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PLANT SEXUAL REPRODUCTION: ASPECTS OF INTERACTION, HISTORY AND REGULATION

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Sexual reproduction in angiosperms is an interactive process involving the sporophyte, gametophytes, embryo and endosperm as well as the environment, aimed at achieving pollination, fertilization and dispersal. This interaction occurs via an interface with nutrients and signals outside the cell and even outside the plant. Sexual reproduction has a history. In water, algae have different types of sex organs and gametes, and in some cases the female gamete stays on the plant. The zygote uses water movement and gravity for dispersal. Some algae have alternation of generations in the life cycle, and only the gametophyte functions in sexual reproduction. On land, ferns and mosses inherited alternation of generations, with oogamy and zygote development on the gametophyte, with wind dispersal of the meiospore. In angiosperms, heterospory and the retention of the megaspore, megagametophyte and embryo on the sporophyte lead to a seed with gravity and biotic dispersal. The history of sexual reproduction is based on sex determination, due to cross-fertilization and recombination. Sex differentiation is manifested in the increasing complexity of interaction in the nutrient supply, the retention of the gametophyte or even the embryo, and the type of vector of dispersal. Regulation of sexual reproduction in angiosperms is governed mainly by the sporophyte, with the expression of new genes for biotic pollination and seed dispersal. In the heterotrophic gametophyte some gene expression is suppressed. The development of sexual reproduction is due to the communication between the organism and a dynamic environment.

Key words: Sex differentiation, dispersal, interaction, regulation, environment.

INTRODUCTION

The organism represents a unit of form, function and biotope. When a plant starts sexual reproduction, its flowering means the origin of new forms and functions in connection with the environment.

Sexual plant reproduction is a complex sequence of different steps leading to a new organism. The genome is renewed through recom- bination in first meiosis and cross-fertilization. In the flower bud of angiosperms the anther and ovule develop to prepare the male and female gametophytes. In the open flower, pollination and fertilization occur, and then seed and fruit development begins.

This review presents sexual reproduction in angiosperms as an ordered and dynamic interaction between the sporophyte and gametophytes during development, the sporophyte and the environment during pollination, the male and female gametophytes during fertilization, the sporophyte and embryo with endosperm after fertilization, and the sporophyte and the environment during seed dispersal. Based on the sequence of ordered steps, the complex regulation of sexual reproduction will be presented.

The process of sexual reproduction in angiosperms can be compared with the process of sexual reproduction in algae, ferns, seed ferns and gymnosperms. Storage in a gamete or megaspore, retention of the gamete or the megaspore on the plant, and the means of dispersal, all determine the development of angiosperm sexual reproduction. Such a com-

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parative approach will give insight into the origin of the interaction and into the regulation of sexual reproduction.

INTERACTION

Interplay between organisms involves interactions taking place outside the organisms. Signaling substances and the production of special nutrients or other substances require cooperation of organisms. The area of cooperation is called the interface and is marked by special products in the functioning of sexual reproduction outside the cell or even outside the organism (Willemse, 1996). Due to its specialized function connected with reproduction, the diversity of products and their occurrence outside of the organism, the interface is not considered as an apoplast (Hong-Yuan Yang, 2001).

In the flower bud, the sporophyte of angiosperms begins the interaction in the sporangium after meiosis. In the anther the microspore develops into pollen, and in the ovule the megaspore develops into a megagametophyte. The sporophyte provides space for the developing gametophytes, attends to nutrient supply and retains the megagametophyte. Signals regulate the interaction between organisms. The interface between the sporophyte and developing male gametophyte consists of the callose wall, the locular fluid, and the remnants of the tapetal cells; these can remain as a coat around the pollen. Pollen can also export signal substances in the pollen wall. The interface between the sporophyte and developing megaspore contains callose and degenerating nucellus cells.

In the open flower the interaction between sporophyte and environment is expressed by way of pollination. In abiotic pollination, pollen is adapted to wind, water or gravity; and in biotic pollination, to animals or to the plant's selfing mechanism. Pollen uptake occurs on the stigma, and is adapted to the means of dispersal. In biotic pollination, the sporophyte elaborates a mode of advertisement, through odor and color and the form of petals and/or sepals. Routing, commonly leading to cross-pollination, takes place in the open flower, with or without guiding and nectar.

