teleomorphic forms (sexual) are known and belong to the class Ascomycetes and, sub-class-Pyrenomycetes (*Clathrospora*, *Leptosphaeria*, *Lewia and Pleospora*). Nearly 44 species of teleomorphic forms have been discovered, but they may be certainly close to a hundred. The most common anamorphic species is *Alternaria alternate.*

In culture, the velvety colonies of *Alternaria alternata* are generally grey to black and dark, having septate branched conidiophores that bear at their ends, chains of simple or branched conidia. Initially, ellipsoid or clavate conidia get divided by transverse walls and soon get divided by longitudinal or oblique septae quite characteristic of different species. The mature multicelled conidium acquires a flask- like or club-shaped structure having indistinct or well-differentiated apical rostrum (depending upon the species), and dark-brown pigmentation. The species of the genus *Alternaria*, are characterized by their dark pigmentation due to melanin. The melanin pigment accounts for the UV-resistance of these moulds, as the melanin pigmentation very effectively absorbs harmful UV-radiation and consequently protects the organism from fatal damage caused by the radiation. Consequently *A. alternata* has a worldwide distribution and the pigmented conidia of this species can be found distributed all over the world.

Figure: Greyish black colonies of *Alternaria* growing on Potato Dextrose Agar (PDA)

Figure: Conidia growing in chains on branched, septate mycelia of *Alternaria alternata*

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/3/3c/Alternaria_alternata_-_SNAP-165447-0005.jpg/800px-Alternaria_alternata_-_SNAP-165447-0005.jpg

Systematic Position

Kingdom	: Mycota
Sub Kingdom	: Eumycotina
Phylum	: Ascomycota
Sub-phylum	: Euascomycetidae
Class	: Ascomycetes (Teleomorphic)
	: Deuteromycetes (Anamorphic)
Order	: Moniliales
Family	: Dematiaceae
Genus	: *Alternaria*

Occurrence and Distribution

As a saprophyte, *A. alternata* can be isolated from decaying organic material, and is predominantly found in soil. Except for the winter time conidia of *A. alternata* can be found almost everywhere in the air which accounts for the further distribution of this species by air currents.

Apart from being pathogenic, *A. alternata* also plays a role in biodeterioration and can be isolated from building materials where it appears as dark olive spotting or staining. The

minimal growth temperature of *A. alternata* is about -2^0 C to 5^0 C, the optimal growth temperature is 20^0 C and the temperature maximum is 32^0 C. *A. alternata* has a broad pH spectrum and can tolerate pH between 2 to 9.

Figure: *Alternaria* sp. growing on building. Appears as dark brown spot and can be confused with dirt also.

Source:http://www.specialchem4coatings.com/documents/indexables/contents/42/images/alternaria-contamination.gif

The species of *Alternaria* are cosmopolitan. They are ubiquitous with a number of plant pathogenic species, causing **blight diseases** of the cultivated plants. They can be found on various substrates such as senescent plants, vegetables, soil, food, wall of old houses, and various organic materials. *Alternaria* species have been isolated from various substrates and habitats including audiovisual (tapes, plate negatives, unvarnished glass), waterlogged wood, rubber, dunes, hydrocarbons, plastics, paper, parchment,paint (natural or synthetic), easel painting, murals, plants, food (fruits, vegetables, grains, nuts), soil (cultivated forests, rhizosphere of many plantations), and textile (cotton, jute, wool). The conidia of *Alternaria* are potent allergens, triggering seasonal reactions during the summer months, both in young and adult humans.

All species of Alternaria are not pathogenic or undesirable; some are used as biological agents to control invasive plants. *Alternaria alternate* saprophytic as well as pathogenic, and is a ubiquitous cosmopolitan species which flourishes in dry arid desert dunes as well as in saline lakes and moist soil along the banks of rivers and drains.

A. alternata serves as food for moths such as *Acarus gracilis*, *A. siro, Tarsonemus waitei* and *Acotyledon redikorzevi*. It is toxic and pathogenic to many crop plants. Its conidia act as allergens and can cause severe allergic respiratory diseases (asthma, chronic sinusitis, rhinitis), mycoses and leukopenia. It also causes fungal disease of skin as well as the

scalp. Its mycotoxins, called **alternaric acid,** altenuene and alternatiol are responsible for leukopenia.

It is a facultative parasite, and causes blight disease on leaves, stems and fruits of members of the family Brassicaceace (=Cruciferae) and the family Solanaceae. Diseases caused by *Alternaria* species are very common and are worldwide in their occurrence. Important host plants include a variety of crops such as apples, broccoli, cauliflower, carrots, potatoes, Chinese cabbage, tomatoes, bok choy,citrus,cereals, many ornamentals and a number of weeds.

Symptoms

Alternaria generally attacks the aerial parts of its host. In the leafy vegetables, symptoms of *Alternaria* infection typically start as a small, circular, dark spot. As the disease progresses, the circular spots may grow to 1.0 cm or more in diameter and are usually gray, gray-tan, or near black in color.

Due to fluctuating environmental conditions, *Alternaria* does not have a uniform growth rate, thus spots develop in a target pattern of concentric rings.

Figure: Leafspot of mustard caused by *Alternaria brassicae* showing the typical target board pattern representing concentric rings

Source: http://anrcatalog.ucdavis.edu/pdf/8040.pdf

Where host leaves are large enough to allow unrestricted symptom development, the target board effect in the infected spots are diagnostic feature for *Alternaria* infection. Apart from the target board pattern, the necrotic lesion is also often covered with a fine, black, fuzzy

growth of the pathogen. This growth is because of the asexual stage of *Alternaria* species produced radially on the dying host tissues.

Many *Alternaria* species produce toxins that diffuse into host tissue ahead of the growth of the fungus. Therefore, it is not uncommon to see a yellow halo that fades into the healthy host tissues that surround the infected target spot.

Alternaria infections on roots, tubers, stem and fruits are associated with the dark-brown, sunken lesions that get hardened to form cankers. The fungus may sporulate in these cankers, causing a fine, black, velvety growth of fungus representing asexual reproductive structures (**conidiophores bearing conidia)** on the affected area.

Alternaria Brown spot of *Citrus* –plants, and leaf blight of Brassicaceae is commonly caused by *A. alternata*, and has been reported in South Africa, Turkey, Israel, Iran, Spain, Italy, Greece, Brazil, Argentina, Peru and Colombia. On citrus plants, this disease does not affect oranges, but causes spotting on leaves and stem-branches.

Figure: *Alternaria* brown spot lesions on a twig and young leaves

Source: http://edis.ifas.ufl.edu/pdffiles/CH/CH01700.pdf

On young lemon fruits, leaves and twigs, it produces brown-to-black lesions surrounded by a yellow halo. The halo is caused by a fungal toxin which rapidly kills citrus tissue. Leaf lesions are generally circular but will often have a tail, following the leaf vein which gives the lesions an eye-spot appearance. The necrosis extends along the veins as the toxin spreads in vascular tissues. On young leaves, lesions can appear as early as 36-48 hours after infection.

Figure: *Alternaria* brown spot lesions on leaves of a susceptible cultivar

Source: http://edis.ifas.ufl.edu/pdffiles/CH/CH01700.pdf

Figure: *Alternaria* leaf spot of lemon caused by *Alternaria alternate*

Source: http://edis.ifas.ufl.edu/pdffiles/CH/CH01700.pdf

Leisions enlarge as leaves mature and can vary in size from 1-10 mm and will be larger if the infection occurred earlier in the season. If *Alternaria* brown spot is severe, the leaves may drop and entire shoot can wilt and die. Severe fruit infections, result in the drop of young fruitlets. Remaining fruit can have lesions that vary in size from dots to large pock marks on the peel. Young lesions form a corky protuberance that can be disloged as the fruit matures, leaving a light tan pock mark.

Figure: *Alternaria* brown spot lesions with the typical corky protuberances on fruit surface.

Source: http://edis.ifas.ufl.edu/pdffiles/CH/CH01700.pdf

Figure: Light tan pock marks on the peel of fruit from where the corky protuberances of *Alternaria* brown spot lesions have been dislodged

Source: http://edis.ifas.ufl.edu/pdffiles/CH/CH01700.pdf

Occasionally, *A. alternata* is able to penetrate the citrus rind and cause localized necrosis. Young fruits are susceptible to *Alternaria* brown spot for 4 months after petal fall. Even when the fruit are no longer susceptible, some fruit may fall as the result of earlier infections, especially if the lesions are near the fruit stem.

Thallus structure and anamorphic(asexual) stage of *Alternaria*

The thallus of species of *Alternaria* consists of multicellular branched brown coloured mycelium that multiplies vegetatively under normal conditions. It produces multicellular branched conidiophores that bear at their terminal ends or tips of branches, multicellular conidia singly or in chains. The conidia of *Alternaria* species are often beaked and always multicelled, having longitudinal and transverse septae. The conidia are dark coloured due to melanin pigment and borne singly or in chains.

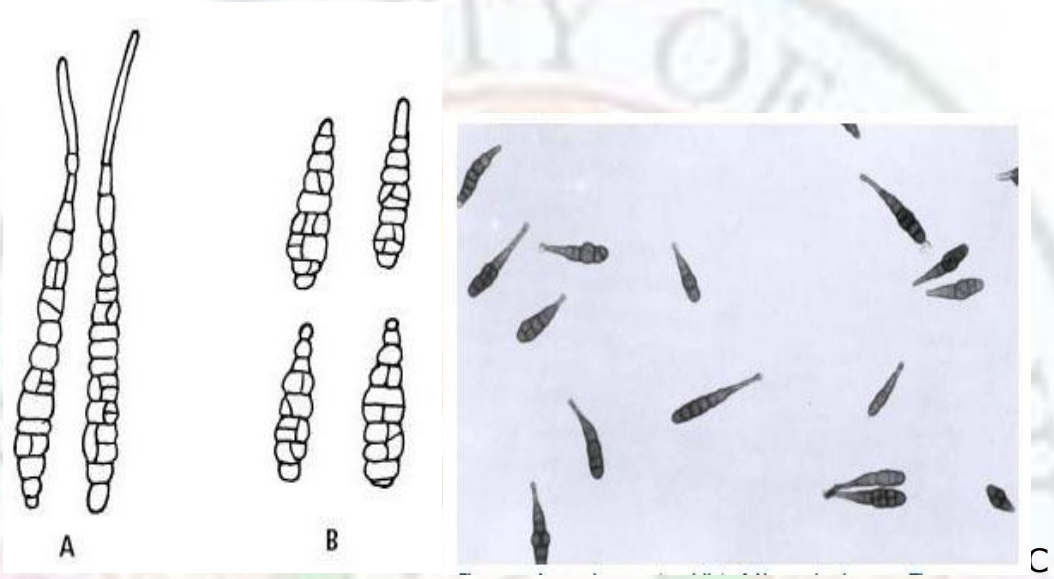


Figure: Conidia of *Alternaria brassicae (A); Alternaria brassicicola* (B) and *Alternaria alternata* (C)

Source: http://anrcatalog.ucdavis.edu/pdf/8040.pdf

Source: http://edis.ifas.ufl.edu/pdffiles/CH/CH01700.pdf

Plant pathogenic *Alternaria* species survive between crops in the form of conidia and dormant mycelium in infected plant residue or in soil, and on seeds. If the fungus is seedborne, it may attack seedlings, causing damping –off, stem lesions, or collar rot . Most often, however, the fungus grows and sporulates on plant residues during periods of rain, heavy dew or under conditions of good soil moisture. Spores are wind blown or splashed onto plant surfaces where infection occurs. The spores must have free moisture to germinate and infect. Penetration of the host can be direct, through wounds or through stomata.

