

# REPRODUCTIVE APPROACHES IN PHYTOPATHOGENIC FUNGI

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## Abstract

Sexual reproduction provides genetic exchange in eukaryotic organisms as varied as animals, fungi and plants. Given its pervasiveness, sex is thought to have evolved once, probably associated with or soon after the origin of eukaryotic organisms. The fundamental principles of sex are preserved, including ploidy changes, the process of meiosis and formation of gametes, recognition of mate and cell to cell fusion leading to the creation of a zygote. Although the basic tenants are shared, sex determination and sexual reproduction occur in myriad forms throughout nature, including outbreeding systems with two mating types or sexes, unisexual selfing, and even examples in which organisms switch mating type. As provided with diverse and robust genetic models, kingdom fungi offer insights into the molecular nature of sex, sexual condition, and evolution to precede our understanding of sexual reproduction and its impact throughout the eukaryotic tree of life.

## Introduction

80000 to 120000 no. of fungal species have been explain till date, although the total number of species is estimated at around 1.5million (Hawksworth, 2001; Kirk *et al.*, 2001). This would render fungi one of the least-explored biodiversity resources of our planet. It is notoriously difficult to delimit fungi as a group against other eukaryotes, and debates over the inclusion or exclusion of certain groups have been going on for well over a century.

Collectively, fungi and fungal-like organisms (FLOs) cause more plant diseases than any other group of plant pest with over 8,000 species shown to cause disease. The importance of fungi as agents of plant and human disease, producers of industrial and pharmacological products, and decomposers has spurred scientists worldwide to study their biology. The impact that fungi have with regards to plant health, food loss, and human nutrition is staggering. Some of the world's great famines and human suffering can be blamed on plant pathogenic fungi and FLOs.

Sexual development is though a mysterious but common phenomenon in eukaryote including animal, plants and fungi. It is an exciting issue that how organisms have evolved partner recognition systems and sexual development. How did sex first evolved and also

maintained throughout all eukaryotic ancestry for billions of years? Fungi provide an excellent system to understand the genetics of sex in several aspects: (i) each fungal group exhibits a variety of sexual development models; (ii) genomic sequence of many fungi are now available, and studies revealed that many fungi earlier classified as “asexual fungi” maintain all the necessary machinery needed for sex. Study on evolution of sex in eukaryote like the fungi has been proved to be informative, since first sex was possibly happened in last common ancestor shared by eukaryotes, an aquatic single-celled microbe from which all eukaryotes are descended.

The event of reproduction is one of the most variable traits for the living organism with giving rise to offspring either by sexually or clonally, and in the former case by selfing or outcrossing. Patterns of reproduction and inheritance significantly affect major ecological and evolutionary processes, as well as the applied fields of breeding and biotechnology (Whitton *et al.*, 2012), conservation biology (Fréville *et al.*, 2007), species invasion (Barrett, 2011) and pathogen evolution and control (Shea *et al.*, 2000; Zuk, 2009), therefore understanding the factors affecting reproductive mode of an organism's is of fundamental importance. The frequency of reproduction by sexual and asexual means is highly variable among eukaryotes, with the vast majority being able to carry out sexual reproduction either exclusively (e.g. mammals) or alternatively with clonality (e.g. aphids many angiosperms and fungi). Sexual reproduction involves the series of haploid and diploid phases, transitions taking place by meiosis, resulting in recombination and segregation, and syngamy, where two haploids fuse.

Mating systems are also highly variable: most species undertake mainly selfing or outcrossing, whereas a few species demonstrate a mixed-mating system in both plants and animals (Jarne & Auld, 2006; Iqbal & Kohn, 2006). Selfing occurs after the syngamy between haploid cells which are produced by the same diploid individual, whereas, outcrossing is the result of the syngamy between haploid cells produced by different diploid individuals (Figs 1 and 2). In animals and plants, selfing occurs through the union of gametes which are produced from meiosis in a single diploid individual, referred to as diploid selfing. In some eukaryotes, particularly with an extended haploid life phase such as ascomycete fungi, some algae, mosses and ferns, possibilities of selfing depend on the union of two mitotic descendants of the same haploid cell, called intrahaploid mating, intragametophytic selfing (Hedrick, 1987) or haploid selfing (Billiard *et al.*, 2011).