The interaction between the sporophyte and male gametophyte consists in the uptake of pollen, including recognition as well as activation of the pistil. After acceptance, the sporophyte guides the pollen tube towards the micropyle and supplies nutrients. The interface is the pollen coat and stigmatic droplet or pellicula, followed by the nutrients present in the pollen tube pathway. Nutrient supply flows between the sporophyte and female gametophyte. The interface consists of degenerating nucellus cells, and in the megagametophyte are transfer cells and a filiform apparatus with pollen tube attraction products. The interaction of the male and female gametophyte is realized by attraction products and the opening of the pollen tube in the synergid.

In the fruit, the sporophyte interacts with the embryo and endosperm by providing nutrients and space. In the beginning the interface has degenerating nucellus cells and inner integument cells. Later, after development of the endosperm, depending on the type of seed, breakdown products supply an interface for embryo development. Again there is interaction between the sporophyte and the environment, defined by seed or fruit dispersal via a vector. Commonly the abiotic vector for seed dispersal is gravity, but wind and water are also used. In biotic transport, the sporophyte elaborates a mode of advertisement, expressed in the odor, color and form of the fruit. The fruits are exposed on the plant, and the sporophyte adds nutrients to the fruit. Again, advertisement and nutrition represent interaction with animals.

From flower bud to fruit there is continuous interaction, with the main role for the sporophyte and a specialized role for the gametophytes. The interaction with the environment leading to pollen and seed dispersal is marked by the open flower and the fruit. The interface has nutrients and signal substances coming from the surrounding tissues but also consists in special cell walls and excretion and degeneration products.

This interaction and the interface mark the flower as an organ of communication. This system is the result of the history of the process of sexual reproduction in plants.

HISTORY

The history of plant life is marked by the transition from water to land.

WATER

In water, unicellular algae have sexual reproduction in the simplest form. Gametes are formed after meiosis and prepared for flagellar movement, attracting and fusing in water. The isogametes fuse to form a zygote, the unit of dispersal. The vector of dispersal is water, and then gravity plays a minor role. Recombination takes place during meiosis, and if cross-fertilization is promoted the zygote has a renewed genome. This renewal is the main goal of sexual reproduction.

The origin of multicellular algae is the result of incomplete cytokinesis, with cell wall contact, as in colonies, or wall and cytoplasm contact forming an organism with symplast and apoplast. Cells begin independent development after meiosis or mitosis followed by complete cytokinesis. For the reproductive function, localized sex organs, the antheridium and oogonium, develop from the epidermis. Morphologically dissimilar gametes are also formed. This is due to enlargement of the cell with storage products. The enlarged gamete is called the female gamete; it can be mobile or immobile. The fusion of such mobile cells is termed anisogamy, whereas the fusion of an immobile oocyte and a mobile male gamete is referred to as oogamy. Enlargement of the female gamete entails greater dependence on gravity for dispersal. Because of this, the fertilized oocyte determines the final position of the zygote and its settlement on the substrate.

The introduction of alternation of generations in the life cycle of algae is the next important step for plant sexual reproduction. The possibility to originate an organism from each unicellular stage during the life cycle results in a sequence of organisms. Each unicellular cell stage is a totipotent cell capable of making a plant. The mitospore and the zygote form a sporophyte, the meiospore forms the gametophyte, and a functional difference between plants develops. Only one plant is able to produce gametes, the gametophyte. Moreover, in a life cycle with alternation of generations there are two moments of dispersal, one by the meiospore and one by the zygote.

The gametophyte has sex organs and can have oogamy. In some algae the oocyte stays on the gametophyte, and the zygote and young plant develop on the gametophyte. With this retention an interaction takes place between the gametophyte and the zygote and even the young plant. In such a life cycle there is only one moment of dispersal by the meiospore.

LAND

A life cycle with alternation of generations, with oogamy and the development of the plant after fertilization on the gametophyte, characterizes the early land plants such as mosses and ferns. All these characteristics were already developed in water.

Ferns have alternation of generations, oogamy depending on water, and the development of the plantlet on the gametophyte. A terminal sporangium develops apically on the stem of primitive ferns, preparing the meiospores as units of dispersal. More advanced ferns have sporangia on their leaves. The meiospores are transported by wind, and land on the ground by gravity.

