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INVITATIONAL ONR LECTURE

Impact of Basie Research on the Practical Application of Fungal Processes

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To what extent basic research may contribute to the improvemet of fungal processes is discussed with regard to tasks, potential, and limitations. Since a main requirement of industry is not only to enhance the yield of microbial products but also to come to stable production levels, the task of basic genetics is to accomplish this through a concerted breeding; that means especially through recombination of the genetic material in order to complement the traditional selection and mutation methods. The potential application of genetics is explained with the aid of specific examples chosen for various organisms and their processes. The first deals with single-cell protein production from yeasts, a problem originated from industry and indeed a challenge to basic genetics. The second is genetic control of morphogenesis of fruit bodies in higher Basidiomycetes, a topic which originated through basic research and has implications for recycling procedures. The third, a joint venture, senescence, was originally of theoretical interest, but is certainly relevant for avoiding the strain-aging occurring in fermentors. Since it is now evident that the causative agent of senescence is plasmid-like DNA, a new field for basic research has opened, because there is a real possibility to apply gene cloning to filamentous fungi which adds even further potential to the practical application of fungal processes. The limitations of basic genetics in this context are discussed briefly through examples of the failure of DNA from different, but often closely related, organisms to coexist and to be expressed through a common physiological machinery after hybridization.

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INTRODUCTION

Too often, the past traditional research activities in universities were devoted mainly to basic problems with little or no concern whether results were or were not applicable to practice. For biologists that meant a better understanding of the basic processes of life, and, in my special field of fungal genetics, this would comprise, for instance, gene structure, mutation, recombination, and function as well as transmission of genetic elements via sexual or parasexual processes.

However, this philosophy of research has changed abruptly during recent years in many countries and especially in my country. Already, by the early seventies, the Federal Minister of Research and Technology initiated, maybe under the shade of the emerging energy crisis, biotechnological research programs such as production of single-cell protein (SCP) on the base of cheap carbon sources and the recycling of plant waste with respect to protein or ethanol production. An additional fact, the shortage of research money from the universities and from grants, has induced many scholars to follow this new trend and to reflect upon their research programs with respect to practical application. I may add that undoubtedly a third factor also was responsible for that change of attitude; I mean the inborn curiosity of all biologists to tackle "problems of the day" and to enjoy that wonderful feeling of success in seeing that all the ambition "wasted" in science is after all good for something practical.

Naturally, this brings up the main question, in what way and to what extent can basic research contribute to the improvement of fungal processes. We shall try to answer this question by considering the nature of the tasks, the challenges, as well as the potential and limitations involved.

DISCUSSION

Task of Basic Genetics in Biotechnology

A main objective of industrial microbiologists is to improve the yield of microorganisms and to develop stable production levels. As may be seen from Fig. 1, this was realized in the past mainly by selection and mutation. (The term selection means both screening of strains and improvement of culture conditions.) However, some time ago, it became evident that in many cases these methods alone were not sufficient. They need to be supported by recombination of the genetic material, allowing for improved exploitation of the gene pool available.

This technique, which we have termed "concerted breeding" (Esser 1971), is, as a matter of fact, very old and has been applied over centuries to animals and higher plants, often by practitioners unaware of the underlying mechanisms. The reason that microorganisms, such as bacteria and fungi, were not so readily manipulated in the early days was because recombination under laboratory conditions was either not known or technically hardly accessible. Recombination in microorganisms is now recognized to concern both the sexual and the parasexual cycles. Of course, if a life cycle is not understood, sexual recombination is impossible; if there is no way to bring nuclei from imperfect fungi to form a heterokaryon, parasexual recombination cannot be realized.

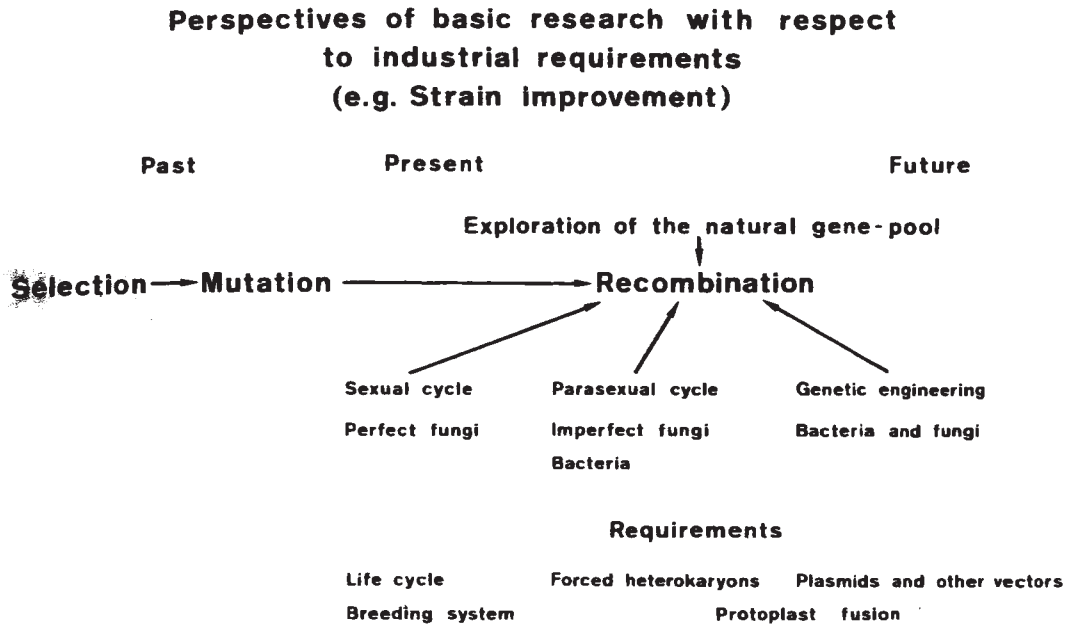

FIG. 1. Flow diagram showing the impact of basic genetics for biotechnology.