Biologists have fought for more than a century to spot the causes responsible for the evolution of such preference for sexual vs. asexual reproduction. Sexual reproduction has it seems that been vanished independently in diverse eukaryotes (in fungi, Billiard *et al.*, 2011; López-Villavicencio *et al.*, 2010; and in plants, Whitton *et al.*, 2008). However, there is rising confirmation that some species thought to be asexual shift to cryptic sex (Schurko *et al.*, 2009;

Lee *et al.*, 2010). Biological classes showing a vast diversity of reproductive approaches offer exclusive chances to recognize the factors affecting the evolution of sex and mating systems. The majority of hypothesis and explanations have been based on insect and plant models, and other eukaryotes, even with more diversity in reproductive approaches, have been regularly excluded from the contest (Birky, 1999). Eukaryotes such as fungi are exceptional models to study these issues (Lee *et al.*, 2010; Billiard *et al.*, 2011; Whittle and Johannesson 2013). There have been a manifold changes from sexuality to asexuality (López-Villavicencio *et al.*, 2010; Coelho *et al.*, 2017) in fungal groups including obligate sexual species (e.g. *Microbotryum*, Giraud *et al.*, 2008), species with both sexual and asexual reproduction and others strictly asexual species (Schurko *et al.*, 2009). Fungi are considerably informative because they show a vast diversity in the degree of diploid and haploid selfing rates (Billiard *et al.*, 2011), and they offer considerable advantages compare to other eukaryotic models for experimental studies (Goddard, 2008). For example, many spp. can be cultivated under laboratory conditions, have comparatively short life cycle and can be revived after storage which is useful in experimental studies.

### **The different approaches in reproduction and mating systems in fungi**

Fungi display a vast diversity in life cycles. Most fungi are able to reproduce through both sexual and asexual reproduction. However, there is a possibility of syngamy in fungi as compared to animals and plants. In heterothallic fungi, haploid selfing is never happens because syngamy can occur only between haploid cells with different alleles at the mating type locus/loci. However, fungi considered to be homothallic, syngamy can occur between genetically identical haploid cells resulting in haploid selfing

### **Asexual reproduction in Fungi**

Fungi show the following methods of asexual reproduction

**Fragmentation** During this process, the fungal mycelium breaks into two or more identical fragments either due to some external force or accidentally. Each fragment gives rise to new mycelium. This occurs frequently in nature and in cultivation of fungi in laboratory.

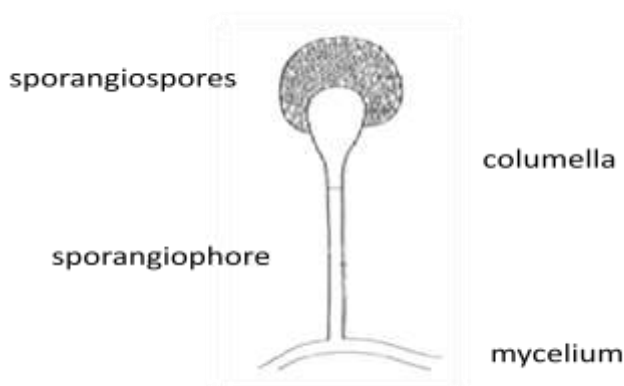
**Budding** The parent cell produces one or more soft zone projections appears on cell wall which swells out, and called buds, which later develop necessary structures and detach to grow into new individuals. Budding is common in unicellular forms like yeast and may occur on spore also.

**Fission** this type of process occurs only in fission yeast e.g. *Schizosaccharomyces*. During this process, the cell divides transversely in to two cells.

**Spores** The most common method of asexual reproduction in fungi is by spores. The term spore is used to describe any small reproductive, propagative or survival unit which after separation from a hypha gives rise to new individuals. Asexual spores are also called mitospores.

Asexual spores are of two main types i.e. **Sporangiospores** and **Conidia**.

Sporangiospores are produced within a saclike structure, called sporangium (Fig 1). The entire contents of sporangium are converted through cleavage into one or usually more spores. Sporangium is borne on a specialized and an undifferentiated hyphal structure, called sporangiophore. Sporangiospores may be motile or non-motile. Nearly all the true fungi produced non-motile sporangiospores, called aplanospores.