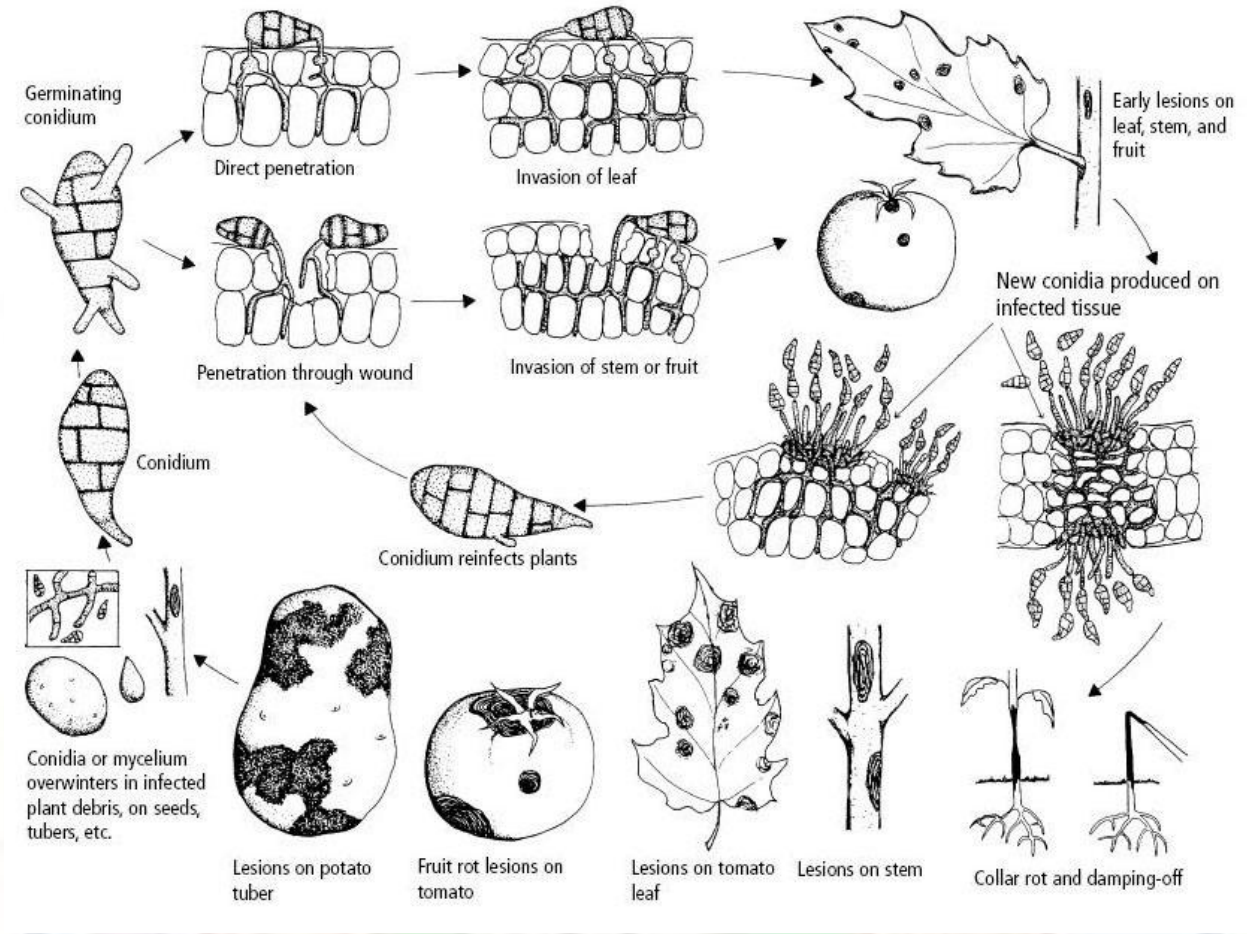


Figure: Development and symptoms of diseases caused by *Alternaria*

Source: http://anrcatalog.ucdavis.edu/pdf/8040.pdf

Tissues that are stressed, weak, old or wounded are more susceptible to invasion as compared to healthy and vigorous tissues.

Anamorphic(Asexual) stage of *Alternaria*

The conidia of *Alternaria* species borne on multicellular branched conidiophores, are thick-walled, multicellular and pigmented and thus tolerate adverse conditions like dry weather. In citrus plantation, they are produced on leaves 10 days after symptoms appear, primarily an old lesions on mature leaves. Spore production continues up to 50 days after infection. In addition, conidia are produced in lower numbers on fruit and twigs remaining on the tree as well as on leaf litter. When there is no susceptible tissue available, such as over the winter, the fungus survives on mature leaves, twigs and fruit. Spore production is greatest when relative humidity is above 85%. Spores are air-borne and release into the air is triggered by rainfall or by a sharp change in relative humidity. Once the spores are released, they are moved by wind to susceptible tissue where they are able to infect. When

temperatures are favorable (20 -29^{o}C), the length of the wetting period required for infection is about 8-10 hours. When temperature drops below 17^{0} C or rise above 32^{o}C respectively, the fungus requires extended leaf wetness durations (>24 hrs) to cause significant infections. On highly susceptible cultivars, as little as 6 hours of leaf wetness can result in infections. Most of the infections probably follow a rainfall event, but dew can be sufficient to bring about infection.

Teleomorphic (Sexual) Stage of *Alternaria*

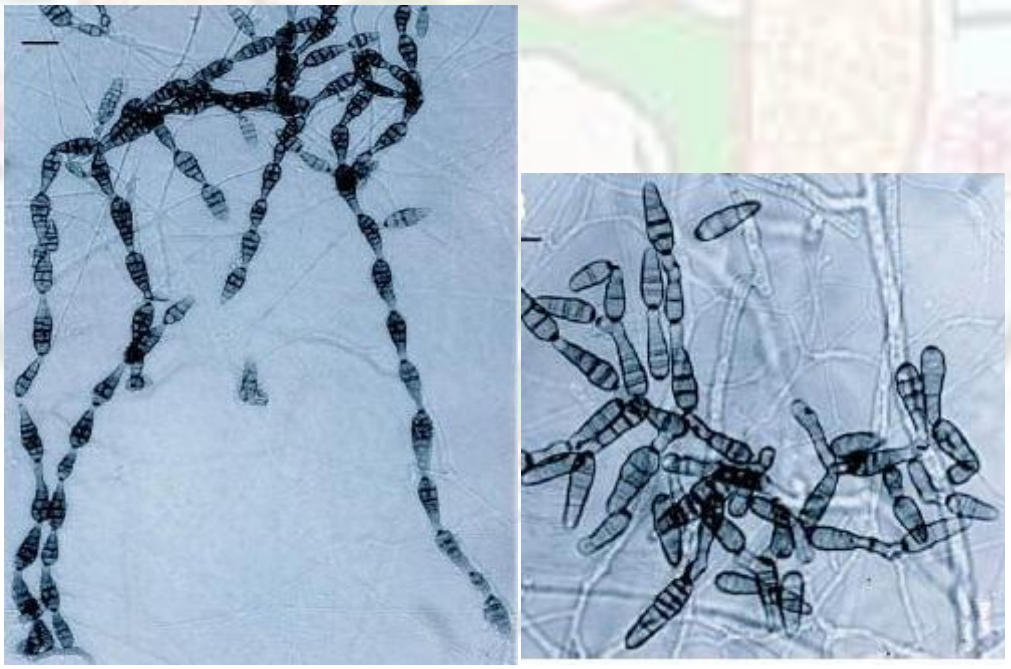
Several teleomorphic genera having their anamorphic form as *Alternaria*, such as *Clathrospora, Lewia, Leptosphaeria and Pleospora*, are known, however, for convenience, we are describing the characteristics of *Lewia hordeicola and L. infectoria* isolated from cereals. Both *Lewia hordeicola and L. infectoria* are homothallic, and produce fertile *ascomata (*Spherical perithecia with ostiole) on synthetic medium after long incubation at 4^{0} C in the dark. The two species differ from each other in the shape and size of ascospores, the conidial sporulation patterns, and the shape, size, septation pattern and roughness of the conidia. Both the species are reported to act as spoilage agent in stored barley, wheat, rice, maize and oats.

The studies were made based on separate cultures raised from single ascospores of each of the above named *Lewia* species. In both the cultures, the fungal colonies were lanose to loosely cottony that were initially yellowish tan and then, turned brownish grey with age. In both the cultures conidial stage appeared within 14 days. Additional structural differences in the above-named anamorphic and teleomorphic stages are as follows:

Conidial Or Anamorphic Stage	***Lewia hordeicola***	***L. infectoria***
	Conidial chains evenly spread on the entire surface of the agar-plate	Conidia grouped in clumps here and there in the agar-plate
	Chains of conidia longer having 7-12 conidia per chain	Chains of conidia shorter, which usually occur singly or in chains of 2 to 4 conidia.
	Conidia longer and wider on the lower side representing	Conidia shorter, clavate, and narrower when compared with

	flask like appearance	conidium of *Lewia hordeicola*
	Highly thick-walled and dark due to more melanin and having distinct apical rostrum	Comparatively thin-walled, less melanin & apical rostrum not distinct.
	Higher number of cells per conidium as longitudinal septation is more common	Fewer cells per conidium as longitudinal septation is rare.
Ascomata or Teleomorphic stage(perithecium)	Produced in culture in low nutrient medium, at low temperature(4^0C) & in darkness	Produced in nature on the infected host plants
Shape of Ascomata	Globose , no beak but indistinct opening present (ostiole)	Ellipsoid, with a short papillate beak with distinct opening (ostiole)
Size of Ascomata	Nearly 400 lm in diameter	500X15 lm
Ascospore size	20X7 lm	20x7 lm

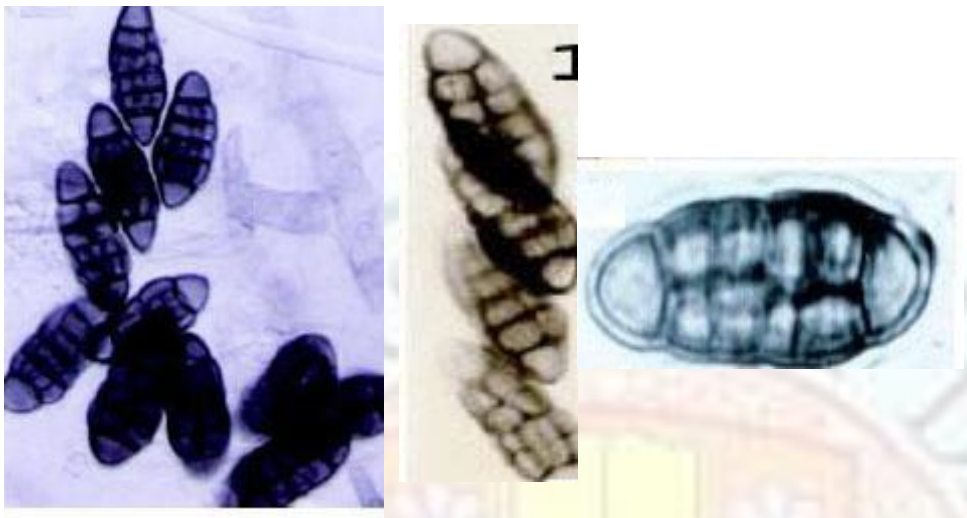
Source: http://www.mycologia.org/content/98/4/662.full



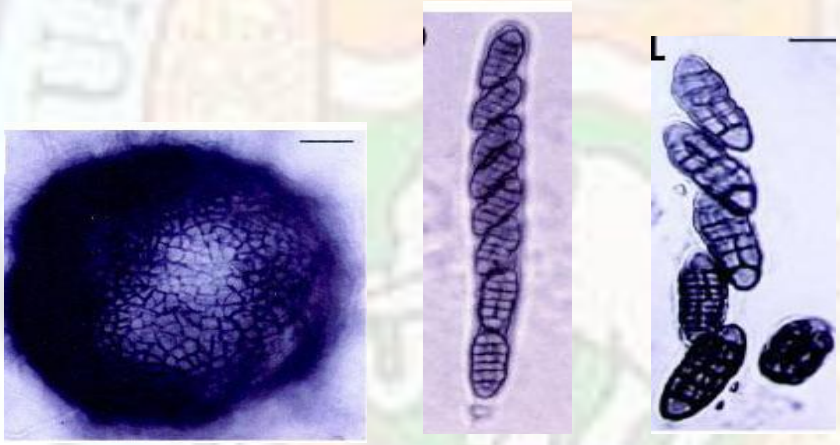
(a) **(b)**

Figures: *Lewia hordeicola* and *Lewia infectoria.* (a) Conidial chains of *L.hordeicola*. (b)Clumps of conidial chains in *L.infectoria*.