The next important change happens in the heterosporic ferns. A functional (as in some horsetails) or morphological difference in the meiospores develops, mainly through an increase of storage in one of the spores, the megaspore. With this development there begins an interaction, with the sporophyte providing extra nutrition to the megaspore. Again, this difference in meiospores has a consequence for spore dispersal. Microspores are transported by wind, and later gravity lets them fall downwards. The megaspore has gravity dispersal and falls on the ground. The use of different vectors presents a risk to fertilization when the spores are far apart, but on the other hand it promotes cross-fertilization.

The extinct seed ferns are heterosporic, with prolonged retention and development of the megaspore in the megasporangium on the sporophyte. The sporangium develops from the epidermis and hypodermis. This long interaction results in the formation of a seed, the unit of dispersal. Megagametophyte development, fertilization and seed development all take place on the sporophyte, which provides space and nutrients. The gametophytes become heterotrophic. The seed is transported mainly by gravity to the ground, as is still the case with modern gymnosperms. In the microsporangium the microspores develop into microgametophytes with a few cells having a simple male sex organ and producing immobile sperm cells, or else into pollen grains. The pollen grains are dispersed by wind and fall on the pollination droplet, which places them at the top of the nucellus. Pollen tubes grow in the nucellus and the sperm cells are transported in the pollen tube cytoplasm towards the megagametophyte. In the macrosporangium the megaspore develops a coenocytic liquid endosperm surrounded by a megagametophyte wall. Cellularization follows, and one or more sex organs develop on the top of the cell mass, each with an isolated oocyte and venter cell, both surrounded by follicle cells. Two or more neck cells form the entrance for the pollen tubes. The pollen tube grows through the neck canal and one of the sperm cells fuses with the oocyte to form the zygote. After fertilization the seed remains on the sporophyte. The mature seed will be dispersed by gravity, or by wind in cases where the seed is extended with a membranous wing.

In some gymnosperms such as Cycadales, highly flagellated spermatozoids are released in water present just above the neck cells of the megagametophyte, a last example of fertilization in water. In *Welwitschia* and *Gnetum*, development of the megagametophyte is delayed and it remains coenocytic. Pollen tube penetration in the coenocyte results in one or even two zygotes. *Ephedra* sp. has double fertilization: one sperm cell fuses with the egg cell and another with the venter cell, and in *Gnetum* sp. both sperm nuclei fuse, each with a megagametophyte nuclleus (Herzfeld, 1922; Friedmann and Carmichel, 1996).

In angiosperms the cell number of gametophytes is very low and sex organs are absent. The two sperm cells are immobile and move in the cytoplasm of the pollen tube. In the megagametophyte an egg cell is selected to fuse with one of the sperm cells ; it is an egg cell initial (Favre-Duchartre, 1984) bordered by two synergids. Before fertilization the development of the megagametophyte stops; it has antipodal cells and a coenocytic central cell which needs fertilization to form the endosperm. The seed is the unit of dispersal. Pollen and seed have abiotic dispersal but in most angiosperms a biotic vector is used and animals become involved in pollen and seed dispersal. The sporophyte begins to function in advertisement and nutrition, and in the case of pollination it also provides routing. This development points to the interaction of the sporophyte with the environment.

Trends

In water the position of the sex organs, commonly developed from the epidermis, is directed to gamete release. The storage in one of the gametes determines the female gamete. This swelling of the gamete has a direct consequence in gravity dispersal. Before and after fertilization, vector transport is influenced by gravity. This manner of formation of the oocyte points to the very strong connection between sexual reproduction and dispersal (Fig. 1a).

The retention of the isolated oocyte on the plant or on the gametophyte means an interaction, which includes nutrition and signaling via an interface. If the zygote develops further on the plant or gametophyte the interaction continues (Fig. 1b). Both gamete storage and retention are effects of nutrition. The introduction of alternation of generations means selection of one organism for sexual reproduction: the gametophyte. In such a life cycle two moments of dispersal are introduced. The meiospore and zygote may use other vectors of dispersal, with the consequence that another biotope can be selected (Fig. 1c).

On land, the life cycle with oogamy, the development of the zygote on the gametophyte, and dispersal only by the meiospore is an inheritance from aquatic plants. The heterosporic ferns develop a microspore and megaspore, as already happens in some plants in water. Sporophyte-gametophyte interaction develops extra nutrition to the megaspore by the sporophyte. The interaction again has a direct consequence for the means of spore dispersal. The megaspore with storage tends to fall downwards but the microspore is dispersed by wind (Fig. 1d).