The technique of genetic engineering, now very well established in prokaryotes, most certainly in the future will occupy its position to manipulate, jointly with the techniques of classical genetics, the genetic material. But apart from all enthusiasm for such, there are still many technical problems to solve in order to secure a full integration of eukaryotic DNA and the eukaryotic cell in this concept of recombination.

However, certain handicaps to improve industrial microorganisms by concerted breeding and through modern genetics have been overcome by knowledge obtained from basic research as will be explained below in outlining some problems dealt with in my own laboratory. (Reviewing literature: Esser 1974a,b, 1977; Hollenberg 1980).

Potential of Basic Genetics in Biotechnology

In the following three examples the interplay of basic genetics and biotechnology are developed differently. In the first, solution of the problem developed from an industrial need. In the second, the converse is true, a problem of basic research interest became of biotechnological application. The third problem is a joint venture, an observation made in the laboratory has practical implications but, moreover, it brings up new possibilities for further developments in basic genetics.

1. *A demand from practice: single cell protein (SCP).* In many countries, especially overpopulated countries, microorganisms are used to produce protein. the organisms are mainly yeasts and bacteria which can be grown in bioreactors (fermentors) on cheap carbon sources such as alkanes or methanol (Prave 1975; Cooney and Levine 1971; Yamada et al.

1968). The yeasts used for SCP production on alkanes belong mostly to the imperfect form genus *Candida*, which under normal conditions produces neither sexual nor asexual spores. Since this is a handicap for strain improvement, it was through an industrial request that my laboratory and others thought of ways for a genetic manipulation. A first step forward was in *Candida lipolytica*, an alkane yeast of particular interest; sexual reproduction requiring the fusion of two opposite mating types was found (Wickerham et al. 1969, 1970). That *C. lipolytica* was classified until quite recently in the Deuteromycetes stems also from the fact that most strains isolated from nature were haploids, belonging to only one of the two mating types, and further that crossing, even when strains of opposite mating type are available, requires special nutritive conditions (Bassel et al. 1971; Gaillardin et al. 1973). The detection of a sexual cycle has led to reclassification of *C. lipolytica* and its integration among perfect yeasts under the name *Saccharomycopsis lipolytica* (Yarrow 1972). Despite these discoveries, other handicaps remained to realize concerted breeding with this organism, thus requiring further research.

Irregular segregation patterns. Random spore analysis of sexual crosses showed abnormal segregations of marker genes which was thought to be correlated with the fact that the spore number in the asci is very variable (Gaillardin et al. 1973). Depending on the cross, instead of the expected four spores, up to 90% of the asci contain two and a few asci, three or even one spore.

A cytological analysis revealed that this variability of the ascospore number results from the absence of a correlation between the meiotic divisions and spore-wall formation. As expected, in the four-spored asci the spore wall is formed around each of the four nuclei produced by meiosis II (Fig. 2, No. 12, 13,2). However, in the two-spored asci the wall formation occurs early (after meiosis I) due to a delay in the onset of meiosis II, leading thus to binucleate spores (No. 10, 11,1), whose progeny become mononucleate after germination (No. 3).

As one might expect from this peculiar mode of spore formation, all spores having more than one nucleus are mostly heterokaryotic because this requires the postreduction of only one allelic pair. The fact that after spore germination the haploid cells are again mononucleate (No. 3) explains that mixed cultures will occur and thus fake an irregular segregation pattern.

However, this disadvantage may be easily overcome if one selects for binucleate spores which, due to the inclusion of two sister nuclei of meiosis II, allow even tetrad analysis, a more sophisticated form of analysis than that from random spores. (Esser and Stahl 1976).

Removal of fruiting barrier between like mating types. Since sexual reproduction is initiated only by the fusion of haploid cells from different mating types, an exchange of genetic material between strains of the same mating type was not possible, as might be required between high yielding production strains. Furthermore, it is known that in yeasts the mating reaction is initiated by glycoproteins located on the cell surfaces (Crandall and Brock 1968). In order to overcome this fruiting barrier between like mating types use was made of the proto-