Source: http://www.mycologia.org/content/98/4/662.full



Figures: Mature ascospores of *Lewia hordeicola*



(a) (b) (c)

Figure: (a) Ascoma (Perithecium) formed in vitro. ;(b) Ascus with immature ascospores; (c) Ascospores just released from an Ascus.

Source: http://www.mycologia.org/content/98/4/662.full

The peridium of ascomata (**perithecium)** is multicellular consisting of not more than two-layered pseudoparenchyma. The asci are sub-cylindrical and bear eight uniseriately arranged multicellular ascospores. The ascospores are ellipsoid, usually tapered at both ends, rarely short-clavate and inequilateral. The young ascospores have 5-transeverse

septae and mature ascospores develop a longitudinal septum in the middle so that both the terminal ends of each ascospore have one-celled compartment.

The asci mature at different times and release their ascospores one by one after emerging through the ostiole of perithecium. The ascospores, after dispersal by wind or water germinate to produce branched multicellular mycelium on suitable substratum on a specific host or any organic material.

Disease Control

Control of *Alternaria* diseases can be accomplished in several ways. It is important to remember to grow plant cultivars that have disease resistance. When seed may be carrying the fungal spores, raising of crop using disease-free seed or seed that has been treated by fungicide, can greatly reduce disease incidence. Rotating crops so that susceptible crops follow non-host crops is useful in reduing disease incidence. Crop residue destruction by burning, and weed control also helps reduce disease.

Ultraviolet light has been shown to be essential for spore formation in *Alternaria* species. Therefore, under greenhouse growing conditions, the use of ultraviolet light-absorbing film can greatly reduce the incidence of some diseases of *Alternaria.*

Finally, there are a number of fungicides that have activity against *Alternaria* species. The commonly used fungicides are Chlorothalonil, captan, fludioxonil, imazalil, iprodione, meneb, mancozeb, thiram, mercuric chloride, and selected copper fungicides (Bordeaux mixture) which have varying degrees of efficacy against *Alternaria* species.

Invitro studies reveal that *A. alternata* is susceptible to amphotericin B, fluconazole, itraconazole and ketoconazole.

Fungicides are the primary means of controlling *Alternaria* brown spot of Citrus plantations. The plantations should be raised by using resistant varieties. The saplings should be planted at a wider spacing to promote rapid drying of the leaf canopy that makes the disease more manageable.

In existing plantings, it is important not to promote excessive vegetative growth. Over watering and excessive nitrogen fertilization should be avoided. Frequent light hedging should be done, rather than less frequent severe hedging. The best time to hedge to control *Alternaria* brown spot of Citrus fruits, is late March. The number of fungicide applications needed for control varies greatly with the susceptibility of the cultivar and the severity of

the infestation. The first spray should be applied when the leaves are at the stage of expansion to prevent buildup of *Alternaria* on the spring flush. The second and subsequent applications may need to be made as often as every 10 days to achieve good control on fruit and foliage.

Economic Importance

Some species of *Alternaria* are obligate saprophytes and have an important role in degradation of organic matter in soil. Because of this property, they are responsible for spoiling, damaging and deteriorating our items of daily utility such as audiovisual item, wood, plastics, paper, paint and paintings, food and textile –products.

The conidia of *Alternaria alternata* have been reported to act as allergens leading to severe allergic respiratory diseases (asthma, chronic sinusitis, rhinitis), mycoses and leucopenia. *A. alternata* also causes disease of skin as well as the scalp. Leukopenia is caused by toxins (altenuene, alternatiol and alternaric acid) produced by *A. alternata.*

Many species of *Alternaria* have acquired the property of inhabiting living tissues of crop-plants, thereby causing great economic loss to agriculturists and horticulturists. Some important diseases caused by *Alternaria* species are:

Carrot leafblight caused by *Alternaria dauci,* Black rot of Carrot caused by *A. radicina,* Leaf spot of crucifers caused by *A. brassicae* and *A. brassicicola,* Early blight of potato and fruit-rot of tomato, caused by *A. solani;* Broccoli headrot caused by *A. brassicae* or *A. brassicicola,* Fruit spot on peppers caused by *A. tenuis* and *A. alternata* and Brown spot of Citrus plants caused by *A. alternata.*

(a) (b)

Figure: Alternaria disease of Carrot (a) leafblight, (b) blackrot

Source:http://s3.amazonaws.com/plantvillage/images/pics/000/000/906/large/Alternaria_leaf_blight.jpg?1372189189,http://upload.wikimedia.org/wikipedia/commons/thumb/0/0d/Alternaria_radicina_on_Daucus_carota.jpg/427px-Alternaria_radicina_on_Daucus_carota.jpg

***Neurospora* species**

Introduction

Neurospora is a fungus wherein, its teleomorph (sexual stage) belongs to the class Ascomycetes. It reproduces both asexually and sexually. Its *Anamorphic (*asexual) stage belongs to the class-Deuteromycetes (*Fungi Imperfecti)* and is placed in the Genus *Monilia/Chrysonilia*.

The Generic name, *Neurospora*, literally means "***nerve spore***" because of the characteristic striations present on the outer surface of **ascospores** produced in its teleomorphic stage that resemble the axons or the nerve fibres. *Neurospora* is commonly called "Red or pink bread mold as when it infests a bakery, it gives pink or red colour to the bread because of the colour of its mycelium and abundant conidia produced in its anamorphic stage produced on the surface of bread or any other organic matter.

The first published account of ***Neurospora*** is found related to its infestation of French bakeries in 1843.

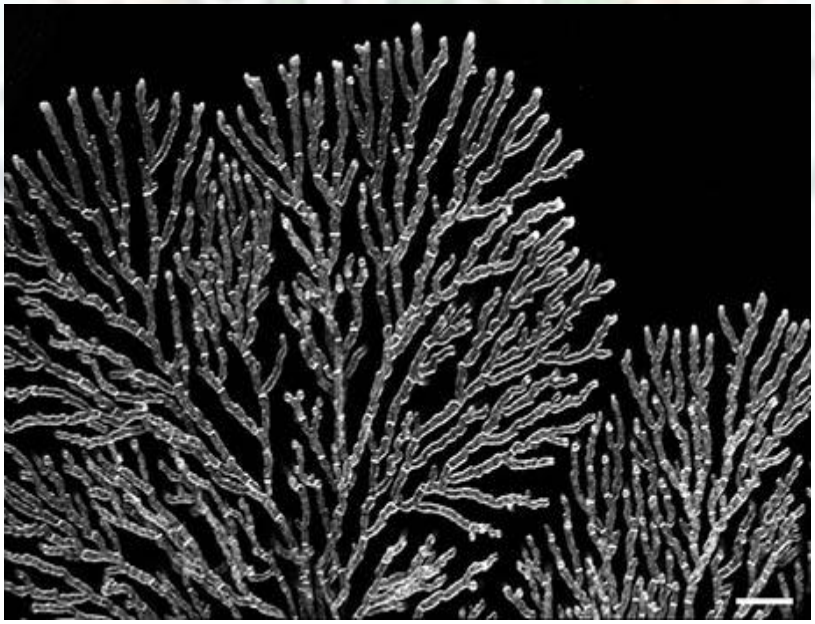


Figure: Thallus of *Neurospora*

Source: http://www.ediblegeography.com/wp-content/uploads/2012/09/Neurospora_spray-460.jpg (CC)

Neurospora is also known as the "Drosophila of the plant kingdom" as it is used as a **Model Organism** for studies in genetic analysis. The advantages of using *Neurospora* as a genetic tool are: (I) It is the fastest growing fungus in vitro**,** and can be grown easily on a synthetic minimal medium (II) It is haploid and will express every genetic change whether **recessive** or **dominant** in the same generation. (III) Analysis of genetic recombination is facilitated by the ordered sequence of products of meiosis, followed by mitosis in the form of eight ascospores arranged linearly in each ascus, involving no random shifting of nuclei or the ascospores. (IV) The release of ascospores per ascus is systematic, one by one at fixed intervals so that single ascospores in orderly sequence can be isolated in successive single spore cultures and analysed for various traits. The asci in each ascocarp **(Perithecium**) mature at different times and release their ascospores systematically. When one ascus has released its ascospores, second ascus will grow into the ostiole of perithecium, and liberate its ascospores in the same way. As per the report published in the issue of "Nature" in April, 2003, the entire genome, representing nearly 10,000 genes on the seven chromosomes of *Neurospora (*n=7), has been sequenced (v) all the seven chromosomes of *Neurospora* are **telocentric** or **achrocentric** so that all the cross-over (leading to genetic recombination) take place involving the longer arms of chromosomes, and the genes can be easily mapped in relation to relative distance from the centromere on each chromosome.

The arrangement of coloured and colourless ascospores in different asci produced in a perithecium of *Neurospora*, representing either a ditype (2+2+2+2) arrangement or tetratype (4+4) arrangement, has helped us to understand that crossing-over during meiosis occurs at 4-stranded stage of chromosomes in meiosis-I; and recombination percentage involving any two linked genes on a certain chromosome, can never exceed 50%.

Neurospora is actively used in genetic studies and molecular research around the World. G.W.Beadle and Edward Tatum, exposed *Neurospora crassa* cultures to X-rays, and isolated nutritional mutants (**auxotrophs**) that failed to grow on minimal medium because of error in their metabolic pathway. After long investigations, they were able to unravel the genetic causes of different metabolic pathways in *Neurospora* based on which they proposed "**One gene, one enzyme**" hypothesis which means that every enzyme (or protein) is coded by a different gene. This hypothesis, now has been changed to "One gene, one polypeptide"

hypothesis because many proteins are made up of two or more polypeptides and each polypeptide is coded by a separate gene.

Systematic Position

Kingdom :	Mycota (=Fungi)
Sub-kingdom :	Eumycotina
Phylum :	Ascomycota
Sub-phylum :	Euascomycetidae
Class :	Pyrenomycetes
Order :	Sordariales (=Sphaeriales)
Family :	Sordariaceae
Genus :	*Neurospora*

Occurrence and Distribution

The species of *Neurospora*, represent obligate saprophytes which grow easily on any organic matter. The baker's bread is the most-preferred substrate where this fungus grows fast and gives pink or red colour to the bread by growing on its surface.

The most common habitat for *Neurospora* lies in subtropical and tropical areas, however, some species do occur in temperate forests. The specific characters of different species vary based on geographical location of occurrence of the fungus. The commonest species are *N. crassa, N. sitophila, N. tetrasperma, N. cerealis, N. indica and N. intermedia.*

Figure: *Neurospora* growing on bark of a tree

Source:http://microbewiki.kenyon.edu/images/thumb/3/38/DJacobN-in-N_1._bark_jpg.jpg/200px-DJacobN-in-N_1._bark_jpg.jpg

The species of *Neurospora,* have the ability to decompose burnt plant and animal matter, and they germinate and multiply fast in the exposed habitats, especially, the tree-bark, after forest fires. Because of its fast rate of asexual reproduction and abundant vegetative growth, *Neurospora* is valued for research purposes, especially, in genetic and molecular biology.

