Genetics and Morphogenesis in the Basidiomycetes

Edited by
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To the memory of John Robert Raper,
scientist, teacher, friend.
Not so much the words or the paper, but the ideas.

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

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Preface

When we first conceived of this symposium we wanted the presentations to consist of more than a recitation of the most recent results. Rather we asked each participant to summarize and analyze the research covered by their topics. Furthermore, we encouraged the expression of new ideas. We believe that our goals have been met and that this volume represents more of the future than the past.

We thank our many colleagues who provided their thought and time. The officials of the Second International Mycological Congress should be congratulated for a well-run meeting. Our special thanks to Eloise Henry and Paula Shatten for their efforts on the IBM Recorder and Composer.

Contents

INTRODUCTION*

Philip G. Miles

Although officials of the Second International Mycological Congress could not give official approval to a memorial symposium, the fact is that this symposium on Genetics and Morphogenetic Studies of Basidiomycetes had its inception in a desire to honor John Robert Raper. Scores of scientists were contacted, and there was overwhelming approval of the idea of a symposium of this congress in memory of Professor Raper. Suggestions were also sought as to the topics to be covered in the symposium, but on this there was more divergence of opinion, as would be expected in view of John Raper's many scientific contributions and broad interests which included the hormonal control of sexual development in fungi, the biological effects of beta radiation, and the genetic control of the incompatibility systems and morphogenesis of sexuality in higher basidiomycetes. We incorporated as many suggestions as possible, but ultimately Dr. Schwalb and I had to make the decisions as to the contents and organization of this symposium, recognizing that it could have been done in many different ways. We are confident, however, that any imperfections in the organizational pattern will be less obvious because of the contributions of this outstanding group of participants.

Mrs. Raper expressed to me some concern that she had been invited to be a participant simply because she was John Raper's wife. Dr. Schwalb and I assured her that she is on the program because of her own specific scientific contributions in this field and that it would not in our opinion have been in keeping with John's strict sense of scientific honesty to have selected a participant for reasons other than that the person was an outstanding representative for the topic to be covered. As John's wife alone, we would have honored her with a front seat at this symposium, but not a place on the program.

Many of you are familiar with John Raper's scientific

* This statement was read at the beginning of the Symposium

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accomplishments and some will be familiar with various phases of his career. For those who are not familiar with this, I would like to give a brief synopsis.

The youngest of eight children, John Raper was born on a farm in North Carolina. He received both the bachelor's and master's degrees from the University of North Carolina, an institution well known to mycologists for the studies of Coker and Couch and their students. What more auspicious introduction to mycological science could one have than to learn about fungi from Professor John Couch? The next stop was Harvard University for another master's degree and the Ph.D. under the tutelage of Professor W.H. Weston, Jr., a recognized master in the training and development of young biological scientists. This was followed by a two year post-doctoral fellowship at Cal Tech where he grew *Achlya* in great quantities and isolated a small but significantly useful amount of hormone A. His first teaching position was at Indiana University, and this was interrupted by a period of research on the Manhattan Project at Oak Ridge where he studied the biological effects of beta radiation. Following the war he took up a position at the University of Chicago where he continued the *Achlya* studies and embarked on the investigations of the genetic control of incompatibility in the tetrapolar basidiomycete, *Schizophyllum commune,* while climbing up the academic ladder from Assistant to Full Professor. In 1954 he became Professor of Botany at Harvard University to succeed "Cap" Weston. While he did not match in numbers Cap's record of guiding over 50 students to the Ph.D., the number of outstanding young scientists whose research was guided by John marks an outstanding contribution he has made to our profession.

The spirit of his active inquiring mind will be very much with us during these meetings, for many of you here have known him and few could meet him even casually without sensing that he was an extraordinary man. If he were here with us physically today, I think that he would be getting a bit restless by now and would probably be saying: "There are excellent people waiting to tell us some exciting things. Let's get on with it." So be it!

CONTROL OF DEVELOPMENT BY THE INCOMPATIBILITY SYSTEM IN BASIDIOMYCETES

Carlene A. Raper*

INTRODUCTION

Many aspects of development in Basidiomycetes fascinated John Raper, but the question of central importance to him was, always, "How do the incompatibility genes do their work?"