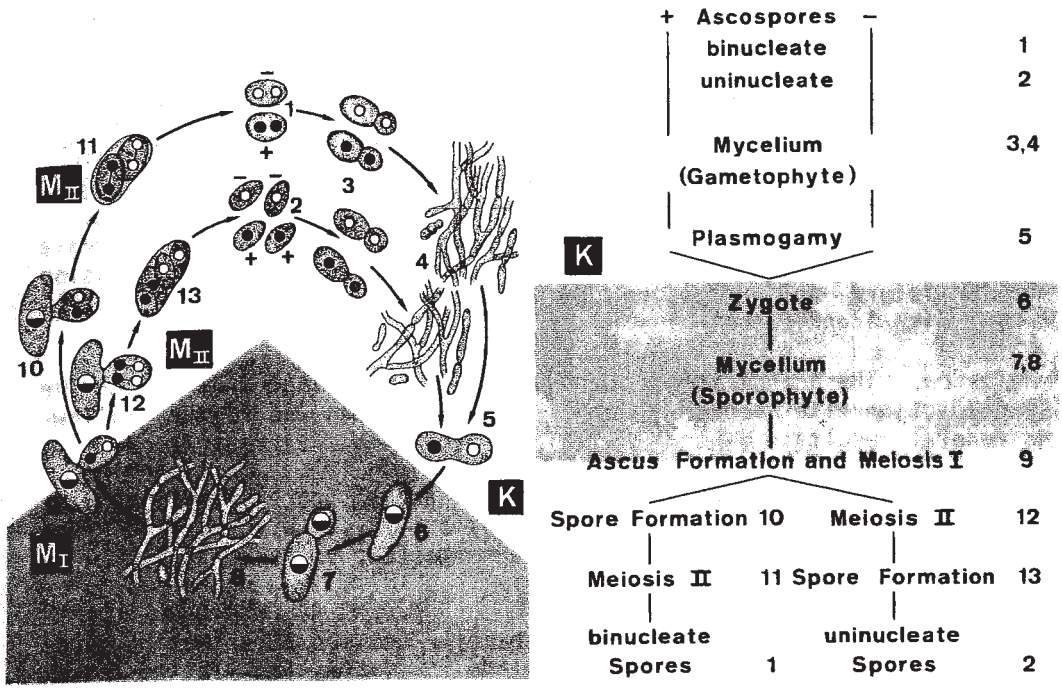

FIG. 2. Life cycle of *Saccharomycopsis lipolytica*. The white and the black nuclei represent the genetic difference caused by the mating type alleles + and -. The diploid phase is represented by the shaded area. For sake of clarity in the haploid phase, the development of three and one spore asci is omitted. For the same reason the +/- nuclei are drawn as prereduced only. (Adapted from Esser and Stahl 1975)

plast technique. My collaborator, Stahl (1978), succeeded in hybridizing protoplasts with an identical mating type. Following regeneration, he obtained stable diploids which, according to the segregation of markers, were able to produce normal ascospores.

Broadening of the genetic bases. In order to widen the possibilities for a genetic manipulation of *S. lipolytica*, we have tried to get a hand on extrachromosomal DNA such as plasmids or mitochondrial (mt) DNA, because in a petite negative yeast such as *S. lipolytica* both might contain valuable genetic information. Plasmid or plasmid-like DNA has not yet been found, but its mt DNA was found to be a circular molecule having a unit length of 14.5 um corresponding to 45.5 kilobase/pairs (kb) (Kück et al. 1980).

Criteria for taxonomic classification. In yeasts, generic classification is based mainly on morphological characters, whereas the definition of a species depends predominantly on physiological properties such as the assimilation of carbon and nitrogen sources. Classifications are performed routinely on agar slants and in negative tests small single colonies are often noticed. These are caused by spontaneous mutations as confirmed by adequate genetic tests. Sometimes these

mutations have altered the nutritional requirements of a yeast in such a way that, according to the classification "laws", a particular mutant must be transferred to a different species (Table 1).

This means that in many cases the classification depends only on single gene differences, whereas the differences in DNA base homology was found to be less than 1%. Certainly it is rather difficult to justify a new species on the basis of a single biochemical gene mutation; therefore, it is necessary in practice to perform at regular intervals an extended series of physiological tests in order to avoid confusion in nomenclature and to ensure that different laboratories are still working with identical strains (Esser and Stahl 1979).

Conclusion. The industrial requirement to facilitate and to improve SCP production with *Saccharomycopsis lipolytica* has led to the following general results:

1. The elucidation through cytology of ascus spore formation has created the possibility to perform genetic analyses with *S. lipolytica* just like with another of the well-known perfect yeasts, e.g., *Saccharomyces cerevisiae.*

2. The circumvention of the fruiting barrier between like mating types has widened the possibility to manipulate *S. lipolytica* via the sexual cycle.

3. The biochemical characterization of mt DNA has broadened the genetic basis in this petite negative yeast.

4. Genetic and biochemical analysis of spontaneously occurring mutations during prolonged vegetative propagation in yeasts has contributed to a better understanding of the species concept of yeasts.

2. *An offer to practice: Genetic control of morphogenesis.* Morphogenesis or differentiation is one of the most intriguing phenomena in biology. Many attempts, following Speemann's classical studies in embryogenesis, have concerned animals, plants, as well as fungi and have described not only morphogenetic features but underlying physiological aspects. Take, for example, sexual hormones causing differentiation in Mucoraceae and *Achlya,* respectively, and the role of cyclic AMP as a morphogenetic substance in slime molds (Esser and Kuenen 1967; Gerisch et al. 1974; Scott 1976; Newell 1978).

However, these studies rarely have considered the genetic aspects of morphogenesis. Although fungi are rich and varied in morphology and are often very excellent tools for physiological studies, the fungi mentioned above, e.g., *Mucor* and *Dictyostelium,* particularly are hardly accessible to genetic analysis.

Better genetic systems are available in higher fungi. From our early work with the ascomycete *Sordaria macrospora,* it became evident that one of the most remarkable morphogenetic events in fungi, the transgression from mycelial to plectenchymatic growth and the subsequent formation of fruit bodies, is under multigenic control (Esser and Straub 1958).