Thallus Organisation and cell structure

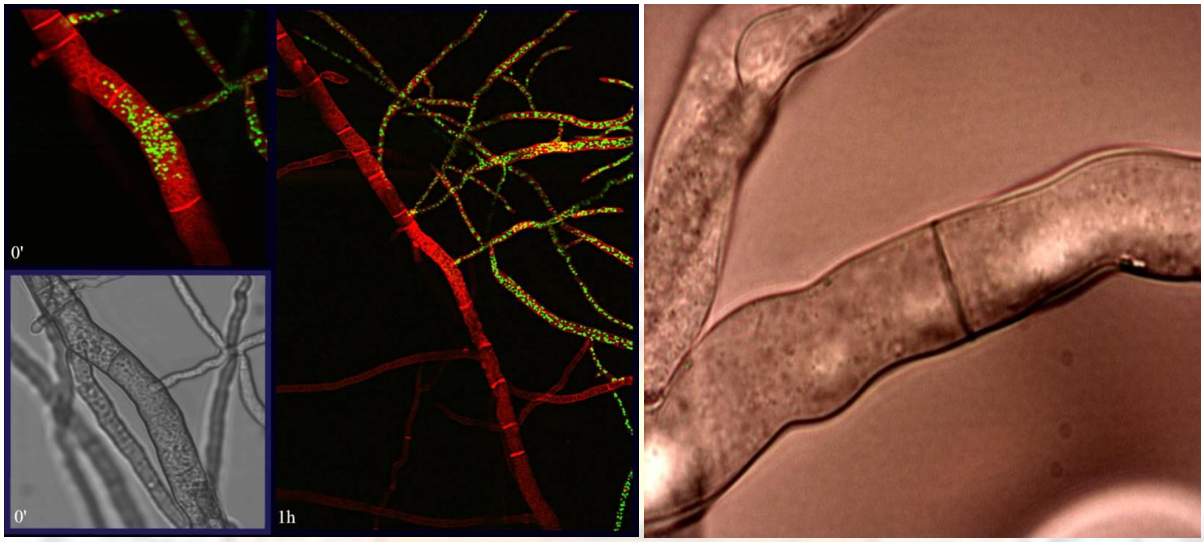


Figure: Branched mycelium of *Neurospora*

Source:http://www.fgsc.net/Neurospora/photos/GlassHypha.jpg,http://upload.wikimedia.org/wikipedia/commons/5/5b/Neurospora_crassahyphae.jpg

The mycelium of *Neurospora* is represented by numerous, branched aerial hyphae which form a pink coloured mass because of pink-coloured spherical or oval conidia borne in chains on multicellular branched pink-coloured conidiophores. The **anamorphic (asexual**) stage represented by micro-and macroconidia is known as *Monilia sitophila* which was known as early as 1843. The **teleomorphic (sexual**) stage representing the Genus, *Neurospora,* was discovered somewhere in 1927. *Neurospora* can propagate itself indefinitely, vegetatively by fragments of its hyphae and asexually, by means of multinucleate **macroconidia** and uninucleate **microconidia.**

The individual hyphal cells are multinucleate with its cytoplasm enclosed in a plasma membrane. The cell walls of hyphae are composed of the polysaccharide, called **fungal cellulose** or **chitin.** The vegetative and reproductive thallus has atleast twelve specialized cell-types of which the most common are "hyphal cells", "micro-and macroconidia", "branched erect multicellular conidiophores", "pseudoparenchymatous cells" forming peridium of perithecium; ascogenous hyphae, " asci " and "ascospores". The hyphal cells are tubular having rigid cell wall except at the tips, where hyphal growth occurs. The

components of plasma membrane and cell walls are synthesized some distance from tip and transported forward to the tip in the form of vesicles.

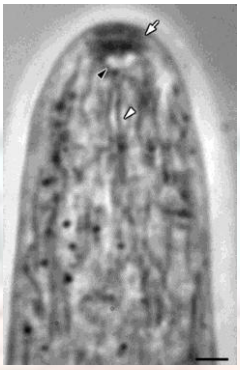


Figure*: Neurospora crassa* wild-type hyphae shown with phase contrast optics.A well-organized Spitzenkörper composed of a core (black arrowhead),secretory vesicles (white arrow) and functional protein (not shown). Mitochondria (white arrowhead) are visible. Scale bar ~ 1 □m. (Image by M. Uchida and R.W. Roberson)

Source: http://www.fgsc.net/Neurospora/photos/Roberson%20Spitz%20PC.jpg

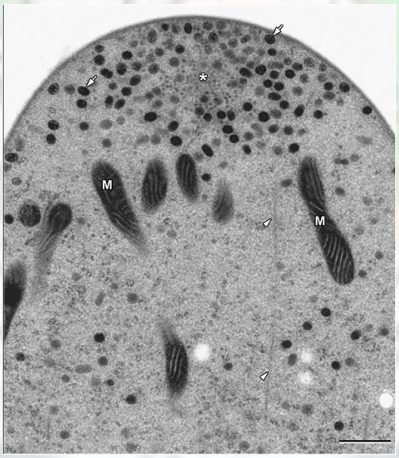


Figure: Transmission electron microscopy of cryofixed and freeze-substituted wild-type hyphae of *Neurospora crassa*. Near-median longitudinal section through hypha illustrating the Spitzenkörper was composed of a cloud of secretory vesicles (arrows) surrounding a core (asterisk). Microtubule (arrowheads) and mitochondria (M) are noted.

Bar = 3.3 □m. (Image by R.W. Roberson from: Riquelme, M., Roberson, R.W**.** McDaniel,

D.P., Bartnicki-García, S., 2002. The effect of ropy-1 mutation on cytoplasmic organization in mature hyphae of *Neurospora crassa*. Fungal Genetics and Biology 37: 171-179)

Source: http://www.fgsc.net/Neurospora/photos/Roberson%20TEMwt.jpg

along the cytoplasmic strands representing **cytoskeletal** "highway". The vesicles, after accumulating at the tip of hyphae in a body, called the "**Spitzenkorper",** are distributed to the apical growing region of extension.

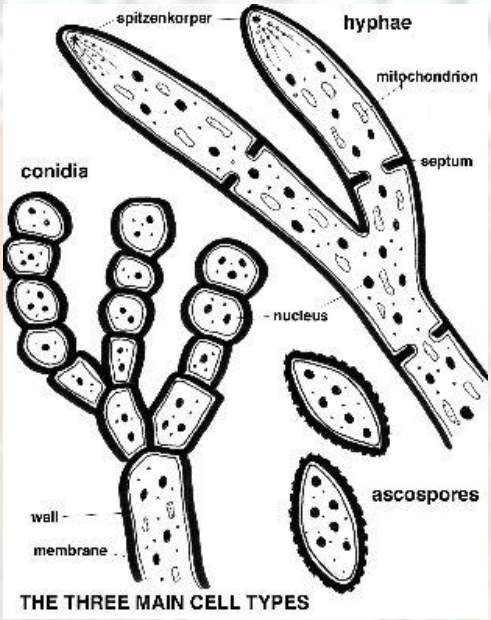


Figure: a) the hyphal "cell", b) the conidium, c) the ascospores

Source: http://www.fgsc.net/Neurospora/photos/sm3MAINCELLTYPES.jpg

At the growing point, a high gradient of Calcium ions is essential for growth and extension of hypha. Branching of a hypha takes place some distance behind the growing point resulting in the production of new growing points responsible for growth and development of new hyphae.

The new hyphae of *Neurospora* are one of the fastest growing filamentous fungi with a rate of about 10.0 cm. per day. The prolonged vegetative phase of mycelium as well as the reproductive phase of *Neurospora* is **haploid**, and the **diploid** phase is extremely short-lived. The **diploid** phase is one-celled and, is noticed only in the teleomorphic stages in the form of **ascus mother cells** which undergo meiosis immediately after Karyogamy, ultimately producing asci, each having generally eight haploid ascospores. In vegetative

growth phase, *Neurospora* utilizes burnt plant or animal matter as a source of food, but they do grow on any organic matter most commonly growing on the bakers bread. They are known to cause great damage if they infest a bakery or if they enter a bacteriological or mycological laboratory as contaminant, wherein, it is difficult to exterminate them because of their fast rate of hyphal growth and easy dispersal of conidia by wind. The conidia and ascospores act as units of dispersal. Ascospores being darkly pigmented and thick walled, are more resistant to stress such as desiccation or high temperature than conidia which are thin-walled and poorly pigmented.

Reproduction:

Anamorphic or Asexual Reproduction:

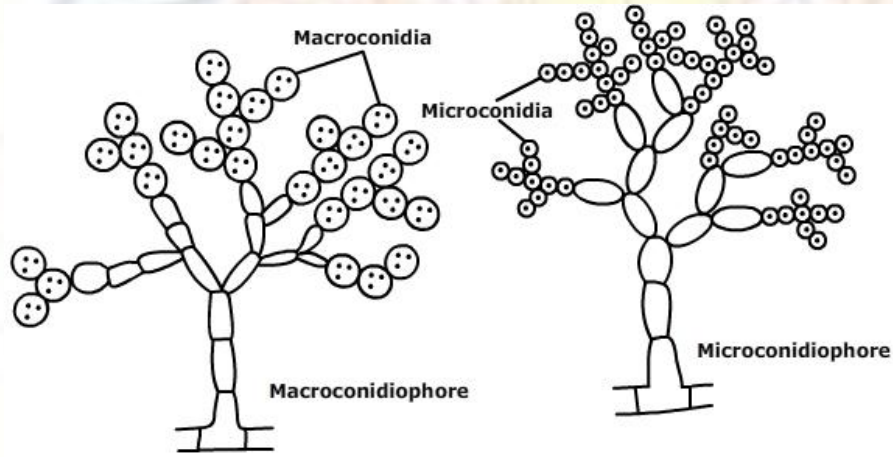


Figure: Anamorphic phase of *Neurospora*

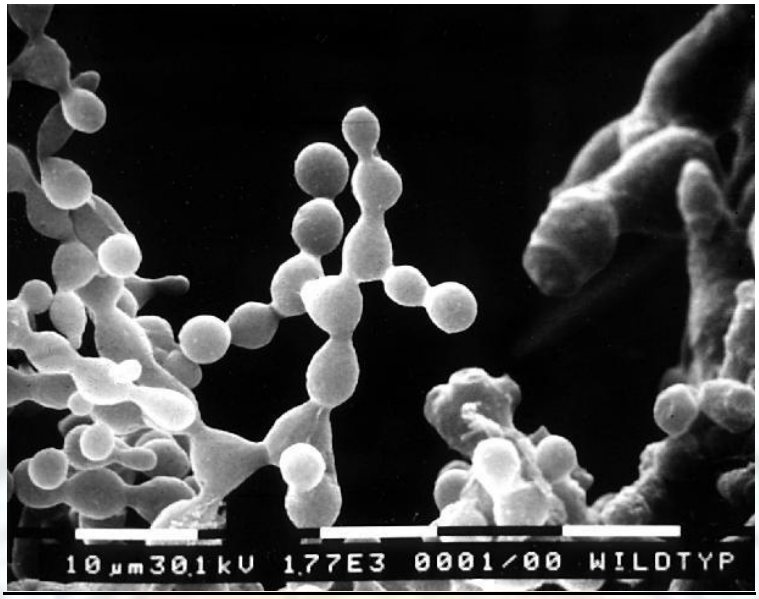


Figure: SEM micrograph of conidia of *N.crassa* showing branched chains of macroconidia

Source: http://www.fgsc.net/Neurospora/74a2.jpg

Asexual cycle of *Neurospora* is represented by the Genus *Monilia/ Chrysonilia*, that multiplies at devastating rates through macroconidia and microconidia borne on branched multicellular conidiophores. The conidiophores originate as erect hyphae from the prostrate hyphae growing on the substrate and after getting branched twice or thrice start cutting conidia in chains in a **basipetal succession**. The first formed older conidia at the terminal end of the chain' get dispersed by wind as new conidia are produced successively. The macroconidia are oval cells each having 2-3 genetically identical haploid nuclei, that can survive for several weeks at room temperature. However, their life can be prolonged if incubated at low temperature. On dispersal, each macroconidium under favourable conditions germinates by sending out a germ tube which turns into the first vegetative hypha of the new thallus.