Despite the decades of effort resulting in considerable information about the incompatibility system -- a signifigant part of which was contributed by John Raper -- the answer to this question is little more apparent now than it was over fifty years ago when, independently, Marie Bensaude (1918) and Hans Kniep (1920) first defined the system in the higher Basidiomycetes. The answer is of relevance, not only to an understanding of mating interactions and sexual development throughout the higher fungi, but also to an understanding of the control of development in eukaryotes generally. Specific analogies in genetic aspects are apparent in the *S* allele system for control of fertilization in higher plants and in the control of histocompatibility in higher animals.

In essence, the products of the incompatibility gene-complexes interact to convert the fungus from one state of differentiation to another through a sequence of morphologically distinct events. The system has been detected in approximately 90% of the Homobasidiomycetes analyzed. This represents about 450 heterothallic and secondary homothallic species of the estimated 5000 species extant (Raper, 1966).

Although research to date has not revealed the nature of the products of the incompatibility genes, the accumulated information makes speculation about this question more tempting than ever. I, as

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In memory of my husband, John R. Raper.

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others previously, succumb to that temptation, with a consideration first of relevant background information, then an explication of two proposed models for the molecular basis of incompatibility gene function, followed by a discussion of these models as they accommodate known facts and as they might be tested.

BACKGROUND

The detailed sequence of events in development as controlled by the genes of the incompatibility system varies among species of Basidiomycetes but a common feature is the conversion of a self-sterile homokaryotic mycelium to a heterokaryon that is capable of forming fruiting bodies.

A generalized scheme of the life cycle of Basidiomycetes is given in Figure 1. Its salient features are the alternation of an indefinite haploid phase with a one-celled diploid phase, usually with a heterokaryotic phase of dikaryotic structure interposed. Under appropriate environmental conditions, the heterokaryon is induced to produce fruiting bodies containing basidial cells in which karyogamy, meiosis and spore formation occur in rapid succession.

The illustrated scheme typifies events in the majority of known species, such as *Coprinus fimetarius* (Bensaude, 1918; Mounce, 1922), *Schizophyllum commune* (Kniep, 1920), *Lentinus edodes* (Oikawa, 1935; Nisikado and Yamauti, 1935), *Flammulina velutipes* (Kniep, 1920; Vandendries, 1923), and *Pleurotus ostreatus* Vandendries, 1933). (See Raper, in press, for comprehensive references on these species). Variations in several of the steps are found in other species. For example, in *Agaricus bitorquis,* the homokaryotic mycelium (step 2) is multikaryotic instead of monokaryotic and the fertile heterokaryotic mycelium (step 4) is dikaryotic without clamp connections (Raper, 1976). In *Agaricus bisporus,* a secondary homothallic form, the fertile heterokaryon not only has no clamp connections but is multikaryotic. There is no apparent dikaryotic structure except for cells just basal to the basidia. Also the spores from the two-spored basidia are dikaryotic at conception with the consequent bypassing of the homokaryotic