The next step in seed ferns and gymnosperms is the prolonged retention of the megaspore on the sporophyte. This again means an interaction via an interface, with nutrition and signaling during spore and gametophyte development, and pollen uptake and guiding. Megaspore dispersal is blocked, and the prolonged retention results in seed after fertilization (Fig. 1e).

In angiosperms the interaction between the sporophyte and gametophytes becomes more intensified. The effect is a gametophyte with a low number of specialized cells. The contribution of the sporophyte to biotic pollination (Fig. 1f) and seed dispersal (Fig. 1g) is elaborated mainly in angiosperms. This includes interaction and signaling with the biotic environment.

Sexual reproduction is connected with nutrition, signaling, interaction and the means of dispersal. Gamete storage and retention or prolonged retention is a historical trend, and it evokes interaction via an interface. A consequence is gravity as the main vector for megaspore and seed dispersal. In cooperation with dynamic changes in the environment, transport via biotic vectors on land becomes a characteristic of the sporophyte and is realized by it.

REGULATION

The history and trends leading to the process of sexual reproduction in angiosperms are associated with changes in the regulation of reproduction and dispersal. In angiosperms many genes associated with sexual reproduction have been isolated, and the functions of some are well known (Raghavan, 1997). The expression of sex differentiation plays an important role in the regulation of reproduction and dispersal.