The microconidia are spherical, uninucleate and smaller in size as compared to macroconidia. The microconidiophores are similar to macroconidiophores. The method of formation of microconidia is also basipetal as during the formation of macroconidia. Functionally, microconidia have the potentiality to germinate on favourable medium and produce germ tube that gives rise to first vegetative hypha representing new thallus. But in heterothallic species of *Neurospora*, the microconidia act as male gametes (**spermatia;** singular, **spermatium**), and function as paternal fertilizing elements to help in sexual reproduction. On dispersal through wind when a microconidium comes in physical contact with trichogyne (fertile receptive hypha from maternal parent); a hyphal branch emerging

from ascogonium (**protoperithecium),** a hormone emitted from the spermatiim (microconidium), helps in plasmogamy between spermatium and trichogyne resulting in transfer of a male nucleus from spermatium to ascogonium to act as a prelude to the sexual cycle leading to the formation of ascocarp, called the **perithecium**. Thus microconidia have a dual function. They can germinate directly to give rise to vegetative mycelium or if they come in physical contact with trichogyne of ascogonium (female sex organ) of the opposite mating type, they act as spermatia or the male gametes. Normally, a + mating type will produce a + type of spermatia and + type of ascogonia and –mating type would produce a – type of spermatia and so also, the – type of ascogonia. During sexual cycle, + spermatium would fertilize a – trichogyne borne on ascogonium produced on the mycelium of (-) strain and a – spermatium would fertilize a + trichogyne borne on ascogonium produced on mycelium of + strain.

However, in homothallic species, the function of male gamete can be performed by a microconidium or macroconidium borne on the same mycelium which has produced ascogonium or even by any other vegetative cell belonging to the same mycelium but representing a hypha other than the hypha that has produced an ascogonium.

Teleomorphic or Sexual Reproduction

Three different modes of sexual cycle are known in *Neurospora*, **heterothallic, homothallic or pseudohomothallic** (secondary homothallic). **Heterothallic** sexual life cycle is exhibited by *N.sitophila* and *N.crassa* where two mating strains, A or + and a or -, are recognized. In these heterothallic species, mating leading to plasmogamy or dikaryotisation can occur only between two physiologically or genetically different strains. That is, a or – spermatium would fuse with trichogyne of ascogonium produced by A or + mycelium and vice versa.

N.galapagoensis is reported to be a **homothallic** species. Here, plasmogamy takes place between a trichogyne of ascogonium, and a micro-or - macroconidium produced by the conidiophores borne on the same haploid mycelium or even involving any vegetative hypha other than the trichogyne of ascogonium produced on the same thallus/mycelium.

N.tetrasperma exhibits **pseudohomothallic** sexual cycle. Here, each ascus formed after completion of usual sexual cycle resulting in the formation of perithecium, bears four ascospores each of which has two genetically different nuclei. Thus , when such a dikaryotic ascospore, after dispersal, germinates producing a vegetative thallus, its each cell would have dual mating type mixture of nuclei. In this species, although uninucleate microconidia

may be either + or -, but the macroconidia would have a mixture of + & - nuclei and each ascospore produced after sexual cycle,has one+ and one – nucleus. Thus, during sexual cycle in *N.tetrasperma*, there is no need of plasmogamy. The + and – nuclei in the ascogonium (protoperithecium) would enter into the process of ascus formation without any need of process of plasmogamy or dikaryotization as the somatic cells and ascogenous cells are already **dikaryotic** (a cell having one + nucleus and one – nucleus) or **heterokaryotic** (a multinucleate cell wherein, out of several nuclei, atleast one nucleus is + type and one nucleus , - type).

The sexual cycle in *Neurospora* reveals that there is gradual degeneration of male sex organ wherein morphologically identifiable "**antheridium**" is missing. A distinct female sex organ, that is, ascogonium (protoperithecium) is always produced by all the species. In *N.sitophila* and *N.crassa*, two physiologically different thalli (+ & - type) are involved in sexual cycle, wherein the function of antheridium is performed normally by uninucleate microconidia (= spermatia) of mating type different from the mating type of ascogonium bearing thallus, and rarely by macroconidia produced by the conidiophores borne on the mycelium belonging to the opposite mating type. In *N. galapagoensis*, trichogyne of protoperithecium undergoes plasmogamy with a microconidium or macroconidium or any other somatic cell produced or borne on the same thallus that comes in its physical contact.

In *N. tetrasperma*, there is no need of plasmogamy because protoperithecium is heterokaryotic and, it bears a mixture of several + and several – nuclei. Thus after the onset of initiation of sexual reproduction,after the differentiation of ascogenous hyphae, the compartmentalization or cytokinesis occurs in such a way that every ascogenous cell is dikaryotic representing one + and one – nucleus. Ascus formation takes place in any dikaryotic ascogenous cell that exhibits karyogamy immediately followed by meiosis, and that is followed by one generation of mitosis so that the young ascus would have 4 + type and 4 – type of nuclei. However,during cytokinesis, it is ensured that the mature ascus has only four dikaryotic ascospores each having one + and one – type of nucleus.

Perithecium Development

The steps involved in the process of sexual cycle in heterothallic species of *Neurospora* are as follows:

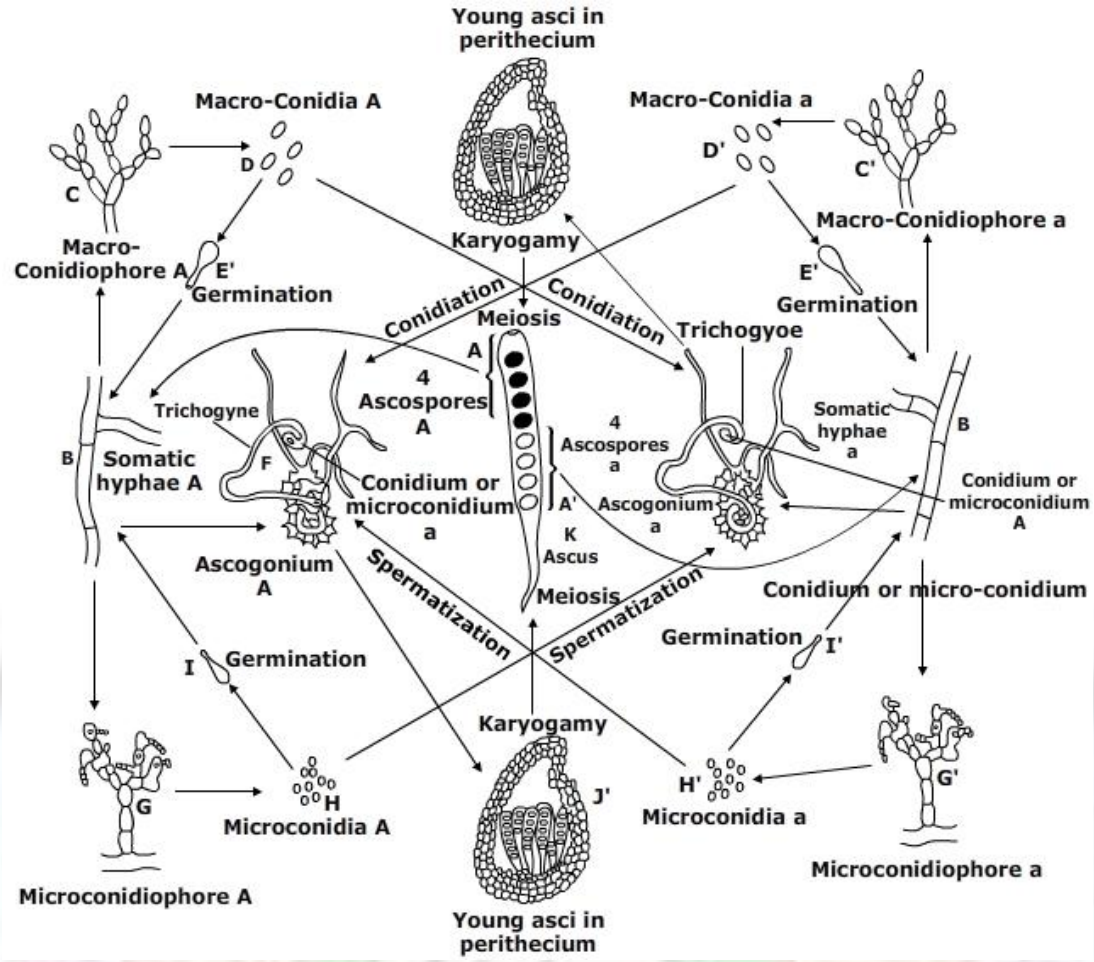


Figure: Anamorphic (asexual) and Teleomorphic (sexual) reproduction cycles of *Neurospora sitophila*

Source: Author

The sexual reproduction is favoured if the fungus is grown in the minimal medium at pH= 6.5 with sucrose as C-source, KNO_3 as the N-source, along with trace-elements and biotin . The female sex organ, **ascognium**, differentiates in the mycelium which is already reproducing asexually with the help of abundantly produced multinucleate macroconidia and uninucleate microconidia. The ascogonium (protoperithecium) has a multinucleate swollen base embedded in the pseudoparenchymatous mass of vegetative cells and it extends into a long aerial hypha that gets branched to produce several aseptate hyphal branches called **trichogynes**. A male organ, **antheridium**, is not produced. The function of male gamete is performed by a uninucleate microconidium **(= spermatium**) that undergoes plasmogamy with any one of the trichogynes of an ascogonium. The function of trichogyne is to act as a receptive hypha that receives a male nucleus and little cytoplasm from the spermatium. After plasmogamy between a spermatium and trichogyne, the remaining trichogynes

produced by the same ascogonium, collapse and degenerate. The male nucleus, migrates to the base of ascogonium and divides repeatedly by mitosis until the number of male (-) and female (+) nuclei becomes equal. The + & - nuclei form pairs to represent dikaryons which get arranged in the periphery of swollen base of ascogonium. Ascogenous hyphae are produced next to each dikaryon each of which continues dividing conjugately repeatedly through mitosis. After cytokinesis, the ascogenous hyphae become septate representing dikaryotic ascogenous cells. Some or most of these dikaryotic ascogenous cells act as ascus mother cells wherein, karyogamy (diploidisation) is followed by meiosis-I and meiosis-II producing four haploid nuclei. Each of these four haploid nuclei undergoes one mitotic division resulting in the production of eight haploid (n) nuclei per cell which is now called the young ascus. Each of these nuclei in the young ascus, gets surrounded by protoplast and cell wall producing eight ascospores per ascus. The ascus acquires cylindrical shape and the arrangement of ascospores in each ascus is linear and is ordered by the sequence of meiotic and mitotic divisions without involving any random shifting of nuclei or the ascospores.

As the process of ascospore and ascus formation is progressing, the vegetative cells surrounding the protoperithecium organize to form pseudoparenchymatous multicellular, 2-3 cell layered peridium, to constitute flask-shaped (pyriform), beaked **ascocarp**, called **perithecium.** The beak of the perithecium has a distinct opening, the ostiole. The perithecia are produced in abundance, and are generally superficial.

The cylindrical asci each having 8 ascospores, and thick persistent wall with a non-amyloid annular structure at the apex, form a basal tuft originating at the base of the perithecium. Although sterile hyphae, paraphyses are present initially among the asci, they collapse and degenerate at maturity. The mature perithecium is dark coloured having distinct opening, the ostiole in its beak. The young ascospores are uninucleate which become multinucleate due to repeated mitosis of its haploid nucleus. As the ascospores mature, they become broadly fusiform, ellipsoidal,and remain unicellular, These ascospores are initially hyaline to yellowish brown, but become dark brown or black at maturity. The ascospores develop longitudinal ridges / ribs.