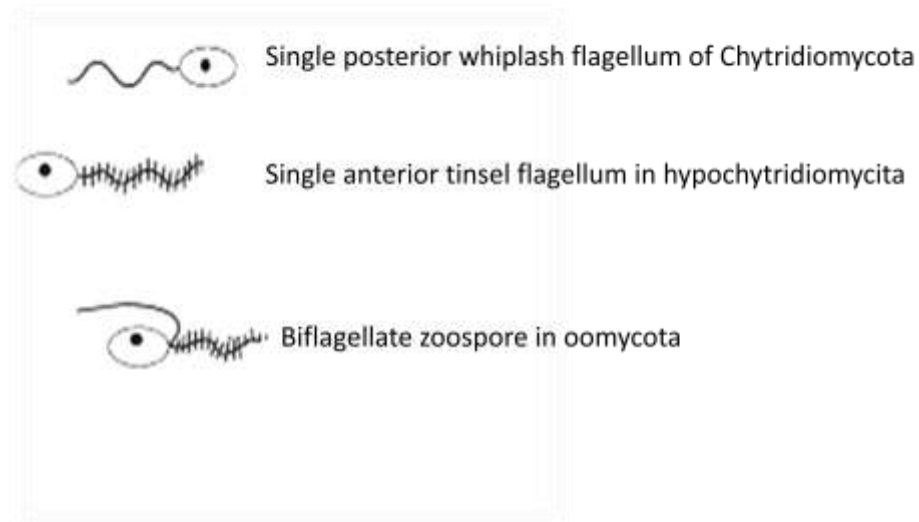


**Figure 1.** Sporangia of fungi

However, motile sporangiospores called zoospores are produced by fungi belong to phylum chytridiomycota. Zoospores are usually equipped with one or two flagella. Pattern of flagellation is of two type (i) tinsel and (ii) whiplash type. Flagella are mostly found in aquatic fungi. However, terrestrial fungi which are rely on water for dispersal or infection may also posses flagella. On the basis of characteristic insertion of flagella, the zoospores are differentiated in following groups (Fig 2)-

- ✓ Single posterior flagellate zoospores with whiplash flagella are characteristic of the Chytridiomycota. Each flagellum has 1 microtubules arranged in the 9 + 2 pattern characteristic of eukaryotes.
- ✓ Zoospores with two whiplash flagella of unequal length are called anisokont and are found in some Myxomycota and the Plasmodiophoromycota, both now classified among the Protozoa.
- ✓ Zoospores with a Single anterior flagellum of the tinsel type are characteristic of the Hyphochytriomycota.

- ✓ Biflagellate zoospores with apically or laterally attached both whiplash and tinsel flagella (heterokont), are characteristic of the Oomycota.



**Figure 2.** Structure of zoospores

Conidia these are most commonly found in Ascomycota and anamorphic fungi. Conidia may be uni- or multicellular and display variety of shape and colour. The most important method of asexual reproduction is by means of exogenously produced conidia. The conidia are produced on the conidiophores in basigenous or acrogenous chains.

### Arrangement of conidiophores:

The conidia are generally produced on conidiophores. The conidiophores may be organized into definite fruiting bodies or produced free from each other. The most common fruiting bodies are of following types (Fig 3).

#### 1. Pycnidium:

This type of fruiting body is a hollow, commonly globose or flask-shaped structure whose pseudoparenchymatous walls are lined with conidiophores. In such case the conidia are known as pycniospores or Pycnidiospores. The pycnidium opens by an opening called ostiole.

#### 2. Acervulus:

This type of fruiting body develops a mat of hyphae, below the epidermis of cuticle of the host plant and giving rise to short conidiophores closely packed together. On maturation such