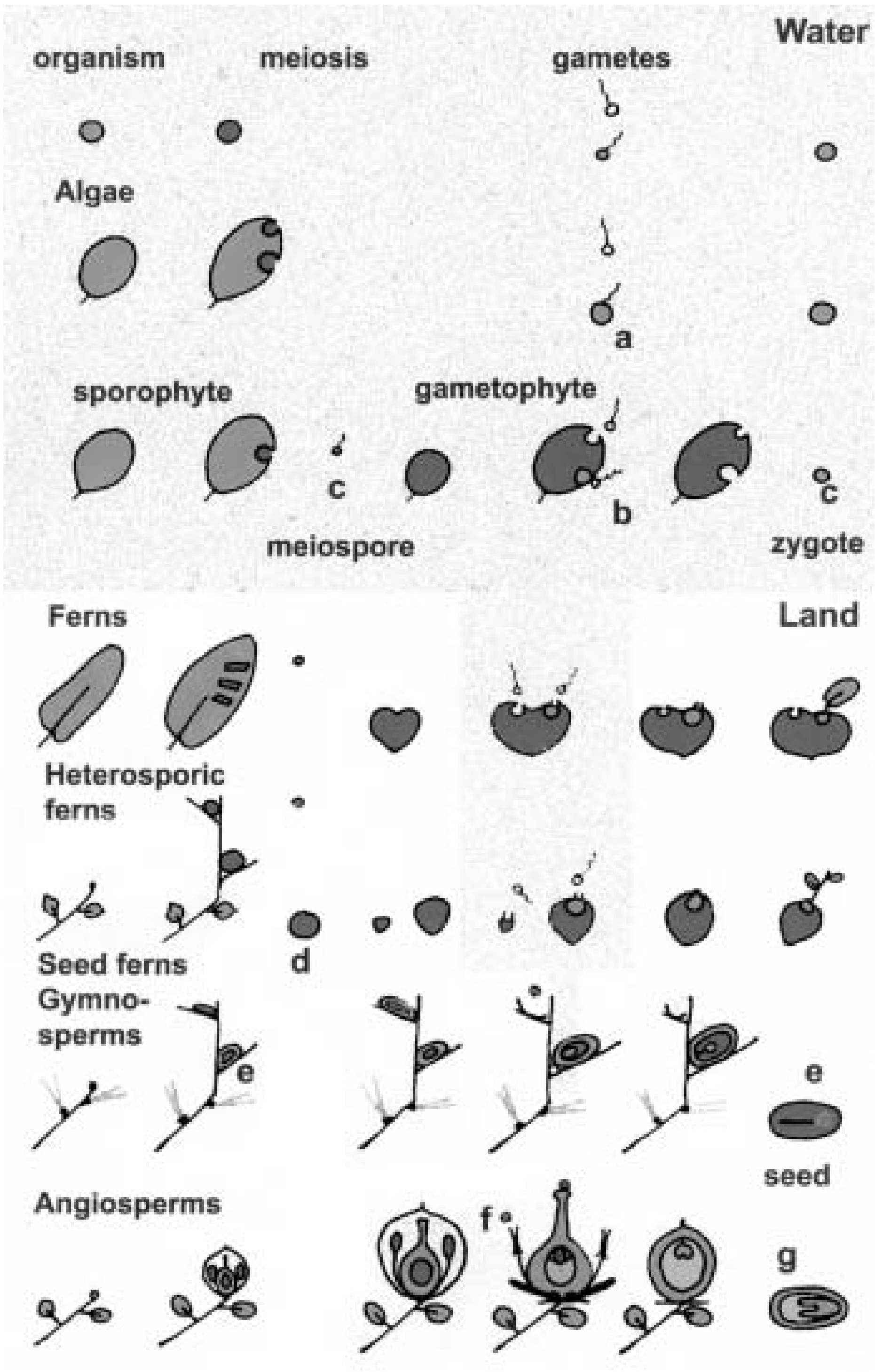

Fig. 1. Scheme representing life cycles, arranged in order of the history of plants. First is the life cycle of unicellular algae followed by multicellular algae, algae with alternation of generations, ferns, heterosporic ferns, seed ferns along with gymnosperms and angiosperms. **a–g** – important steps during the history of sexual reproduction. **a** – storage of gamete with the consequence of gravity dispersal; **b** – retention of the gamete on the plant, with the consequence of nutrition; **c** – two moments of dispersal by meiospore and zygote, with a fit to different biotopes possible; **d** – storage of the megaspore, with the consequence of gravity dispersal; **e** – prolonged retention of the megaspore and megagametophyte, leading to seed dispersal; **f** – biotic pollination, involving a strong interaction between sporophyte and environment; **g** – biotic seed dispersal, involving strong interaction between sporophyte and environment.

SEX DIFFERENTIATION

The sex of an organism is determined through sex differentiation. Each organism is totipotent and has genes for the expression of both sexes, but during sex differentiation only one sex is developed. This is an effect of the male- or female-determining factors activating the related genes. The simplest example of the process of sexual reproduction is found in unicellular algae. Gene expression with respect to sexual reproduction in unicellular plants involves at least meiosis and gamete differentiation. Gamete differentiation includes cell isolation and preparation for mobility, attraction and fusion. The amount of storage and degree of immobility have consequences for the way gametes are supplied with nutrition and dispersed. A gamete can be small, light, large or heavy as a consequence of nutrition; it can be mobile or immobile, which has an effect on dispersal by water or gravity. Thus, sex differentiation includes interaction with the dynamic environment. Another expression of differentiation is the retention of the female gamete and even the fertilized oocyte on the plant. This retention involves interaction through nutrition and signaling via an interface.

In life cycles with alternation of generations, sex differentiation is first expressed in the gametophyte only. On land, sex differentiation is also expressed in the sporophyte in a specialized sporangium as the organ producing the microspores and megaspores, as in heterosporic ferns and seed plants. The interaction between sporophyte and gametophyte becomes more intensive. Sex differentiation again relates to the means of dispersal by abiotic or biotic vectors, and this includes a further interaction with the dynamic environment.

SEX DIFFERENTIATION AND REGULATION IN ANGIOSPERMS

In angiosperms the sporophyte is very involved in sex differentiation. This has consequences for sex differentiation in gametophytes. These get a reduced number of cells, but each with a higher level of differentiation. The sporophyte plays a dominant role in nutrition of the gametes, embryo and endosperm. The sporophyte also governs pollen and seed dispersal.

Sporophytic gene action in the flower bud

During flower development, macro- and microsporangia, ovules and anthers develop. The composition of these sporangia is comparable, with a common structure having a multi-layered wall and sporogenic tissue prepared for meiosis. Anthers and ovules are heterosporic sporangia, and are related to the terminal sporangium of a primitive fern, the organ for meiospore dispersal on land. The role of the sporophyte in nutrition of heterotrophic gametophytes results in more intensive interaction, which includes other gene actions.

For pollination, the sporangia become surrounded with petals and sepals for advertising and for routing pollen to be deposited on the stigma, and with nectaries for nutrition. In the sporophyte new genes become involved to develop this advertisement, routing and nutrition, as well as the release and uptake of pollen. Genes are selected during flower induction and the formation of petals, sepals, stamen and pistil, represented in the ABC model (Coen and Meyerowitz, 1991) and its revisions (Gutierrez-Cortines and Davis, 2000; Maheswari et al., 2001; Soltis et al., 2002). If the anther and ovule are sporangia their origin should be expressed in the model. It is suggested that the ovule can be considered an independent sporangium (Colombo et al., 1995), but the same can be postulated for the anther. Regulation of the important nectar glands commonly present in the flower also should be incorporated in the model.

