Theor. Appl. Genet. 60, 265-268 (1981)

Genetic Studies of the Basisdiomycete *Agrocybe aegerita*

Part 2: Genetic Control of Fruit Body Formation and its Practical Implications

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Summary. In the edible white rot fungus *Agrocybe aegerita* the threshold from mycelial growth to fruit body formation is under control of a single gene in both monokaryons and dikaryons.

The allele *su* opens the pathway for fruiting and allows the subsequent expression of the fruiter genes *fi*$^+$ (fruit body initials) and *fb*$^+$ (fruit bodies). Its allele, *su*$^+$, suppresses monokaryotic fruiting completely and restricts dikaryotic fruiting drastically.

The detection of this threshold gene *su*$^+$/*su* and its action and interactions has practical implication in that an opportunity for concerted breeding is created.

First results indicate that the fruiter genes are involved in two essential parameters of productivity. Both time of fruiting and biomass production depend on the two fruiter genes *fi*$^+$ and *fb*$^+$.

Comparable results obtained with two other basidiomycetes suggest that the genetic control of fruiting in *Agrocybe aegerita* is a general mechanism which may be made use of in breeding work with other basidiomycetes of economic value.

Key words: *Agrocybe* – Genetics – Fruiting – Biomass

1 Introduction

Because of their culinary value, mushrooms have been cultivated by empirical methods for centuries. In spite of the extensive experience of mushroom growers with the induction of fruit bodies through varying environmental conditions (Lelley et al. 1976; Chang and Hayes 1978), the genetic basis of fruiting morphogenesis has remained unknown. Of the numerous genetic studies on the higher basidiomycetes, most have concentrated on the elucidation of breeding systems. The few which have dealt with morphogenetic problems have been concerned with the mycelial morphogenesis involved in the transition from the monokaryon to the dikaryon bearing clamp connections (Raper 1966). Until recently it was generally accepted that once a dikaryon is established, initiation of fruiting depends only on the presence of suitable environmental conditions. However, it has become evident that the transition from mycelial to plectenchymatic growth and subsequent fruit body differentiation is under genetic control (Esser 1978). This came to light indirectly through analysis of a widely distributed phenomenon called monokaryotic fruiting (Stahl 1975; Stahl and Esser 1976). Accordingly, most higher basidiomycetes are able to produce fruit bodies asexually on monokaryotic mycelia in the absence of conditions considered essential for sexual fruiting. Surprisingly, the practical implications of monokaryotic fruiting have been overlooked.

In the first paper of this series, evidence that the fruiting potency of normal dikaryons is markedly enhanced by crosses with monokaryotic fruiters was presented (Esser et al. 1974). Later it was shown that monokaryotic fruiting is brought about by the presence of two unlinked genes, *fi*$^+$ and *fb*$^+$ (Esser and Meinhardt 1977). Only those strains which carry the active alleles of both genes produce monokaryotic fruit bodies. Although smaller in size, they are identical in shape with the dikaryotic fruit bodies. Strains having the *fi*$^+$*fb* genotype form only spherical fruiting initials. Fruiting does not occur in the presence of the inactive allele, *fi* (genotypes *fi fb* or *fifb*$^+$). Further, at least one active allele of the two morphogenetic genes is required for the fruiting of the dikaryon (*fi*$^+$*fb*$^+$ × *fi fb* or *fi*$^+$*fb* × *fi fb*$^+$). While this hypothesis seemed to account for the genetic basis of fruit body formation, more extensive crossing carried out in breeding this mushroom revealed irregularities which could not be explained by the action of the two genes alone. The work here described is the basis for a more precise understanding of fruiting in *Agrocybe*, its genetics and its productivity.

0040-5752/81/0060/0265/$1.00

2 Materials and Methods

Strains

The race BR is native to the region of Brünn (CSSR) (incompatibility factors A_1B_1/A_2B_2); for details see the first paper of this series (Esser et al. 1974). The ST race (incompatibility factors A_3B_3/A_4B_4) was collected near Strasburg (France) and kindly given to us by Dr. A. Braun.