Therefore, it was tempting for us to decipher the genetic control

TABLE 1. Taxonomic consequences of physiological inconsistencies found in yeast after prolonged vegetative growth and relevant data on DNA base composition

Genus	Species	Designation	Physiological Alterations: Compounds Relevant for Taxonomical Classification		Effect on Classification	Mol % G + C: 'Wild' Strain	Mol % G + C: Mutated Strain
			Yes	No			
Candida	*boidinii*	70028	Cellobiose Salicin	Sorbose	*Candida berthetii*	34.5±0.20[a]	34.0±0.35
Pichia	*lindnerii*	70719		Ribose Sorbose Succinic acid	None	52.3±0.27	51.8±0.22
Saccharomycopsis	*lipolytica*	H-5027		Ribose Sorbose	None	54.2±0.34	54.4±0.19
Saccharomycopsis	*lipolytica*	KW 4		Sorbose Ribose Glucitol	None	53.8±0.43	53.9±0.26
Saccharomyces	*cerevisiae*	F (diploid)		Trehalose α-Methyl-D-glucoside Melezitose	None	42.9±0.23	43.0±0.33

[a]Mean ± standard deviation.

of the fruiting morphogenesis of higher Basidiomycetes, especially of those which, as wood-rotting fungi, may be used either for recycling procedures or production of edible mushrooms or for both. Typically, the genetic control on the mating reactions beginning with the fusion of two monokaryotic hyphae of opposite mating type and leading to morphological differentiation of a dikaryon was known to require mostly a

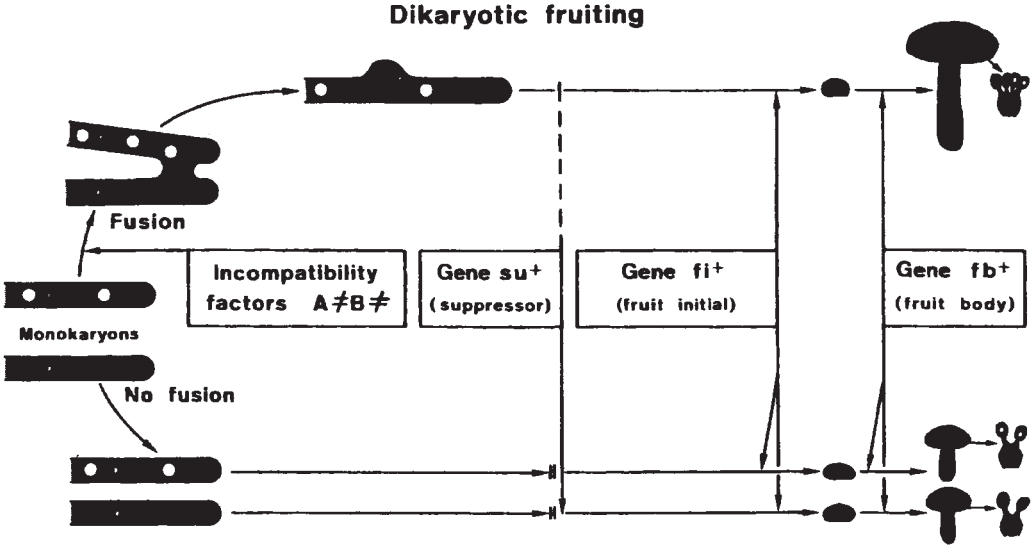

FIG. 3. Genetic control of fruit-body formation in *Agrocybe aegerita* (Agaricales). Both dikaryotic fruiting (sexual cycle) and monokaryotic fruiting (asexual cycle) start from monokaryotic mycelia originating from single basidiospores. The genetic difference of nuclei due to the different incompatibility factors is marked by black and white color. Monokaryotic fruit bodies are smaller and their basidia have two spores only. (Adapted from Meinhardt 1980)

heterogeneity of incompatibility factors (Fig. 3 upper left) (Raper 1966). However, until quite recently, no genetic factors were known to control the initiation and differentiation of the ultimate fruiting structure, the sporangium or basidium, in which the sexual reaction (karyogamy with subsequent meiosis) takes place. Only the environmental conditions were known for this essential morphogenetic sequence in some mushrooms.

A comparative genetic analysis of three, not too closely related species, *Polyporus ciliatus* (Poriales), *Agrocybe aegerita* (Agaricales), and *Schizophyllum commune* (Poriales or Agaricales according to the attitude of the particular taxonomist) has revealed a general principle which will be exemplified for *Agrocybe aegerita*, an edible mushroom found in the southern parts of middle Europe (Esser 1978; Esser and Meinhardt 1977; Esser and Stahl 1973; Esser et al. 1974, 1977, 1979; Hoffmann and esser 1978; Meinhardt 1980; Meinhardt et al. 1980; Stahl and Esser 1976).

As may be seen from Fig. 3, we arrived at an understanding of

fruit-body morphogenesis in dikaryotic mycelia via the detour of a genetic analysis of the so-called monokaryotic fruiting, the ability of monokaryons produced from single basidiospores to form asexual fruit bodies or fruit-body-like structures. It was found that there is one main gene which has the function of a switch. If this gene, called "suppressor," is present in its active form su^+, no differentiation occurs at all and there is no passing over the threshold from mycelial growth to fruit-body initiation and subsequent development, irrespective of all other morphogenetic genes present in the genome.

