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Genetic studies of the *b* incompatability locus of *Ustilago maydis*

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SUMMARY

The *b* locus of *Ustilago maydis* is both an incompatibility locus and a pathogenicity locus. There are several different *b* factors, and they are randomly distributed and equally frequent in the natural population. A sample of 62 lines contained 18 *b* groups and permitted an estimate of no more than 25 *b* groups in the natural population. All lines within the same *b* group are indistinguishable in their mating behaviour. No linkage was detected between *b* and any of the four biochemical markers tested. No intrafactor recombinants at *b* were found among more than 6700 progeny. Differences in degree of pathogenicity between solopathogenic diploids and comparable haploid by haploid crosses were detected.

1. INTRODUCTION

The corn pathogen *Ustilago maydis* has two incompatibility loci, *a* and *b*. The *a* locus has two factors, a_1 and a_2, and controls haploid fusion (Rowell, 1955; Holliday, 1961*b*). The *b* locus appears to control the remainder of the sexual cycle. Since completion of the sexual cycle is inseparable from disease development in corn, the *b* locus has also been considered a pathogenicity locus (Holliday, 1961*b*). The *b* locus is multifactorial; Rowell & DeVay (1954) claim to have isolated 15 different *b* factors from natural infections. Disease is produced when two haploid strains carrying different *a* and *b* factors combine in the corn plant. Tumour-like growths on the host, called galls, are formed which subsequently become filled with a black sooty mass of diploid brandspores (teleutospores). Upon germination the brandspores undergo meiosis and produce the haploid yeast-like progeny.

Few direct genetic analyses of the *b* locus have been made. Rowell (1955) cited his study of the distribution of *b* factors in natural populations, but he never published the data. Rowell & DeVay (1954) reported no recombination within the *b* locus among a total of 1305 progeny from 62 crosses. Holliday (1961*a*) found no linkage between the *a* and *b* locus, but this report was based on very limited data. It is the purpose of this paper to clarify certain questions about the genetics of the *b* locus: the distribution of *b* factors in the population, the frequency of recombination within the *b* locus and the linkage of other known gene markers to the *b* locus.

2. MATERIALS AND METHODS

General laboratory techniques have been described elsewhere (Holliday, 1961*a*, *b*; Puhalla, 1968). Several wild-type cultures as well as the source strains for the markers

ad-1, *me-15*, *inos-3* and *nic-3* (requirements for adenine, methionine, inositol and nicotinic acid respectively) were supplied by Dr Robin Holliday. Other wild-type cultures were monosporidial isolates from brandspore samples sent to the author by various scientists throughout the United States and southern Canada. Mating type determinations were made in corn or on double strength complete agar medium (DCM) (Puhalla, 1968).

3. RESULTS AND DISCUSSION

(i) *Distribution of* b *factors in the population:* b *groups*

A total of 62 monosporidial lines from 33 separate infections were tested for the incompatibility factor carried at both the *a* and *b* loci. Only two *a* factors were found, thus confirming the earlier findings of Rowell & DeVay (1954). On the other hand, the 62 isolates could be assigned to 18 different *b* groups. All the *b* factors within one *b* group were indistinguishable on the basis of mating and were therefore considered to be identical. No instances were found where a strain fell into more than one such *b* group. Table 1 shows the distribution of the *b* groups in the sample.

Table 1. *The frequency of the number of occurrences of* b *groups in a sample of 62 lines*

	No. of occurrences of specific *b* groups					
	1	2	3	4	5	6
Frequency	1	5	3	5	2	2

The distribution of frequencies in Table 1 satisfactorily fits ($P = 0{\cdot}21$) a truncated Poisson distribution in which the 0 frequency class cannot be measured. Such a distribution requires that all the *b* groups are equally frequent and that they are distributed at random in the population. The total number of *b* groups in the population sampled was estimated at 21 (95 % confidence interval: 17–25) using the formula of Wright (Raper, 1966).

Of the 62 isolates, 23 were recovered from one small field in Connecticut, U.S.A. Even this limited subsample, however, contained 13 different *b* groups and showed only a slightly higher frequency of multiple occurrences than the entire sample. This attests to the wide and thorough distribution of the *b* factors in nature.

(ii) *Linkage between the* b *locus and known gene markers*

Several crosses were analysed in which the gene markers *ad-1*, *me-15*, inos-*3* and *nic-3* were segregating. Examination of at least 94 progeny from each cross revealed no linkage between any of these markers and the *b* locus. Moreover, the *b* locus was also independent of the *a* locus. This confirms the earlier finding that there is no linkage between *a* and *b* (Holliday, 1961*a*).

(iii) *Recombination within the* b *locus*

Meiotic recombination within the *b* locus could be detected if the recombinant mated either with both parental *b* types (dual mater) or with neither. Table 2 lists the progeny of four crosses tested for such behaviour.