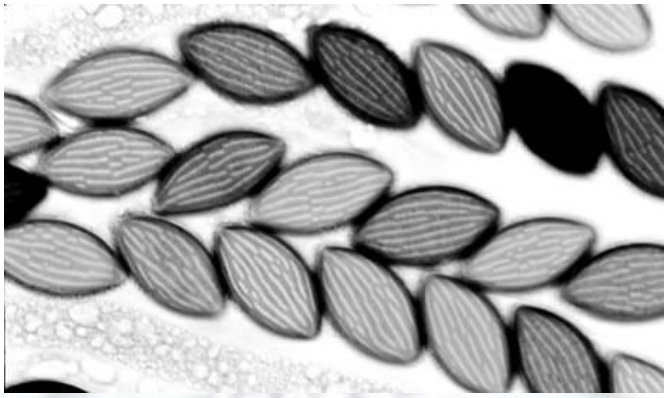


Figure: Ascospores of *N. crassa* showing longitudinal striations

Source: http://www.fgsc.net/neurospora/raju2_files/image002.jpg

on the outer wall (that give it its name, *Neurospora*) and bear one or more germ pores to facilitate germination. Like the conidia, ascospores also act as unit of dispersal for *Neurospora*. However, they are more resistant to environmental stress (such as desiccation) as compared to conidia because they are thin-walled and less pigmented. Since they are heavier, they travel shorter distance in air. On reaching suitable substratum, ascospores germinate exhibiting polarized growth forming a germ tube that gives rise to first vegetative hypha to start the new thallus.

The peridium of perithecium is though multicellular and dark, yet the basal tuft of cylindrical asci within the perithecium, can be seen distinctly through light/dissection microscope. The maturation of asci is gradual. As soon as an ascus matures, it grows towards, and into the ostiole of perithecium and releases its linearly arranged ascospores one after the other. Thus, single haploid ascospores which are the products of one meiosis followed by one mitosis in each ascus can be isolated to raise separate single spore cultures that can be analysed for their genetic traits. This is called **tetrad analysis**.

Economic Importance of *Neurospora*

1. As decomposer of Organic Matter

Neurospora being an obligate saprophyte has a role in degradation of organic waste. By causing decay of leaf litter, dead wood and tree bark especially after forest fire, *Neurospora* enriches the forest soil. Due to abundant vegetative growth because of fast rate of asexual reproduction, it contributes lot of organic matter to fertilize the agricultural soil. The asexual stage of *Neurospora* is known to spoil the grains, especially, barley, wheat and rice in storage.

2. As hazardous in bakery

The first published account of *Neurospora*, finds its place because of the heavy infestation of Bakeries throughout the country in France during the year 1843. The bakeries had to be closed continuously for more than six months to ensure that there is no chance of presence of conidia or hypha of *Neurospora* in any corner in the factory that could create fresh infestation. The fumigation was practised daily in the morning & evening for several days before starting the factory afresh.

3. As contaminant in research laboratories

Once the conidia of *Neurospora* enter a research laboratory as contaminant of some **in-vitro** cultures, it is difficult to exterminate it because of its fast rate of conidial production. The laboratory has to be fumigated daily atleast twice for atleast a month, before it can be used to raise fresh cultures for research purpose.

4. As Model Organism in Genetics and Molecular Biology

Neurospora has been and is being used as a "**Model Organism**" for studies involving metabolic pathways leading to the discovery of concept of "One gene one enzyme hypothesis" which has now led us to the new hypothesis of "One gene one polypeptide hypothesis". Its fast rate of asexual growth has helped the researchers to get larger amount of plant body in short period. Its unique method of linear and ordered ascospore arrangement per ascus and systematic release of ascospores at fixed intervals has been extremely useful in raising single haploid spore cultures for easy genetic analysis.

Scientific Achievements by using Neurospora as a "Model Organism"

Single spore cultures of *Neurospora* have helped us to understand a relationship between the enzymes(proteins) and genes responsible for synthesis of essential metabolites needed for growth and development by the living organisms. The ideal method of arrangement of products of meiosis in separate ascus mother cells leading to formation of separate asci each having eight ascospores linearly and sequentially arranged without involving random shifting of nuclei, followed by systematic release of haploid ascospores, has been useful in gene-mapping by analysis of single spore cultures of each ascus, through the process called, **"tetrad analysis**".

(i) One gene one enzyme hypothesis

G.W.Beadle and Edward Tatum were responsible for the discovery of "One gene one enzyme hypothesis" for which they received Nobel Prize in Physiology and Medicine in 1858.

They performed studies by using *Neurospora crassa.* First, they exposed haploid, asexual conidia of *Neurospora* to X-rays, to cause mutations.The mutants were identified and isolated, that were found to have some errors in their metabolic pathways. The identification of the cause of error, ultimately, led to the discovery of the above named hypothesis. An outline of classical experiments performed and methodology adopted by

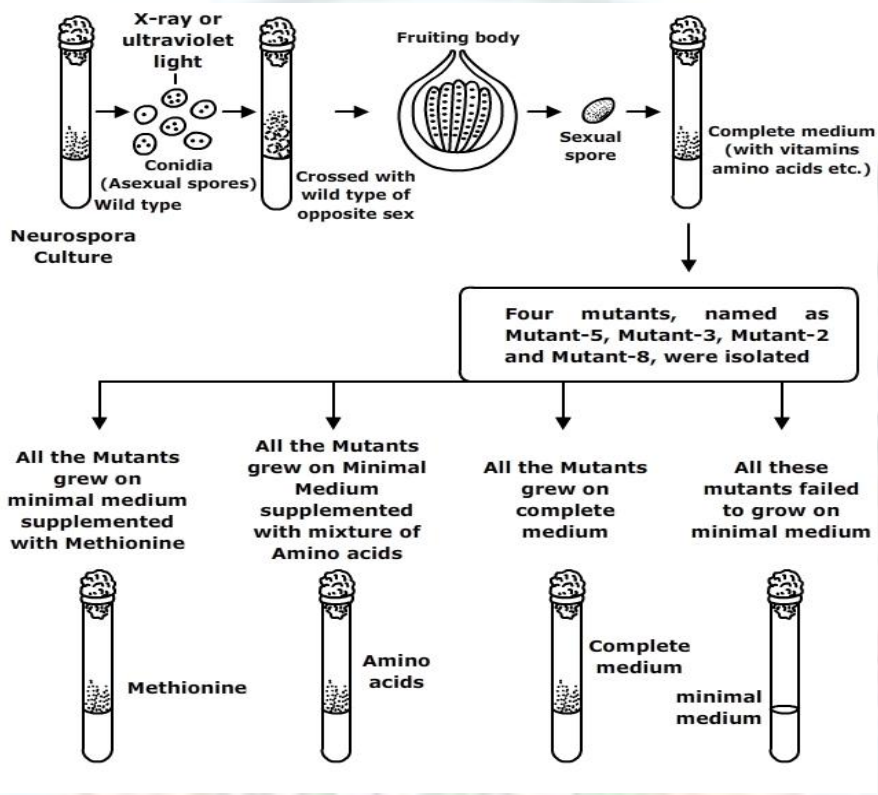


Figure: Diagrammatic representation of classical experiment performed by Beadle & Tatum that helped them in elucidation of biosynthesis of Methionine in *Neurospora crassa*

Source:Author

Beadle and Tatum that helped them to elucidate various metabolic pathways (**Table-1**) leading to the above discovery is as follows:-

1. The haploid conidia(asexual spores) isolated from wild type strains of *Neurospora* are irradiated with X-rays to induce random mutations.

2.The X-ray-irradiated conidia are cultured on complete medium(Minimal medium supplemented with vitamins and amino acids) to raise clones of various mutants that resulted due to irradiation by X-rays.

3. The clones of mutants are mated with the opposite mating type wild strains of *Neurospora* to produce asci containing ascospores produced as a result of segregation of mutant & wild type gene during meiosis in Ascus mother cells.

Table-1

		Result after Conidia were grown on Minimal Medium supplemented with			
Source of Conidia	Minimal medium	O-Acetyl Homoserine	Cystathionine	Homocysteine	Methionine
Wild type	+ Growth	+ Growth	+ Growth	+ Growth	+ Growth
Mutant 5	No Growth	+ Growth	+ Growth	+ Growth	+ Growth
Mutant 3	No Growth	No Growth	+ Growth	+ Growth	+ Growth
Mutant 2	No Growth	No Growth	No Growth	+ Growth	+ Growth
Mutant 8	No Growth	No Growth	No Growth	No Growth	+ Growth

4. Single ascospores per ascus are isolated and grown on complete medium.The order of cultures as per the Order of ascospores of each ascus was maintained by sequentially numbering the cultures for Tetrad analysis. Thousands of such single spore cultures are raised and they are allowed to reproduce asexually with the help of asexual haploid conidia.

5. The conidia from these cultures are grown on minimal medium. The conidia of some cultures fail to grow on minimal medium indicating that these cultures are mutants which represent mutations because of which the fungus (mutant) is not able to synthesise a certain essential metabolite from the raw material available in minimal medium. The tubes containing mutant clones are numbered.Let us consider the experiment that led to the discovery of biosynthesis of methionine.

6. In this experiment the conidia from culture tubes numbered as Mutant-5, Mutant-3, Mutant-2 and Mutant-8 fail to grow on minimal medium, grow on complete medium, grow on minimal medium supplemented with a mixture of all the amino acids, and all these conidia grow on separate minimal medium supplemented with the amino acid, **methionine**. This indicates that the conidia from each of these four culture tubes carry a mutation due to

which the fungus has failed to synthesise methionine. Consequently, all these conidia grow on the minimal medium containing the amino acid methionine.

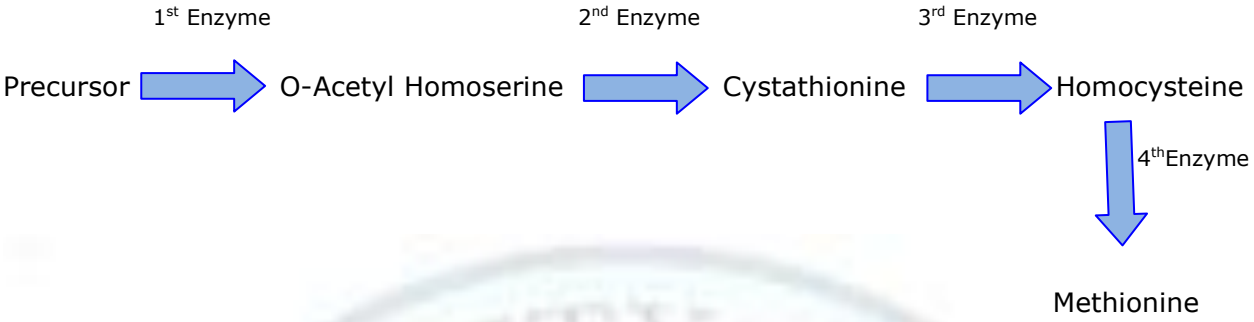
7. Let us remember that these conidia were isolated from wild type that could grow on minimal medium. When conidia from culture tube 8(mutant-8) were either grown on minimal medium or on minimal medium supplemented with Homocysteine or Cystathionine or O-Acetyl Homoserine, they cannot grow, but they grow separately on minimal medium supplemented with methionine. This means that in all these conidia, a mutation affects some step in methionine synthesis starting from a precursor, with Homocysteine, Cystathionine and O-Acetyl Homoserine, as intermediates. The next step is to determine the correct sequence of these intermediates starting from the precursor upto the synthesis of methionine in the last step.