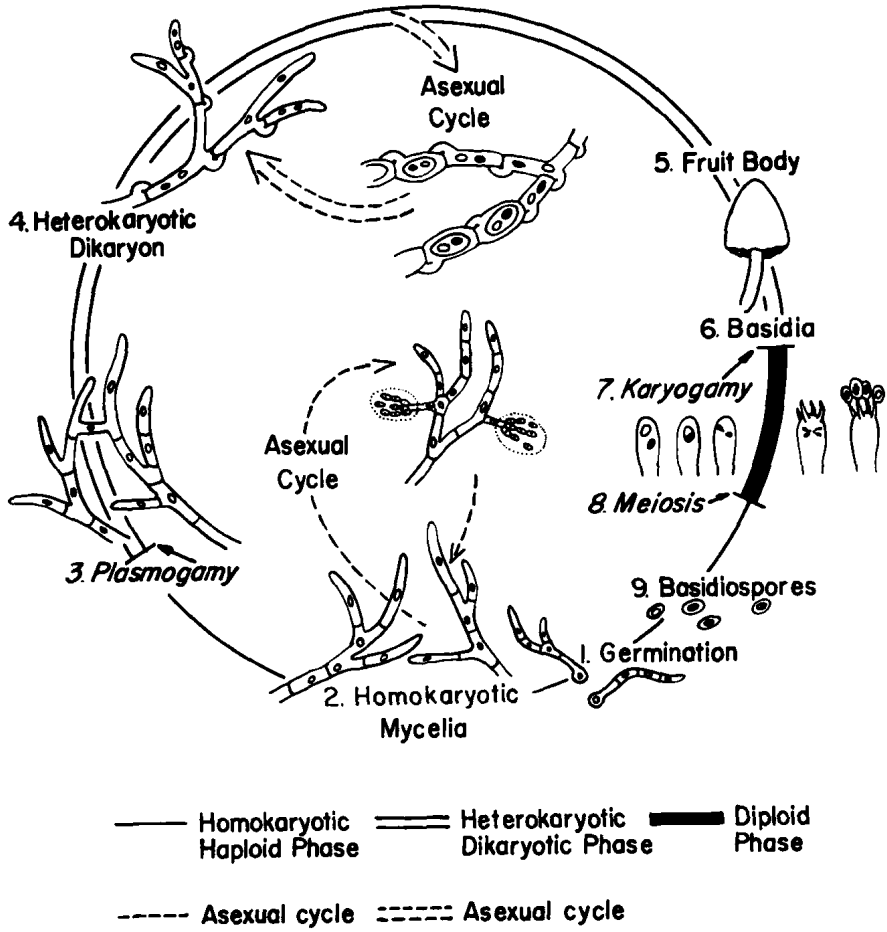

Fig. 1 A generalized scheme of the life cycle of Basidiomycetes.

phase (step 2) (Raper et al, 1972). *Armillaria mellea* represents still another variant in its regular sectoring of the dikaryon into monokaryotic mycelium with presumably diploid cells, and without clamp connections (Korhonen and Hintikka, 1973, J.B. Anderson and R.C. Ullrich, personal communication). A small percent of the species studied have no alternation of the self-sterile, homokaryotic phase with the heterokaryotic phase but are true, so-called primary, homothallics, in which the homokaryon is self-fertile and an incompatibility system is lacking. Morphology of the fruiting body varies widely from the unelaborate, as in resupinates such as *Sistotrema brinkmannii,* to the elaborate, as in Agarics such as *Amanita sp.,* and asexual cycles of various types may or may not occur in the homokaryotic and heterokaryotic phases.

These variations in the expression of development, however, do not obscure the signifigance of the genes of the incompatibility system as the primary controlling elements, and it is this I wish to focus on.

Sexual morphogensis in Homobasidiomycetes is controlled by extensive series of multiple alleles of either a single incompatibility factor, *A* or of two incompatibility factors, *A* and *B*. Because the details of its operation have been examined most comprehensively in the bifactorial species, *Schizophyllum commune* Fr., I will review the relevant information available from studies with this organism as a background for discussion.

The *A* and *B* incompatibility factors are unlinked and complex genetic factors that control distinct but coordinated parts of the sexual cycle. In bifactorial species such as *Schizophyllum,* the *A* and *B* factors segregate at meiosis to produce four types of basidiospores with respect to incompatibility genotype: *Ax Bx, Ay By, Ax By, Ay Bx*; Each type develops into a self-sterile, cross-fertile homokaryon which, when paired in matings with the other three types gives three distinct patterns of sexual morphogenesis. The entire progression leading to the development of the fertile dikaryon (*A-on B-on*) occurs only when there are allelic differences in both *A* and *B* factors (e.g. *Ax Bx* X *Ay By*). Only part of the series of events (*A-on*) occurs when the *A*'s are different but the *B*'s are the same (e.g. *Ax Bx* X *Ay* Bx): and only another part (*B-on*) occurs when the *B*'s are different

but the *A*'s are the same (e.g. *Ax Bx* X *Ax By*). The *B*-sequence of events (*B-on*) involves the reciprocal exchange and migration of nuclei into and throughout the mycelium of each mate and, at a later stage, the fusion of hook cells (clamps). The A-sequence of events (*A-on*) involves the pairing of nuclei, one from the donor mycelium and one from the acceptor mycelium, in each cell, the formation of the specialized hook cell at each septum, conjugate nuclear division, and the septation of hook-cell and hypha immediately following nuclear division. (See Raper, 1966 for further details.) Fruiting normally occurs only when both the A- and B-sequences are operating (*A-on B-on*) (see Schwalb, this symposium). The events and their genetic controls are illustrated in Figure 2.