Gametophytic gene actions before fertilization

In the open flower the heterotrophic gametophytes are partially developed. In the anther the two-celled microgametophyte (pollen grain) has no sex organ but forms two immobile sperm cells, which are moved in the cytoplasm of the pollen tube. In the ovule the megaspore forms a simple female gametophyte, a coenocyte which is converting into a few highly differentiated cells. No sex organ develops, and there is no cell isolation but only an egg cell initial with some storage. The sister cells next to the egg cell initial attract the pollen tube. This means interaction between the male and female gametophyte. Fertilization of the egg cell initial is the beginning of the embryo, and fertilization of the central cell is the beginning of the endosperm; both depend on pollination and fertilization. In the microgametophyte and the megagametophyte of angiosperms, gene expression becomes suppressed and the sporophyte dominates gametophyte development and the pollination mechanism.

Sporophytic gene actions during pollination and after fertilization

The sporophyte directs pollen uptake and guides the pollen tube to the ovules. This interaction at the interface consists of nutrition and signaling, such as recognition and pistil activation, and is regulated by a complex of genes. Probably the whole system of nutrition and signals is oriented toward promotion of cross-pollination.

Double fertilization can also be considered a signal to start embryo and endosperm development. Fertilization of the central cell is a condition for endosperm development. Unlike autonomic endosperm development as happens in gymnosperms, endosperm development in angiosperms depends partly on a fertilization stimulus. In fact the primary endosperm is formed partly by the antipodal cells, but the main part is organized as new tissue with a higher ploidy level from the fertilized central cell. However, autonomous endosperm development occurs after culture in vitro of young pollen (Chatelet et al., 1999), embryo sacs (Mol, 1999) and in some apomicts (Koltunow, 2000), and can be considered gametophyte tissue, developed through some suppression of gametophytic genes. The endosperm is nutritive tissue for embryo development. Endosperm decay will supply the interface with nutrients around the embryo. The sporophyte develops the seed coat from the sporangium wall and the integuments, and the fruit from the surrounding carpels. New genes will become engaged in regulating this great diversity of activities, which are directed toward fulfilling the abiotic or biotic means of dispersal of the seed or the diaspore. In biotic dispersal of the diaspore a similar system of advertisement and nutrition is involved. Less is known about the regulation of the fruit.

The sporophyte dominates the gametophyte in angiosperms, eliciting a number of new gene actions and suppressing gene expression in the gametophyte. The main role of the sporophyte is directed toward nutrition, providing space, uptake and guiding of the pollen tube, and diaspore dispersal. The vigor of the sporophyte determines whether the organism can bear the cost of reproduction, which is measured in the number of pollen, ovules and seeds.

THE PROCESS OF SEXUAL REPRODUCTION

Sexual reproduction is the result of changes in sex determination, differentiation and interaction with the environment. This change is influenced by recombination during meiosis and cross-fertilization, both major events connected with sexual reproduction. It results in a new organism, and is expressed in sex determination. Sex determination involves the formation of new genes as well as the suppression or perhaps loss of genes. Sex is determined by sex differentiation, the formation of sex organs and gametes. Sex differentiation is connected with interaction between organisms, nutrition via an interface, and the means of dispersal.

In water, sex differentiation is expressed in the plant, or in the gametophyte in the case of alternation of generations. Here sex differentiation becomes expressed in storage of the gamete and its retention on the organism, with consequences for the manner of dispersal.

On land, to beome independent of the medium of water and to use the vectors of dispersal, the sporophyte becomes an actor in sexual reproduction. Similarly to the events in water, sex differentiation becomes expressed in storage of the meiospore and its retention on the organism, with consequences for the manner of dispersal: intensified interaction. In angiosperms the sporophyte dominates the gametophyte, and the sporophyte implements biotic dispersal through pollen and seed.

The changes in the process of sexual reproduction and the variety of its expressions are the result of the creation of new organisms through recombination and cross-fertilization in a dynamic environment. This development is possible because of the unity of the organism and its environment. Sexual reproduction is an expression of dynamic communication between organisms and the environment.

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