If the morphogenetic pathway is open, due to the presence of the inactive allele *su*, the action of at least two other genes is needed to complete monokaryotic fruiting: The gene fi^+ is responsible for the formation of fruit-body initials, small pin-head-like bodies. The shape of the fruit body is determined by a second gene fb^+. The subsequent action of both genes (fi^+fb^+) is needed for the formation of a fertile fruit body. A genotype carrying either one or both of the ineffective alleles will have fruit-body initials only (fi^+fb) or never start differentiation at all ($fi\ fb^+$, $fi\ fb$).

It was further found that these genes detected in the so-called monokaryotic fruiters are equally effective and needed for the "normal" dikaryotic fruiting. The minimal requirement is one dose of each gene, either in the *cis* configuration (fi^+fb^+ x $fi\ fb$) or in the *trans* configuration (fi^+fb x $fi\ fb^+$). Otherwise, regardless of external conditions no fruiting occurs.

The practical implication of these morphogenetic studies became evident from some other experiments showing a correlation between the action of morphogenetic genes and productivity. Fruit-body formation as well as the production of biomass depends on the number of active alleles from both fi^+ and fb^+ in the dikaryon.

As may be seen from Fig. 4, the first fruiting flush occurs only

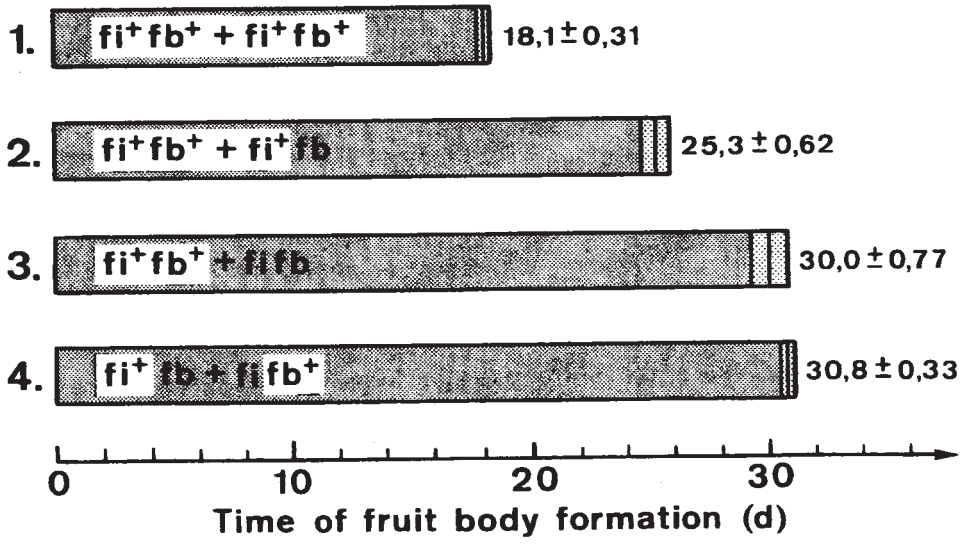

FIG. 4. Correlation between time of dikaryotic fruit-body formation (first flush, rupture of velum) and dose of the morphogenetic genes fi^+, fb^+. (Adapted from Meinhardt 1980)

after 30 d when the minimal dose (fi^+fb^+) is present in the dikaryon, whereas under optimal conditions (two alleles of each fi^+ and fb^+) fruit bodies are completed after only 18 d. It becomes clear from Fig. 5 that the yield of biomass is only about 50% under minimal genetic conditions (fi^+fb^+).

In order to show the practical application of these findings, the compilation of Table 2 may be used as a conclusion. In this context it

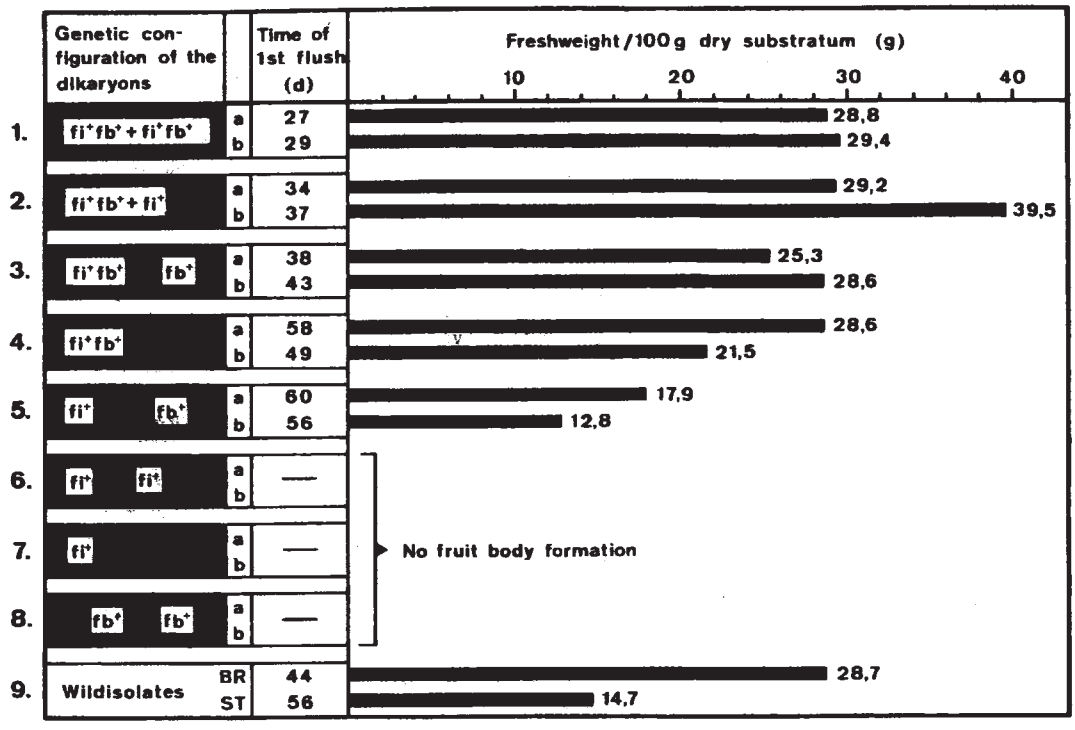

FIG. 5. Correlation between production of dikaryotic fruit-body biomass and dose of the morphogenetic genes fi^+ and fb^+ on straw; a and b stay for two subsequent experiments. (Adapted from Meinhardt 1980)