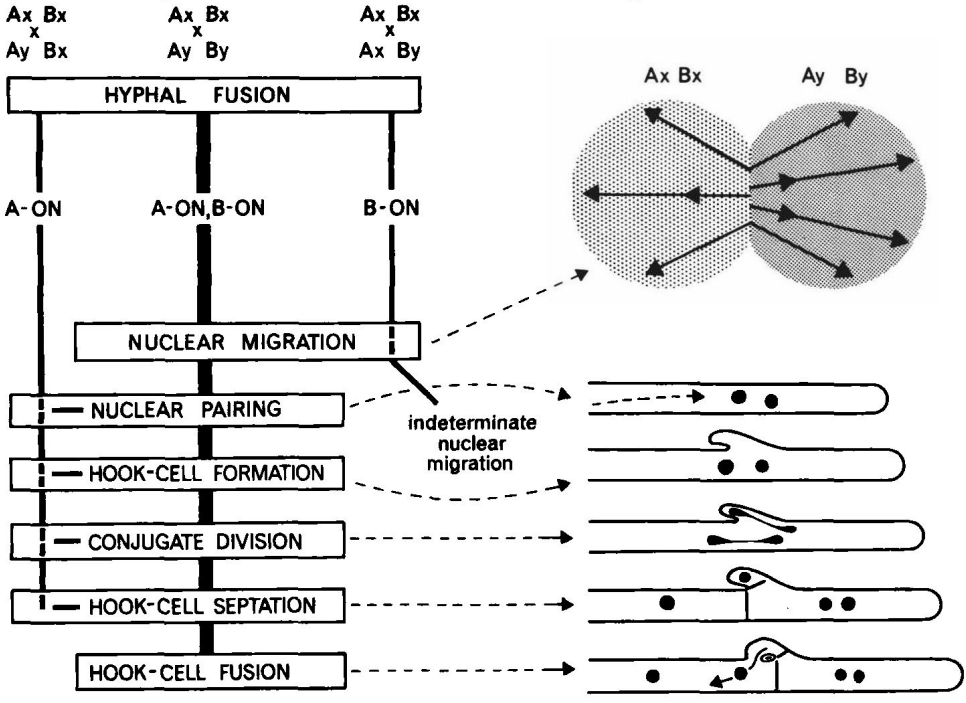

Fig. 2. Control by the *A* and *B* incompatibility factors of sexual morphogenesis in *Schizophyllum*. The progression comprises two distinct and complementary series of events, the A-sequence and the B-sequence, traced by vertical lines at left and right and regulated by the *A* and *B* factors, respectively. Operation of either sequence requires interaction of compatible factors, e.g. *Ax Bx* X *Ay Bx* which is *A-on*, or *Ax Bx* X *Ax By* which is *B-on*. Morphogenesis is completed only when both sequences are operative as traced by the central, heavy, vertical line, *Ax Bx* X *Ay By* which is *A-on, B-on*. (From Raper, J.R. and C.A. Raper. 1973. Brookhaven Symp. Biol. No. 25).

Distinct physiological and biochemical characteristics have been identified as correlates of these three patterns of morphogenesis (see Wessels, this symposium). Differences in the spectra of total soluble proteins were first shown to correlate with sexual morphogenesis by serological tests (Raper & Esser, 1961) and later by polyacrylamide gel electrophoresis (Wang & Raper, 1969). Different isozyme patterns for many enzymes were also demonstrated to be associated with specific states of sexual differentiation (Wang & Raper, 1970).

In the *B-on* phenotype, metabolism is shifted to catabolism with the concomitant elevation of several hydrolytic enzymes (Wessels & Niederpruem, 1967; Wessels, 1969), and energy conservation is highly inefficient (Hoffman & Raper, 1971, '72, '74). The production of large amounts of a specific hydrolytic enzyme known as R-glucanase has been shown to be essential for the dissolution of septa, a necessary prelude to the continuous movement of nuclei from cell to cell that is characteristic of the *B-on* phenotype (Wessels & Marchant, 1974). A preliminary study indicates the involvement of cyclic AMP in the formation of the specifically differentiated hook cells in the *A-on B-on* phenotype (Gladstone, 1973).