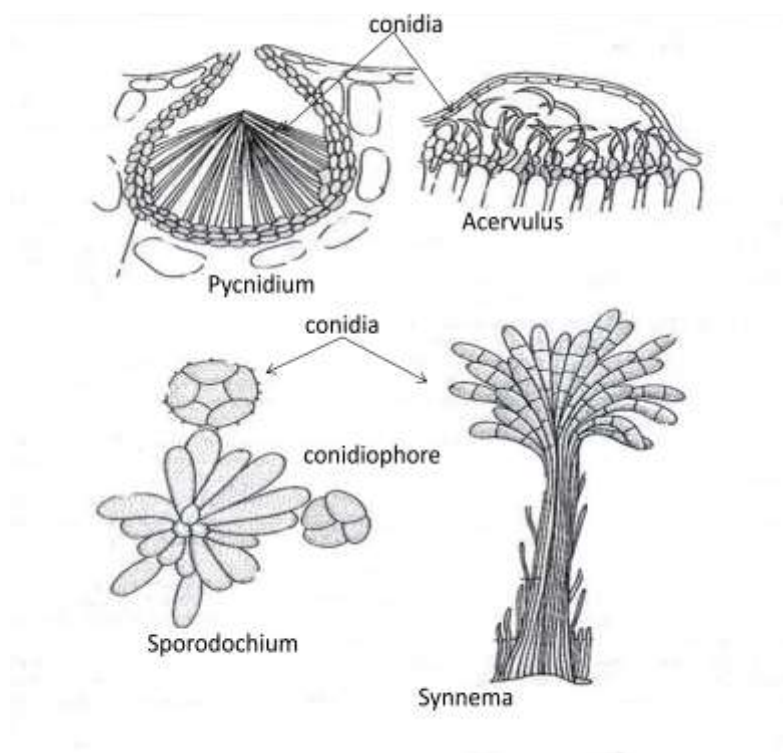
structure becomes saucer-shaped and looks like a flat open bed of conidiophores with conidia at their tips. The acervuli are usually formed by the parasitic fungi.

### 3. Sporodochium:

The conidiophores may also be cemented together to form complex structures known as sporodochia. This is a hemispherical or barrel-like asexual fruiting body. The lower part of sporodochium consists of a cushion-like stromatic mass of hyphae. The conidiophores arise from the surface of the stroma. The conidiophores make the upper part of the sporodochium.

### 4. Synnemata:

Here the conidiophores remain arranged very close to each other. The conidiophores may be branched or unbranched. They are generally united along greater part of their length to form the asexual fruiting body known as synnema (e.g., *Arthobotryum* sp.).



**Figure 3.** Common fruiting bodies of fungi

The conidia vary in shape, size and colour. The conidia of *Aspergillus* and *Penicillium* are spherical and smoky green. They may be smooth (e.g., *Penicillium*) or echinulate (e.g.,

Aspergillus). They may be uninucleate (e.g., Penicillium) or multinucleate (e.g., Aspergillus). In Erysiphe the conidia are barrel-shaped. The conidia germinate immediately after their detachment from the conidiophores when they fall upon suitable substratum in appropriate atmospheric conditions producing germ-tubes. They remain viable only for a short time..

### Sexual Reproduction

Like in other living organism, sexual reproduction in fungi involves the union of two compatible nuclei. The process of sexual reproduction involves three distinct events occur in a cyclic pattern: Plasmogamy, karyogamy and meiosis. In first event, protoplasts of two different sexual cells fused together, called plasmogamy, resulted in two genetically different nuclei coming together within same cell and bring a binucleate cell which carries one nucleus from each parent (dikaryon). Later, fusion of nuclei, called karyogamy results in diploid zygote nucleus. Nuclear fusion is sooner or later followed by meiosis which reduces the chromosomes number to haploid and production of genetically different individuals. Sexual reproduction in most of the fungi involves the production of specialized spores. Four types of spores have been given special names viz. oospores, zygosporos, ascospores and basidiospores (Richards, 2003; Busch, 2011).

### Fungal sexual organs

In general the sex organs of fungi are called gametangia (sing. gametangium). The term isogametangia and isogametes are used to designate morphologically indistinguishable gametangia and gametes. Likewise, heterogametangi and heterogametes are used to designate male and female gametangia which are morphologically different. The male gametangium is called antheridium and female counterpart is called oogonium or ascogonium depending on the group of fungi.

**Sexual compatibility in fungi-** On the basis of sex most of the fungi are classified in following groups:

- A. Hermaphroditic-** In this group each thallus of fungi produces distinguishable male and female sex organs which may be compatible or not. They are also called monoecious. A single thallus of monoecious fungi can reproduce sexually by itself if self-compatible.
- B. Dioecious- Fungal spp** in this group consist of thalli bear only male or female sex organs.
- C. Sexually undifferentiated-** In majority of the fungi morphologically indistinguishable as male or female sex organs are produced.