is certainly of interest that a comparable correlation between morphogenesis and productivity is known for another fungus of great industrial importance. The ergot fungus *Claviceps purpurea* shows a dimorphic hyphal growth: the ascospores or conidia germinate to a normally branched typical ascomycetous mycelium (called sphacelial growth), but there is no production of alkaloids. These secondary metabolites are formed only when the hyphal growth switches over to the so-called sklerotial growth (hyphae with short vesicular compartments) (Mantle 1974). It certainly would be interesting to reveal the genetic background for this phenomenon, especially since only quite recently the general genetics of *C. purpurea* has been worked out (Esser and Tudzynski 1978 and unpubl. data).

3. *A joint venture: Strain-aging or senescence.* Now let us consider a bit about aging, something that creeps up on us all. Senescence or

TABLE 2. Genetic control of fruit-body formation in higher basidiomycetes

Basic Research	Applied Research
A single gene (su) allows to switch on the morphogenesis, i.e., the passing over the threshold from mycelial to fruit-body initiation and subsequent differentiation.	Concerted breeding is facilitated: Strain improvement is possible via the sexual cycle.
Mutation of su to its allele su^+ leads to an imperfect fungus.	Sterile strains may regain fertility when crossed with su strains.
Other genes act subsequently to determine fruit-body morphology: fi^+ fruit-body initials fb^+ fertile fruit bodies	This can be made use of in biotechnology: 1. Simple procedures: Production of mushrooms from cellulose and lignin wastes (recycling).
Productivity (e.g., biomass, time of fruiting) depends on the dose of these genes.	2. Complex procedures: Metabolites only occurring in fruit bodies may be produced in mycelial cultures in fermenters, when the morphogenesis is "switched on."

aging is, of course, a syndrome which accompanies all forms of life. With different levels of biological organization, this syndrome has different phenotypes. In filamentous fungi, senescence is characterized by the following symptoms: decrease of growth rate due to a diminution of mitotic divisions, morphological alteration of the hyphae, eventually stoppage of growth, and finally cellular death. A compilation of fungi (at least 18 species) for senescence is found in Esser and Tudzynski (1980). Certainly senescence in fungi is widespread and in many cases is responsible for the well-known strain-aging during fermentation procedures. Thus, a better understanding of this rather complicated syndrome is of practical interest.

The best investigated case is the coprophilous ascomycete *Podospora anserina* (a close relative of the well-known fungus *Neurosporacrassa)*. Senescence was observed in *P. anserina* almost three decades ago (Rizet 1953) and described in some detail (Marcou 1961). It became evident that the onset of senescence depends on both environmental and genetic conditions and that once the process of aging was in progress, the syndrome could be transferred by an infective principle of then unknown nature via hyphal anastomoses. However, only quite recently was it possible to specify the genetic control of senescence and to identify its causative agent.

There are a number of pleiotropic genes which not only exert their effects on mycelial morphology but are able to postpone considerably the onset of senescence (25 d in the wild strain) in a pairwise synergistic action (Esser and Keller 1976; Esser and Tudzynski 1979). An extreme example is presented in Fig. 6, where, in practice, immortality is gained in the double mutant *incoloris/vivax*.

As a result of a comparative analysis of DNA of juvenile and senescent strains, it became evident:

1. The infective principle which, after cytoplasmic contact, transforms juvenile hyphae into senescent ones is identical with a plasmid-