The primary genetic controls of this pleomorphic array of characteristics are the four loci of the two independently assorting incompatibility factors, *A* and *B*. Each factor is constituted of two linked genes, α and β , and each gene has multiple states with respect to specificity. In the *A* factor there are 9 and 32 alleles of the $A\alpha$ and $A\beta$ genes respectively (Raper et al, 1960; Stamberg & Koltin, 1973) and in the *B* factor there are 9 alleles each of $B\alpha$ and $B\beta$ (Parag & Koltin, 1971). There is no evidence that the products of the two genes within a factor interact with each other but they do appear as functional equivalents. A mating between two haploid homokaryons heterozygous at either or both of the loci within a factor results in the *on* phenotype for that factor – a minimum of a single difference at either locus versus no difference at both loci within a factor has widely different developmental consequences. It is the combined specificities of both α and β genes that confers specificity to the factor. The *A* factor therefore has an estimated 288 specificities and the *B* factor 81 making a total of approximately 23,328 mating types

with respect to combined *A* and *B* genotypes.

Extensive multiple specificities for the incompatibility factors in both unifactorial and bifactorial forms is common throughout the Homobasidiomycetes. For example, the unifactorial form of *Sistotrema brinkmannii* has an estimated 100-300 *A* specificities (Ullrich & Raper, 1974) and the bifactorial *Pleurotus ostreatus* has an estimated 63 *A* specificities and 190 *B* specificities (Eugenio & Anderson, 1968). A complex structure for the single incompatibility factor in unifactorial forms has not yet been demonstrated, but recent studies with *Agaricus bitorquis* revealed not only multiple allelism for its single incompatibility factor but indications of recombination for mating type specificity within the factor (Raper, 1976).

The genetic components of the incompatibility system have been studied primarily through mutational analysis. A study of mutations affecting the expression of morphogenesis has revealed, in addition to the incompatibility genes, a large array of loci scattered throughout the genome that determine specific aspects of the developmental process. They are expressed only during sexual morphogenesis and are viewed as secondary controls of the process. They are recognized in impairment as "modifier" mutations and constitute that component of the system that is regulated by the incompatibility genes. Over 80 mutations representing 12 phenotypes with respect to specific effects on morphogenesis have been analyzed. Several are expressed as specific blocks or alterations to the A-sequence of morphogenesis; others to the B-sequence and some to both sequences (Raper & Raper, 1964, '66, and unpublished). Most appear to be not linked to the incompatibility genes, but a cluster of nine modifier mutations expressed as blocks to nuclear migration in the *B-on* phenotype are linked by 10-20% recombination with the *B* factor (Dubovoy, 1975).

The complex nature of the regulatory components has become apparent from the variety of mutations obtained in the incompatibility loci. Although exhaustive attempts to derive one allele from another through mutation have failed, many phenotypes with respect to alterations in the control of sexual morphogenesis have been generated by mutations within the locus. Mutations have

been obtained in three loci, $A\beta$ $B\alpha$ and $B\beta$, with the latter most intensively studied. In a combined sample of approximately 10^9, some 50 mutations in both *B* loci were obtained as a primary event. They occur with the application of various mutagenic agents in a frequency of 5×10^{-8} and are of two types regardless of locus. Both are constitutive for the operation of the B-sequence of morphogenesis (*B-on*) but one has retained its ability to recognize the parental type as identical to itself and the other has lost that ability (Raudaskosky et al, 1976, and Koltin, this symposium). Mutations as secondary events in the $B\beta$ locus, i.e. mutations of a primary $B\beta$ mutant, are 1000 times more freqeunt than primary mutations and represent at least 10 types. All express a *B-off* phenotype and have varying degrees of deficiency in effecting the *B-on* phenotype when combined with wild type alleles in matings. Among these deficiencies are failures in the acceptance of nuclei, the donation of nuclei, and hook cell fusion. The mutant phenotypes range all the way from reversion to the parental wild-type allele to complete lack of all functions even extending to the adjacent $B\alpha$ locus. All of these secondary mutants, including the latter, are recessive to the parental, constitutive, primary mutant (Raper & Raper, 1973).

The variety of discernable alterations achieved in this single incompatibilty locus indicates a complex gene of two major parts, one for specificity concerned with self versus nonself recognition in allelic interaction, and one for the function of initiating and regulating the B-sequence of sexual morphogenesis. The primary mutations are interpreted as alterations within the specificity region and the majority of the secondary mutations are interpreted as alterations within the regulatory region, sometimes extending into the specificity region. Both regions appear to be subdivided. The specificity region has at least two parts, one for allelic interactions, and one for permitting expression of the regulatory region once a nonself allelic interaction has occurred. In the primary mutations obtained, the latter is always altered to permit constitutive expression; the former is sometimes retained as the parental type and sometimes destroyed. The region for regulatory function appears to be subdivided into three parts, one for the acceptance of nuclei, one