Media and Culture Conditions

These were as described in Esser et al. (1974). In addition, fruit body production was obtained on sterile, chopped straw supplemented with a nitrogen source (KNO_3) according to Takacs (1974). Tetrads of basidiospores were isolated according to the method of Epp (1977).

3 Results and Discussion

3.1 Further Analysis of the Genetic Control of Fruiting

Unexpected segregation patterns occurred only in those crosses involving non-fruiters. Fruiters and strains producing fruiting initials were crossed in all possible combinations with the questionable non-fruiters. The application of tetrad analysis to assay the offspring offers the advantage that from the number of different tetrads the number of genes involved may be derived (for details see Esser and Kuenen 1967).

The following conclusions may be drawn from the data in Table 1:

1) Each of the four non-fruiters must have a different genotype because each shows a different segregation pattern when crossed with the known genotypes (*fi⁺fb⁺* and *fi⁺fb*).

Table 2. Crosses testing the influence of the *su⁺/su* gene on dikaryotic fruiting. All dikaryons were produced using different monokaryotic strains (105 in all). Fruit body formation requires the presence of at least one dose of *fi⁺* and *fb⁺*

Number	Cross Phenotype Genotype		Number of Dikaryons tested	Fructification in %
1	✳ *fi fb su⁺*	🍄 *fi⁺fb⁺su*	14	100
2	✳ *fi⁺fb su⁺*	🍄 *fi⁺fb⁺su*	7	100
3	✳ *fi⁺fb⁺su⁺*	🍄 *fi⁺fb⁺su*	6	100
4	✳ *fi⁺fb⁺su⁺*	○ *fi⁺fb su*	15	20
5	✳ *fi⁺fb⁺su⁺*	✳ *fi fb su*	10	20
6	✳ *fi⁺fb⁺su⁺*	✳ *fi fb⁺su*	20	15
7	✳ *fi fb su⁺*	✳ *fi⁺fb⁺su⁺*	30	6,6
8	✳ *fi⁺fb su⁺*	✳ *fi⁺fb⁺su⁺*	15	6,6
9	✳ *fi⁺fb⁺su⁺*	✳ *fi⁺fb⁺su⁺*	15	13,2

Table 1. Crosses elucidating the genetic constitution of questionable non-fruiters. Four non-fruiters were each crossed with a fruiter (*fi⁺fb⁺*) and a strain forming initials (*fi⁺fb*): various combinations of mating types were used for all eight crosses. The mating types (not shown in the table) segregated as expected. Symbols designating phenotypes of monokaryons: 🍄 fruit bodies; o fruit body initials; ✳ mycelia only. X^2 test gave P values of > 0.2 for the segregation pattern of the tetrads

Crosses			Tetrad analysis								Evaluation		
No.	Phenotypes of parents I	II	Σ tetrads isolated	evaluated	Classification according to phenotype 🍄🍄 ✳✳	○○ ✳✳	🍄○ ✳✳	✳✳ ✳✳	🍄✳ ✳✳	○✳ ✳✳	Segregation pattern of progeny	Genotypes of parents I	II
1	🍄	× ✳	20	17	17	–	–	–	–	–	1:1	*fi⁺fb⁺su*	*fi⁺fb⁺su⁺*
2	○		23	19	5	1	13	–	–	–	1:1:4	*fi⁺fb su*	
3	🍄	× ✳	85	76	4	5	8	10	23	26	1:1:4:6:12:12	*fi⁺fb⁺su*	*fi fb su⁺*
4	○		–	–	–	–	–	–	–	–	–	*fi⁺fb su*	
5	🍄	× ✳	50	33	4	7	22	–	–	–	1:1:4	*fi⁺fb⁺su*	*fi⁺fb su⁺*
6	○		–	–	–	–	–	–	–	–	–	*fi⁺fb su*	
7	🍄	× ✳	45	35	6	–	–	7	22	–	1:1:4	*fi⁺fb⁺su*	*fi fb⁺su⁺*
8	○		80	72	3	4	3	5	29	28	1:1:4:6:12:12	*fi⁺fb su*	

2) Depending upon the number of tetrad types (1, 3 or 6), the non-fruiters differ from the tester strains by 1, 2 or 3 genes, respectively. Therefore, in addition to the *fi* and *fb* loci, a third gene is required to explain the segregation patterns. Since no other phenotype was observed among the offspring, a suppressor gene (su^+/su) is postulated. A statistical evaluation of the crosses indicates that the *su* locus is not linked to either *fi* or *fb*.