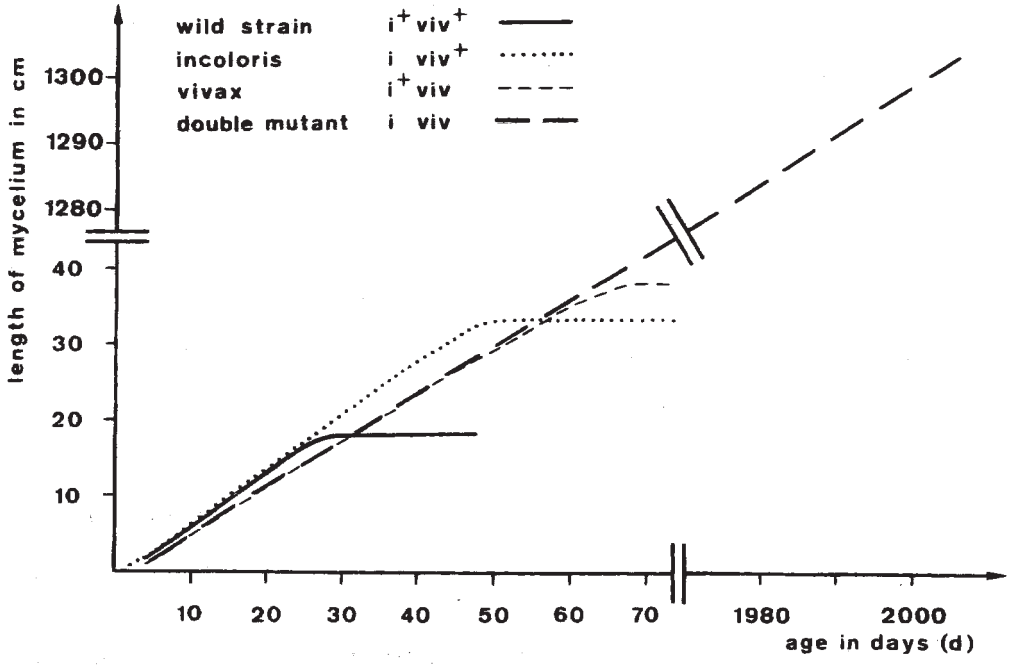

FIG. 6. Growth curves of a wild strain and some mutants of *Podospora anserina*. (From Esser and Keller 1976; the values for age and length were adapted to the present age of the double mutant).

like (pl) DNA having a covalently closed circular (ccc) structure with a basic length of 0.75 µm and a molecular size of 2.4 kb (Fig. 7) (Esser et al. 1980; Stahl et al. 1980).

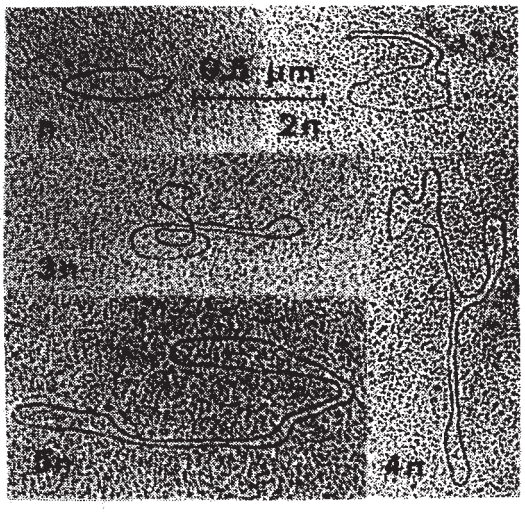

FIG. 7. Electron micrographs of pl DNA of *Podospora anserina:* (n) monomer, (2n-5n) dimer, trimer, tetramer, pentamer. (From Stahl et al. 1980)

2. Further evidence shows that in juvenile hyphae this pl DNA is integrated in the mitochondrion in a way comparable to lysogenic phage in bacteria. Only after disintegration of the mitochondrial (mt) DNA is

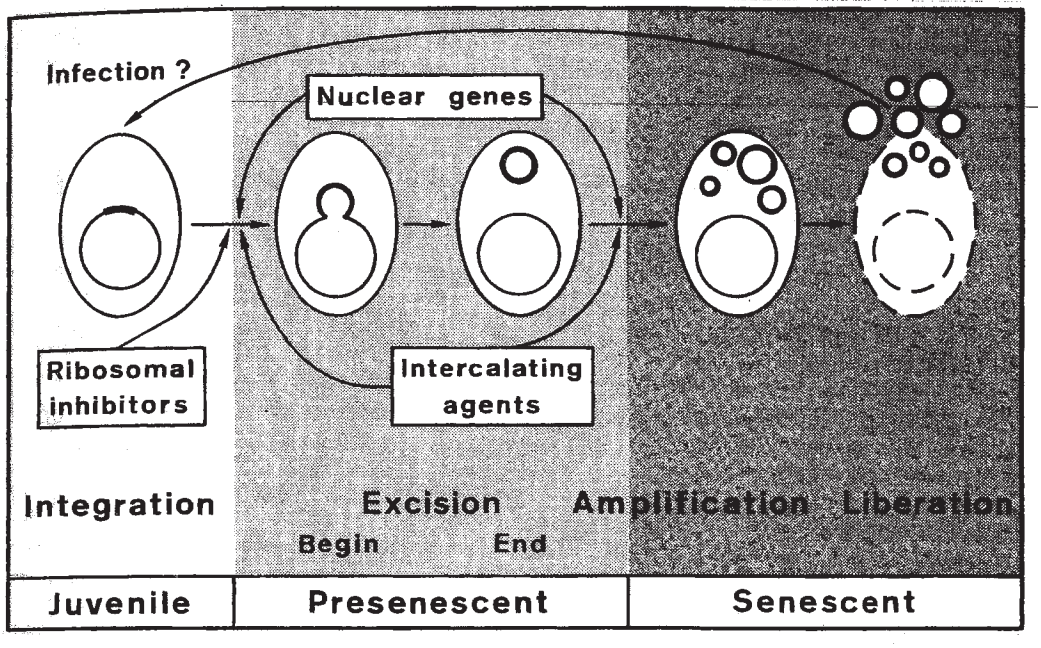

FIG. 8. Scheme of the proposed "life cycle" of the plasmid-like infective principle as correlated with senescence in *Podospora anserina*. The large ovals represent mitochondria, mt DNA is represented by thin circles and plasmid-like DNA by thick bars or circles. The disintegration of the mitochondrion and mt DNA is shown by broken lines. For further explanation see text.

it set free, becomes self-replicative, amplifies, and becomes infective (Fig. 8) (Esser et al. 1980).