8. If conidia from mutant-5 are grown on minimal medium supplemented with either O-Acetyl Homoserine or Cystathionine or Homocysteine, they can grow without methionine. This indicates that mutant-5 has a defect in the first step wherein , precursor is to be acted upon by an enzyme that would convert the precursor to anyone of the above three intermediates to be converted ultimately to methionine.

9. If conidia from mutant-3 are grown on minimal medium supplemented separately either with Cystathionine or with Homocysteine, they can grow without methionine. It suggests that mutant-3 has a defect in the gene responsible for enzyme production that would convert O-Acetyl Homoserine, to Cystathionine or Homocysteine leading to synthesis of Methionine. It also leads us to conclude that mutant-5 had a defect in the gene that was responsible for conversion of precursor to O-Acetyl Homosereine.

10. If conidia from mutant-2 are grown on minimal medium supplemented with Homocysteine, it does not need supplementation of medium with methionine suggesting thereby that mutant-2 has a defect in the gene responsible for converting Cystathionine to Homocysteine leading to synthesis of methionine. It is now clear that mutant-8 has a defect in the gene responsible for enzyme needed to convert Homocysteine to Methionine. It is also clear that mutant-3 has a defect in the gene responsible for enzyme production that would convert O-Acetyl Homoserine to Cystathionine only

11. We can now conclude that methionine synthesis is controlled by 4 enzymes in succession, schematically shown below:



(ii) Tetrad Analysis in Neurospora as an aid to Gene Mapping

As we gained the knowledge related to mixing of characters in the offsprings of sexually reproducing organisms, we learnt that mixing of paternal and maternal characters occurred because of crossing over between paternal and maternal chromosomes (which bear genes responsible for controlling the corresponding characters) during gamete formation in sexual reproductive organs. It was debatable for a long time that during meiosis, whether the paternal and maternal chromosomes of a bivalent, first cross-over (**crossing-over at two-strand stage**) and then get duplicated; or they first get duplicated and then crossing over takes place between any two of the four chromatids (**crossing over at four- stranded stage)** formed after duplication of maternal and paternal chromosomes forming pairs(bivalents) during the process of meiosis-I.

The cytological studies on *Drosophila melanogaster* and tetrad analysis in *Neurospora* have confirmed that crossing-over involving paternal and maternal chromosomes takes place at Four-stranded stage (**tetrad stage)** of chromosomes at meiosis-I, and out of the four chromatids only two non-sister chromatids(one paternal and one maternal chromatid) are involved in exchange of genetic material at a particular locus involving specific genes. The remaining two non-sister chromatids will not have genetic recombination involving the same genes at the same locus. Thus, a maximum of 50% gametes formed after meiosis will exhibit recombination of a specific pair of parental genes, and the rest of gametes will have parental combination(**exhibiting linkage**) involving the said gametes in the individuals of the next generation. This type of study provides a clever way to determine the relative distance between the locus of a gene with centromere of the respective chromosome so that genes present on a chromosome can be mapped. Let us illustrate this based on analysis of

segregation of alleles of a gene of responsible for ascospore-colour in the ascus

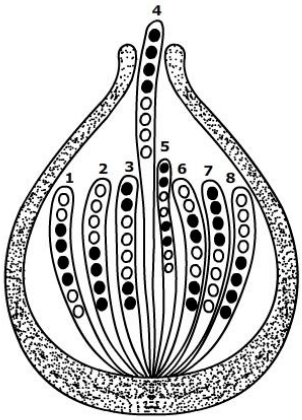


Figure: Diagramatic representation of two patterns of ascospore arrangement in *Neurospora,* representing eight asci. Asci numbered as 1,3,5 & 6 show 2+2+2+2, that is, ditype arrangement of ascospores; and asci numbered as 2,4,7 and 8, show 4+4 or tetratype arrangement of ascospores of a perithecium in *Neurospora*.

Source:Author

It is very common to find that ascospores in various asci of a perithecium exhibit three patterns of ascospore arrangement based on spore-colour. Fifty percent of the asci(numbered as 2,4,7 & 8 in above above figure) in a perithecium have 4+4 (**Tetra type**) arrangement of ascospores wherein, first four ascospores in a sequence are coloured and next four ascospores are colourless or vice versa. The remaining asci have 2+2+2+2 (asci numbered as 5 and 6 in above above figure) or 2+4+2 (asci numbered as 1 and 3 in above above figure) arrangement of ascospores based on spore colour. Both these patterns of ascospore arrangement, viz., 2+2+2+2 and 2+4+2, can be categorized as part of **ditype** arrangement, if we divide the middle group of four ascospores into 2+2.

Let us explain this, diagrammatically based on the following assumptions:-

1. Let there be a gene having its two alleles,'A & a' responsible for spore colour, coloured (A) and colourless (a) in *Neurospora*.
2. Let us consider three ascospore mother cells (AMC), I,II and III, each heterozygous (Aa) for spore colour as shown in Figure below, bearing one pair of homologous

chromosomes, representing one chromosome from '+' strain and one chromosome from '-' strain.

3. During the ascus development , let us consider that AMC-I produces ascus without crossing-over; AMC-II produces ascus involving crossing-over at 2-strand stage of chromosomes and AMC-III produces ascus involving crossing-over at 4-stranded stage of chromosomes.
4. If you follow the diagrammatic scheme, you would find that AMC-I and AMC-II produce mature ascus having **tetratype** arrangement of ascospores; and AMC-III produces an ascus having **ditype** arrangement of ascospores.

It can be easily concluded that ditype ascus is the result of crossing–over at 4-stranded stage of chromosomes wherein, the allelic genes for spore colour (Aa) segregated into separate nuclei (A,a,A,a) during meiosis-II(**second divisional segregation)** and that, after mitosis got arranged in pairs(AA, aa, AA, aa), so that after cytokinesis, the ascus exhibited ditype arrangement of ascospores as shown in Ascus no.5 in above Figure. You would note that in the given diagram, crossing over has been considered involving inner two nonsister chromatids out of the four. The ditype arrangement of ascospores as shown in Ascus nos. 1, 3 and 6 in Fig.9, can be visualized if crossing over is considered involving other three permutations & combinations involving outer two chromatids on one occasion and one inner and one outer chromatids on the other two occasions.

The tetratype ascus produced by AMC-I is the result of no crossing over wherein, genes for spore colour (Aa) segregated into separate nuclei at meiosis-I (**First divisional segregation**), so that mature ascus after meiosis-II followed by mitosis and cytokinesis exhibited **tetratype** (AAAA aaaa) of ascus as shown in Ascus nos.4 and 7 in Figure.

However, tetratype of ascus as produced by AMC-II and as shown in Ascus nos.2 & 8 as in above Figure, could have resulted due to another possibility, wherein, if at **diad** stage in the above diagram, chromosome from 'a'-strain would have been above and that of "A" strain would have been below, followed by sequence of events as in AMC-I. And, in fact, this is the actual reason for the tetratype ascus as produced by AMC-II, and not because of the assumption as depicted here.

It is very significant to remember in the light of our present knowledge of chromosomal behavior during meiotic division that, the chromosomes(DNA) duplicate during the S (synthetic) phase of interphase so that the chromosomes are already longitudinally double (**except at centromere**) when the diploid nucleus enters into **leptotene** stage of meiosis-I. Thus, when homologous chromosomes are involved in the process of pairing before crossing over(at **Pachytene** and **Diplotene**), they are already four-stranded. Thus, in the

present case, the assumption related to AMC-II, wherein, crossing over has been considered at two-strand stage of chromosomes, **is wrong;** and, it can be concluded that tetratype ascus produced by AMC-II is, because of the fact that crossing over did not take place involving non sister chromatids bearing A and a alleles for spore-colour.

Thus it is very clear that ditype of asci in *Neurospora* are the result of **crossing over** or **Second divisional segregation** during meiosis, and tetratype asci are the result of **no crossing over** or **First divisional segregation** during meiosis in Ascus mother cell that gives rise to mature ascus. We can also conclude that crossing over involving the alternate alleles of a gene, say "A" and "a" during meiosis, causes delay in their segregation, that ultimately takes place in meiosis-II leading to second divisional segregation. Segregation pattern of different mutants representing known mutations have been analysed for almost all the genes of *Neurospora*, that have been identified and mapped relative to their distance from the centromere of each chromosome in *Neurospora*. It has been revealed that *Neurospora* (n=7) has nearly 10,000 genes, collectively on its seven chromosomes.

Assuming that the locus of gene in question is closed to centromere of a given chromosome so that chances of multiple crossing overs can be ignored, the map distance between centromere and the locus of gene in question, can be determined by counting the number of asci per perithecium showing second divisional segregation divided by total number of asci as given below:

$$\frac{\text{Number of Asci per perithecium having second divisional segregation X 100}}{\text{2X total number of asci per perithecium}}$$

The above mechanism of crossing over in *Neurospora* supports the view that recombination% can never be more than 50% even if there is chiasma in every tetrad involved. It is because each crossover involves only two of the nonsister chromatids out of the four chromatids of each of the bivalents representing homologous chromosomes.

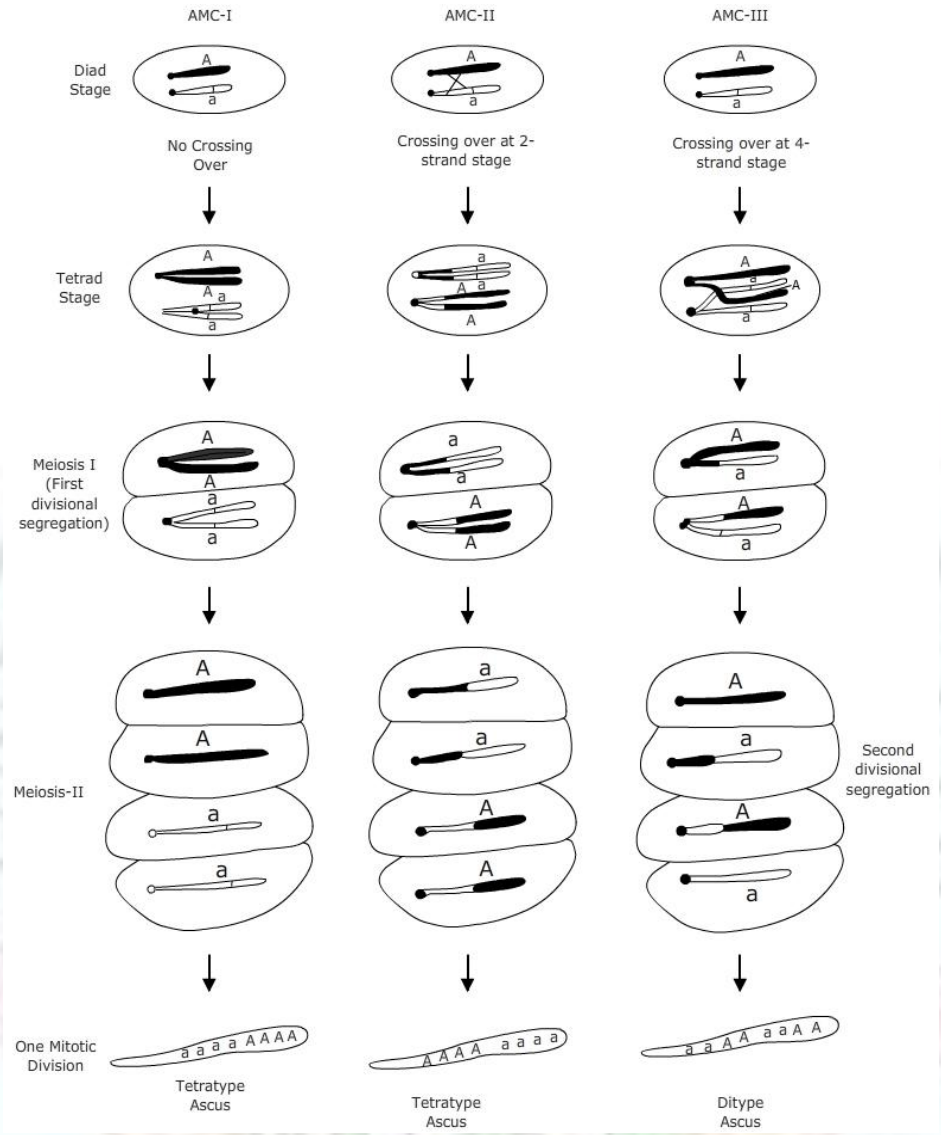


Figure: Schematic representation of cause of tetratype and ditype arrangement of ascospores in the asci of *Neurospora*