On the basis of compatibility, fungi in the categories mentioned above belong to one or other following three groups:

1. **Homothallic Fungi-** every thallus is sexually self fertile and therefore, can reproduce itself without help of other thallus. Fungi in this group do not exhibit mating type and are non-outcrossing.
2. **Heterothallic Fungi-** Every thallus is sexually self-sterile, apart from of whether or not it is hermaphrodite and requires the help of a compatible thallus of a different mating type.
3. **Secondary Homothallic Fungi-** An interesting mechanism is works in some heterothallic fungi, where, two nuclei of different mating types are integrate regularly into atleast some spores. Therefore, offsprings arising from such type of spore are self-fertile and act as they are homothallic but actually they are heterothallic. This phenomenon has been referred as secondary homothallism, also called pseudohomothallism.

Both the terms homothallism and heterothallism are also used to explain the condition in other groups such as oomycetes. These terms are used to explain how sexual reproduction is started in oomycetes, which are phylogenetically closer to brown algae: heterothallic oomycetes never produce gamete and cannot undergo sexual reproduction unless there is an individual of the opposite mating type is present, while homothallic oomycetes can. In both, homothallic and heterothallic oomycetes, outcrossing and diploid selfing are possible after the production of gametes. Thus, the terms, homothallism and heterothallism used to communicate different events in different organisms. This condition is confusing, particularly as the terms homothallism and heterothallism are also used interchangeably with 'selfing' and 'outcrossing' in both oomycetes and fungi.

There are five basic mode of sexual reproduction or sexual fusion which bring the compatible nuclei together.

1. **Planogametic copulation** It involves the union of two naked free gametes which may be motile. Motile gametes are called planogametes. On the basis of size and motility of the gametes there are three type of planogametic copulation- Isogamy, anisogamy and heterogamy.

**Isogamy-** The fusing gametes are similar in shape and size and gametes are called isogametes.

**Anisogamy-** Occurs only in *Allomyces*, where the two fusing planogametes are morphologically similar but different in size.

**Heterogamy-** Occurs only in one genus, *Monoblepheridomycetes* where motile male gamete fused with a non-motile female gamete.

2. **Gametangial contact-** During this event of plasmogamy, the male gametangium, consists of a nucleus inside the antheridium migrates into the oogonium after the sex organs come in contact. The male gametic nucleus migrates through a specialized fertilization tube developed by male sex organs or through a pore at the contact site. Bothe sex organs do not fused but rather retain their identity.



3. **Gametangial copulation-** During plasmogamy, the entire content of both male and female gametangia fused together and become one. The process of gametangial copulation occurs in two ways, either through direct fusion of gametangia or by migration of entire protoplast of one into other gametangium through a pore.
4. **Spermatization-** In some ascomycetes and basidiomycetes (*Puccinia*), the male gametes called spermatia are produced externally on spermatophores or in cavities called spermogonia. After contact with female organ, through a pore the spermatia empty their content. The female organ may be a specialized receptive hypha, a female gametangium or even a somatic hypha.
5. **Somatogamy-** Fusion between two undistinguishable somatic cells or spores is called somatogamy or somatic copulation.

## Conclusion

Sexual reproduction in fungi is characterized by a variety of methods and factors. The fundamental feature of sexual reproduction in all organisms is characterized by fusion of two compatible nuclei. Nevertheless, fungi are mostly haploid, and nuclear fusion is followed by meiotic division. The fusion of two compatible nuclei is pulled off in various ways, viz. production of distinct sex organs and exogenous fusion of gametes and as in many situations where mating involves the fusion of vegetative or somatic cell and none sexual structures are not produced. Reproduction in fungi shows difference in both the number of loci controlling compatibility (one or two) and in the number of alleles that are located at each locus. Growing information on reproduction in fungi through whole-genome sequencing efforts, as well as intensive species screenings, makes it possible to inspect the different hypotheses. Population genetic studies are critically needed to assess whether selfing or outbreeding most often occur in nature and in association with various genetic modes of inheriting mating compatibility factors.

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