3) The action of the su^+ allele is to suppress the initiation and subsequent development of fruit bodies.

4) On the basis of this hypothesis the genotypes of both tester strains and non-fruiters are indicated in the last two columns of the Table. All tester strains must carry the inactive allele *su* since they produce fruiting structures; the non-fruiters must all carry su^+ in all possible combinations with the fi^+/fi and fb^+/fb alleles to explain the various segregations.

5) Further, the fruiting obtained in crosses 2 and 8 is explained through the action of the suppressor. A cross between a strain producing initials and one producing only mycelium normally fails to fruit because the fb^+ allele is absent. However, in these crosses, the fb^+ is furnished by the suppressed non-fruiter.

The involvement of a suppressor gene in fruiting raises the question of its interaction with the morphogenetic loci, fi^+/fi and fb^+/fb. A series of crosses of various monokaryons which allow fruiting suggests an answer to this question: these are summarized in Table 2 and lead to the following conclusions:

1) Normal fruit bodies are formed in crosses, 1, 2 and 3 involving a monokaryotic fruiter.

2) The same holds true to a limited extent in cross 4, in which one partner is also able to form fruiting body initials.

3) In crosses 5 and 6, in which one monokaryon carries the inactive *su* allele, about 15-20% fruiting was observed.

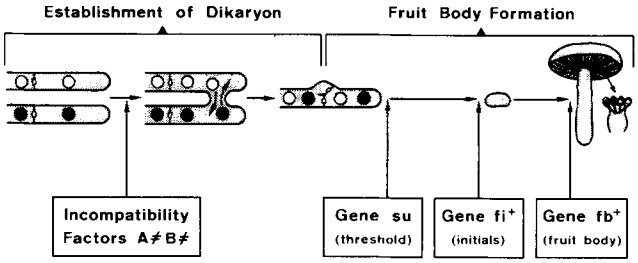


Fig. 1. Genetic control of morphogenesis in *Agrocybe aegerita*. In nature fruiting generally occurs only on dikaryons. The establishment of a dikaryon depends upon the heterogeneity of the two incompatibility factors A and B of the monokaryons. Fruit body formation is under the control of at least 3 unlinked genes. The threshold gene *su* switches on the fruiting pathway; fruiting initials and subsequently, fruit bodies are induced by the fi^+ and fb^+ alleles, respectively

4) In crosses 7 and 8, in which both partners are postulated to carry the su^+ allele, the fruiting is drastically reduced.

5) The relatively high percentage of fruiting in cross 9, in which both monokaryons carry the su^+ allele, is explained by the presence of the fi^+ and fb^+ alleles in both partners.

6) Thus, the *threshold of morphogenesis is controlled by the suppressor gene* in such a way that only the allele *su* is *able to open the pathway*, allowing therewith the expression of the morphogenetic genes, fi^+ and fb^+ (Fig. 1).

3.2 Control of Productivity by the Fruiter Genes

In the dikaryon the fruiter genes have both a qualitative and quantitative effect on the time of fruiting and biomass production. In the experiments for analyzing these parameters, monokaryons, each carrying the *su* allele, were utilized for determining the effects of varying doses of the fi^+/fi and fb^+/fb alleles. (Tables 3, 4).

Table 3 permits the following generalizations:

1) Under optimal genetic conditions, i.e. both nuclei of the dikaryon carrying the fi^+ and the fb^+ alleles, the first flush occurs within 20 days (No. 1).

2) The absence of one active allele causes a delay in time of fruiting; the first flush does not occur until 25 days (No. 2).