3. The pl DNA, when integrated experimentally in a bacterial plasmid, pBR 322, can be cloned in *E. coli* and after a retransfer again be expressed in *Podospora* (Tudzynski et al. 1980 and unpubl. data).

Conclusion. The findings in *Podospora* are interesting from two points of view:

1. It should be possible to use this pl DNA of *Podospora* as a eukaryotic-derived vector which could be used for cloning genes in eukaryotes. This latter aim becomes even more interesting for many reasons, such as toxicology of *E. coli* or instability of strains of *B. subtilis* transformed with eukaryotic DNA. The argument that a vector causing aging is of little use can be devalued because in *Podospora* there exist mutant strains which are able either to suppress senescence (such as the double mutant *i/viv)* or in which aging is reversible.

2. The fact that in *Podospora* the coordinate control of senescence by

nuclear genes and extranuclear pl DNA is understood has created a basis for analogous research in other fungi. This again has an industrial implication and could help to breed production strains with extended longevity or "external life". That is the reason why I have called this example a joint venture which may become more of a joint "adventure" for both basic and applied research.

Limits of Basic Genetics in Biotechnology

As already suggested above, the application of genetics for practical and concerted breeding may not lead to miracles. One must be very careful not to overestimate the real chances for success. This holds especially true for genetic engineering. Because of all the fascination and publicity on the molecular level, the expectation will be difficult to fulfill. A rational perspective is needed rather than all out enthusiasm.

Therefore, in the balance of this paper I shall detail some of the limitations to be expected for classical as well as molecular procedures. I am not referring to technical limits most of which may be overcome by improvement and refinement in methods. Furthermore, the problems of biohazard will not be discussed. This subject area is fraught with a good deal of emotion.

1. *Coexistence of different genetic materials in a common cell.* During the last decade evidence was accumulated that DNA's of closely related organisms are often incompatible when brought together in one cell. This phenomenon has been termed "heterogenic incompatibility" (Esser 1962) and, according to our knowledge, was first described and genetically analyzed in the ascomycete *Podospora anserina* (Fig. 9)

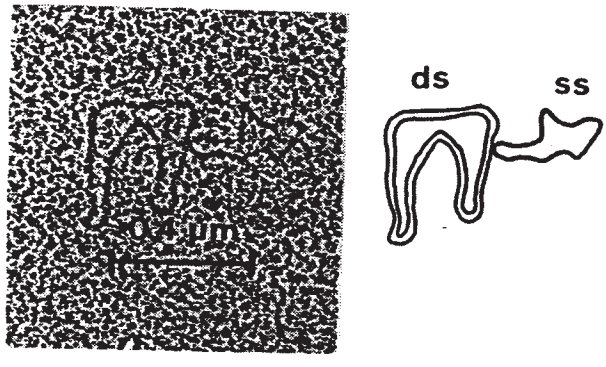

FIG. 9. Heteroduplex analysis of hybrid plasmid: bacterial vector pBR322 with pl DNA of *Podospora anserina*. The photograph shows as diagrammed to the right a double-stranded circular vector molecule (ds) with a single-stranded kinky insertion loop of pl DNA (ss). (From Stahl et al. 1980)

(Rizet and Esser 1953; Esser 1954, 1956, 1959a,b). Meanwhile, comparable phenomena have been found in many other organisms (Esser and Blaich 1973). The failure of coexistence of genetic material in close proximity occurs on all levels, ranging from the restriction phenomenon in the bacteria/bacteriophage systems or via cell incompatibility in

fungi, higher plants, and lower animals and even tissue incompatibility in mammals (Esser 1974a,b). Certainly the mechanisms by which the unilateral or mutual exclusion of the incompatible DNA, cells, or tissues is brought about may be very different, but such differences are of no importance in practice because the effect is the same, impairment of gene exchange through incompatibility, whether chromosomes or DNA are involved. The important question is will hybridization occur and will it last. From this it becomes evident that even when all the requirements, such as life cycles, mating types, or hybrid plasmids are available, for the performance of recombination, concerted breeding and therewith strain improvement is not possible under certain conditions by established genetic procedures.

2. *Expression of genetic material in a foreign cell.* Such limitations occur one step further, even when coexistence of foreign genetic material may be possible. As found mainly in bacteria which were transformed with foreign DNA, there is evidence that while this DNA is expressed faithfully, partial digestion of the product will occur. These questions, which at the moment are not relevant for fungi because there are too little experimental data, were discussed recently by Macleod (1980).

3. *Stability of recombinants.* Even if there are no restrictions for the mechanism of exchange, many cases are known where hybrid chromosomes or hybrid DNA are rather unstable during prolonged propagation. In fungi this "demixing" may show up as sector formation, as quite recently has been observed in hybrids between *Cephalosporium acremonium* (Cephalosporin C producer) and *Emericellopsis glabra* (Penicillin N, an intermediate of Cephalosporin biosynthesis) (Minuth, pers. comm.). Similar phenomena are known especially when DNA is cloned in *Bacillus subtilis* (Lovett and Keggins 1979; Dubnau et al. 1980; Gryczan et al. 1980).

In conclusion. Certainly limits exist for the application of basic research in practice on fungal processes, those which are expected and those which are not foreseen.

However, under the heading of this lecture this had to be mentioned, because genetic engineering turns out to be a promising technique in the future with fungi as well. The evolution of genetics as a science and in practice has, and most certainly will, make successful contributions to process improvement. Much needs to be done; let us do it, but let us not expect too much.

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