Summary

The species of *Alternaria* are anamorphic (reproducing asexually) and belong to the class, *Deuteromycetes*. Teleomorphic genera of *Alternaria* are placed in the sub-class, *Pyrenomycetes* belonging to the **class-Ascomycetes**. All the species of *Alternaria* are saprophytic, and can be easily cultured on synthetic medium. However, some species act as facultative parasites, and are known to cause many plant diseases e.g. early blight of potato is caused by *Alternaria solani* and Brown spot of Citrus-plants is caused by *A. alternata*. The most characteristic symptom of *Alternaria*-infection is circular or eccentric target-board

pattern with a yellow halo external to the infected spot, especially on large infected leaves. The asexual reproductive stage in *Alternaria* is represented by clavate or flask-shaped multicellular, dark brown coloured conidia, borne on brown-coloured multicellular and branched conidiophores. In culture, the conidia may be borne in chains of 3 or more conidia per chain. Since the mature conidia of *Alternaria* have transverse as well as longitudinal septation, they are called **muriform conidia.** Some teleomorphic genera of *Alternaria* species are *Lewia, Clathrospora, Leptosphaeria* and *Pleospora.*The sexually produced perfect reproductive bodies of teleomorphic genera are perithecia, which are generally, spherical with a pore or flask-shaped with a small beak. The asci are cylindrical or subcyclindrical and mature ascospores are always multicellular and dark-brown due to melanin pigment. The most common method to control diseases caused by *Alternaria* is by spraying fungicides at fixed intervals, and cultivate varieties that are genetically resistant to infection by *Alternaria*. The species of *Alternaria* , that occur as obligate saprophytes, have an important role in degradation of organic waste in soil leading to soil fertility.

Neurospora is an obligate saprophyte representing a teleomorphic genus that belongs to sub class-**Pyrenomycetes**, sub-division of the class-**Ascomycetes.** Its anamorphic state is placed in the Genus, *Monilia* or *Chrysonilia,* that is grouped among Imperfect Fungi representing the class-**Deuteromycetes**. *Neurospora* has its name because of longitudinal striations found in the ascospores that resemble the markings on the neurons or the nerve fibres. The fast rate of multiplication of *Neurospora* due to production of abundant pink coloured conidia has made it a "**Model Organism"** to get quick scientific results. The systematic ordered arrangement of ascospores in each ascus followed by their release at fixed intervals has made it suitable organism for studying the process of segregation and recombination of genes through the technique, called "**tetrad analysis".** The entire genome of *Neurospora,* representing nearly 10,000 genes on its seven chromosomes, has been mapped. It has been confirmed that the ditype asci are the products of Second divisional segregation, whereas tetratype asci are the products of First divisional segregation.

The asexual or imperfect state of *Neurospora* multiplies by microconidia and macroconidia borne on branched pink-coloured conidiophores. The macroconidia are oval, larger than microconidia and each has 2 or more haploid nuclei, the microconidia are spherical, smaller than macroconidia, and each has only a single haploid nucleus. During sexual reproduction, microconidium plays the role of male gamete(**spermatium**) and helps in dikaryotisation during the formation of ascogenous hyphae leading to the development of asci and formation of flask-shaped **ascomata**, called Perithecia .

Neurospora exhibits three types of teleomorphic sexual cycles. *N.Crassa* and *N.sitophila* exhibit strictly heterothallic sexual cycle. *N.galapagoensis* exhibits homothallic sexual cycle and *N.tetrasperma* exhibits pseudohomothallic or secondarily homothallic sexual cycle. *Neurospora* reflects a case of gradual degeneration of male sex organ where a distinct male organ (**antheridium**) is absent. In *N.Crassa* & *N.sitophila*, function of male organ is performed by microconidium, that for this reason, is called **spermatium**(= sperm-like).In *N.galapagoensis,* function of male organ may be performed by microconidium or macroconidium or any somatic hyphae borne on the same thallus but having genetically different nucleus. In *N.tetrasperma*, the need of plasmogamy has been completely eliminated because every somatic cell has atleast two genetically different nuclei because the thallus is the product of a dikaryotic ascospore. However these thalli do produce + conidia or – conidia but the ascomata develop from dikaryotic hyphae that give rise to dikaryotic ascogenous hyphae each cell of which is potentially an ascospore mother cell that later exhibits karyogamy and meiosis.

The haploid nature of the thallus, accompanied by fast rate of vegetative growth, ability to grow on minimal medium, expression of genetic trait after irradiation to X-rays in the same generation, associated with quick detection of metabolic errors, followed by easy tetrad analysis, resulted in discovery of "**One-gene-One-enzyme"** hypothesis discovered by Beadle & Tatum for which they were awarded Nobel Prize in Physiology and Medicine in 1958.

Being an obligate saprophyte, *Neurospora*, just like most other fungi is helpful in increase of soil-fertility by its ability to degrade organic waste in soil. It is known to create nuisance in research laboratory as a contaminant in cultures, and when it happens to infest a bakery. The laboratory & the bakery have to be closed for several months to make them free of *Neurospora*, once it happens to invade these two places by chance.

Glossary:

Achrocentric : A chromosome having slightly subterminal centromere

Anamorph: The asexual state of a fungus.

Apothecium: A saucer or cup shaped fruiting body, bearing a single layer of asci generally with or rarely without paraphyses.

Ascocarp or Ascoma (Pl. Ascomata): Fruiting body of Ascomycetes, with its peridium consisting of pseudo-parenchyma or tightly interwoven hyphae and generally containing millions of asci.

Asexual reproduction: Reproduction without the process of karyogamy and meiosis.

Auxotroph : A nutritional mutant that fails to grow in a minimal medium due to development of a metabolic error

Bordeaux Mixture: Fungicide representing mixture of CuSO4, Ca(OH)2 and water, in the ratio 4:4:50 or 8:8:50

Canker: Infected host tissue that gets hardened and gets raised above the general surface of infected organ.

Deuteromycetes: An artificial subdivision in Kingdom Fungi to accommodate those fungi where only the asexual state is known.

Ditype Ascus: Ascus in *Neurospora*, having 2+2+2+2 arrangement of ascospores due to second divisional segregation of an allelic pair.

Facultative Parasite : Saprophytic fungus having potentiality to infect healthy plants and causes various diseases

Fungi Imperfecti : an anamorphic group of fungi belonging to ascomycetes or basidiomycetes, also called conidial fungi or Deuteromycetes .

Heterokaryotic: A cell having more than two genetically different nuclei

Heterothallic: An organism that requires two morphologically or physiologically different thalli for sexual reproduction.

Homothallic: An organism wherein, the single thallus is sufficient for sexual reproduction

Macroconidium (pl. macroconidia): The larger of two different types of conidia produced by a fungus in asexual reproduction.

Microconidium (pl. microconidia): The smaller of two different types of conidia produced by a fungus in asexual reproduction.

Model Organisms: Organisms which are extensively studied to understand various biological phenomena.

Muriform Conidia: Multicellular conidia having transverse as well as longitudinal septation e.g. *Alternaria*

Perithecium: Flask shaped or spherical fruiting body with or without beak having basal tuft of asci with a pore through which asci discharge the ascospores.

Protoperithecium: A female sex organ(ascogonium) produced in some ascomycetous fungi which gets branched to produce hair-like receptive hyphae(**trichogynes)** to act as female organs; which at a later stage gives rise to an ascoma, called **perithecium.**

Spermatium: An asexual spore(conidium) which functions as male gamete in the process of fertilization, called **spermatisation**

Symptom: Any morphological or physiological evidence of a disease in the diseased organism.

Teleomorph: The sexual state of a fungus.

Telocentric: A chromosome having terminal centromere

Tetratype Ascus: Ascus in *Neurospora* having 4+4 arrangement of ascospores due to First divisional segregation of an allelic pair.

Exercises/ Questions for Practice:

1. Describe the vegetative thallus of *Neurospora*.

2. Write a short note on sexual stage of *Alternaria*.

3. Why is *Neurospora* considered as a good model system to study genetics?

4. Name four diseases caused by *Alternaria*?

5. Write short note on:

   i. Spitzenkorper
   ii. *Neurospora* in tetrad analysis
   iii. Early blight of Potato
   iv. *Neurospora* as nerve spore
   v. Perithecium development in *Neurospora*

6. Describe the different types of sexual cycles in *Neurospora.*

7. What is the cause of ditype and tetratype arrangement of ascospores in *Neurospora*?

8. Describe an experiment performed by Beadle & Tatum that served as an aid to propose "One –gene- one-enzyme" hypothesis.

9. Fill in the blanks.

(i) The literal meaning of the Word, *Neurospora* is…….

(ii) The conidia of *Alternaria*, are……celled, but the conidia of *Neurospora*, are ……..celled

(iii) Botanical name of Red Bread Mold is…….

(iv) Early blight of Potato is caused by……..

(v) Two mycotoxins produced by *Alternaria* are,……..and……

(vi) Two heterothallic species of *Neurospora,* are ……..and…….

10. Tick(√) mark the most appropriate alternative

(i) The cause of ditype arrangement of ascospores in *Neurospora* is

(a) First divisional segregation in ascus mother cell

(b) Second divisional segregation in ascus mother cell

(c) Crossing over during meiosis during ascus development

(d) both (b) & (c)

(ii) In the perithecium of *Neurospora*, asci mature

(a) at the same time

(b) at different times

(c) at fixed intervals and release ascospores one by one

(d) simultaneously and release four ascospores at a time

(iii) The number of nuclei in a macroconidium of *Neurospora* is

(a) One (b) two (c) two or more (d) always three

(iv)The Characteristic symptom of *Alternaria* infection on leaves is

(a) Oval chlorotic spots (b) Long brown streaks

(c) Circular or eccentric target board pattern

(d) always circular target board pattern

(v) The following is the Generic name of teleomorph of *Alternaria* species:

(a) *Leptosphaeria* (b) *Pleospora*

(c *)* *Lewia* (d) All the above

(vi) The anamorphic genus for *Neurospora* is

(a) *Clathrospora* (b) *Monilia*

(c *)* *Curvularia* (d) *Cladosporium*

(vii) The ascospores in teleomorphic stage of *Alternaria*, are

(a) always one-celled (b) 2 or 3-celled

(c *)* 3 to 5 celled (d) always multicelled

(viii) The following is/are allergic disease/s caused by *Alternaria*

(a) asthma (b) mycoses (c) leucopenia (d) all the above

(ix) The functions of microconidium in *Neurospora* are/is:-

(a) to act as asexual spore only (b) to act as a male gamete only

(c) to act as female gamete only (d) both (a) and (b)

(x) Minimal medium for *Neurospora* culture, contains

(a) source of Carbon, all vitamins, inorganic nutrients and all amino acids

(b) source of Carbon , all amino acids and all vitamins

(c) source of Carbon and inorganic nutrients

(d) source of carbon, inorganic nutrients and all amino acids.

References/ Bibliography/ Further Reading

1) Agrios G. N. 1988. Plant pathology, 3rd edition, Academic Press, New York, Berkeley, Boston, London, Sydney, Tokyo, Toronto.
2) Brooks F. T. 1953. Plant diseases. Geoffrey Cumberlege.Oxford University Press, London, new York, Toronto.
3) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3713888/

Web links

http://edis.ifas.ufl.edu/pdffiles/CH/CH01700.pdf