3) When only one dose of fi^+ and fb^+ is present, fruiting is even more retarded (30 days). This is true in the cis as well as the trans configuration (Nos. 3, 4).

From Table 4 the following can be concluded:

1) The time of the first fruiting flush corresponds to that observed in the experiments summarized in Table 3.

2) The data from the two wild strains (Exp. No. 1) suggest that biomass production is highly variable in nature. The yield obtained with the Strasburg strain is only about half that of the Brünn strain. The character thus seems to be under polygenic control.

Table 3. Relation between time of fruit body formation (first flush) and dosage of fi^+ and fb^+ alleles. Rupture of the velum was used as an index of completion of fruiting. Ten dikaryons were tested in each experiment

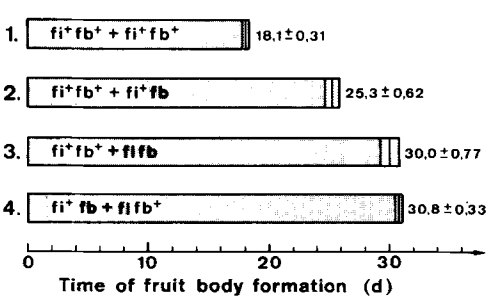
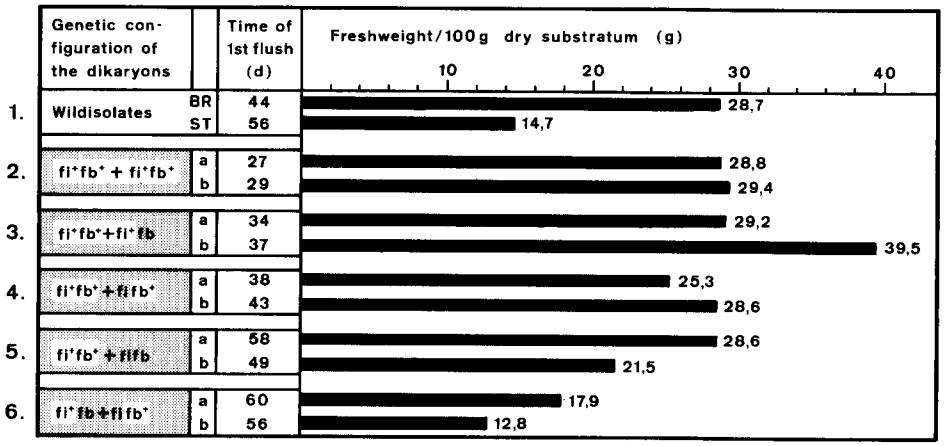


Table 4. Relation between the production of fruit body biomass on straw and dosage of the fi^+ and fb^+ alleles; a and b indicate two different experiments



3) The results obtained with the genetically defined strains show that the fruiter genes, fi^+ and fb^+, play a role in the control of biomass production.

4) While the correlation of number of fruiter alleles with biomass yield is not as pronounced as that with the time of fruiting (see Table 3), the trend is similar: Fewer fruiter alleles give less biomass.

5) The variation in biomass production in these experiments is evidence that control of yield involves additional, as yet unidentified, genes.

The productivity data show that both biomass production and time of fruiting are subject to genetic manipulation.

4 Conclusions

The elucidation of the genetic control of fruiting through detection of the threshold gene, su^+/su, provides a basis for a breeding program in this species. It should be possible to apply this information in breeding other economically important mushroom species. Since comparable genetic mechanisms have been found in *Polyporus ciliatus* (Stahl and Esser 1976) and in *Schizophyllum commune* (Esser et al. 1979), in spite of the fact that the three fungi are not closely related, it is reasonable to predict that the genetic control of fruiting in basidiomycetes in general is essentially the same as in *Agrocybe aegerita*.

5 Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (Bonn-Bad Godesberg). We thank Prof. E. Steiner for reading the manuscript and the technical staff of the laboratory for their assistance, especially Frau G. Lembke and Frau S. Puppe.

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Received April 8, 1981
Communicated by H.F. Linskens

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