With few exceptions half the progeny of these crosses were of one parental *b* type and half were of the other. A small proportion of 'self maters' were also recovered. These are strains which on DCM become covered with a heavy white mycelium. (Most strains of *U. maydis* are yeast-like on DCM.) Such strains are invariably solopathogenic. Presumably they are diploids which have failed to undergo meiosis at brandspore germination

and are therefore heterozygous at both the *a* and *b* loci. Most crosses tested in this laboratory have yielded such diploid progeny at frequencies of less than 1 %. Solopathogenic diploids induce smaller and fewer galls than comparable haploid by haploid combinations, and few brandspores are produced. One of the self maters of cross (1) in Table 2 formed sufficient brandspores to be analysed, cross (1*a*). It was heterozygous at the *me-15* and *ad-1* loci; segregation of these markers and the incompatibility factors in the progeny was similar to that of the original haploid by haploid combination.

The three dual maters listed in Table 2 mated with strains carrying either parental *b* factor in combination with a_2 factor. *A priori* they might be haploids carrying recombinant *b* factors. The dual maters from cross (1) and (2) were prototrophic and solapathogenic; but like unreduced diploids they produced few galls or brandspores. Random

Table 2. *Mating reactions of cross-progeny*

Cross	No. progeny tested	Single maters	Dual maters	'Self maters'
(1) $a_2b_D \times a_1b_A$*	4996	$2490b_D$:$2493b_A$	1	12
(1*a*) Self mater from cross (1)	499	$234b_D$:$264b_A$	0	1
(2) $a_2b_G \times a_1b_A$	625	$319b_G$:$299b_A$	1	6
(3) $a_2b_I \times a_1b_A$	640	$311b_I$:$326b_A$	1	2

* *b* Groups are given letter designations: the 18 *b* groups found so far are labelled b_A through b_R.

The a_1b_A parent in the crosses above carried the gene markers *ad-1* and *me-15*.

progeny from these brandspores showed segregation of the two parental *b* types but no segregation for *a*. In addition the progeny of the dual mater from cross (1) showed segregation for adenine and methionine requirements. It is likely that these dual maters are not *b* recombinants but rather unreduced '*common a*' diploids, i.e. diploids homozygous for the *a* factor but still heterozygous for the *b*. These are known to be solopathogenic (Holliday, 1961*b*, Puhalla, 1968). The same conclusion has been reached for the dual mater of cross (3), even though it is not solopathogenic. In this case lack of pathogenicity is due to the fact that the dual mater requires adenine. Holliday (1961*a*) has shown that paired compatible haploids which carry non-complementing adenine requirements are not pathogenic. Proof of the diploidy of this dual mater is indicated by the generation of methionine requiring and parental *b* phenotypes following irradiation with ultraviolet light. These are expected as the result of induced mitotic recombination in a heterozygous diploid (Holliday, 1961*b*).

It was reported earlier in this laboratory that common *a* diploids are mycelial on DCM (Puhalla, 1968). The dual maters examined here are yeast-like. The discrepancy may be related in part to age. Common *a* diploids after prolonged incubation on DCM or after frequent subculture often change from a yeast-like to a mycelial growth habit.

U. maydis shows tetrapolar incompatibility, but only one of the two loci is multifactorial. In *Coprinus lagopus*, *Schizophyllum commune* and several other Hymenomycetes both loci are multifactorial. Intrafactor recombination has been detected at the *A* locus of *C. lagopus* (Day, 1960) and at both loci of *S. commune*. In *S. commune* recombination frequencies ranged from 3·2 to 22·8 % at the *A* locus (Raper, Baxter & Ellingboe, 1960) and from 0·1 to 8·0 % at the *B* locus (Koltin, Raper & Simchen, 1967). These variations in frequency are controlled by independent genetic systems (Stamberg, 1969). Each locus was found to be made up of two 'subunits', and each subunit carried one of several 'alleles'. Recombination between subunits resulted in new incompatibility factors. A change at only one of the subunits was sufficient to specify a new factor.

No recombination within the *b* locus of *U. maydis* was detected in over 6700 cross-progeny. If the *b* locus has two subunits like *Schizophyllum*, lack of recombination in any cross of Table 2 could simply mean that the *b* factors of the two parents carried one subunit in common. However, this seems an unlikely explanation for all three crosses. Considering the cross with the fewest progeny the upper 5 % fiducial limit for the frequency of recombination within *b* is 0·47 %. In other words, this cross should have detected recombination between two subunits as close together as 0·47 map units. If recombination does occur, it is rare. In this respect the *b* locus is more like the incompatibility locus of the bipolar forms. Rather extensive analyses of *Polyporus palustris* (Flexer, 1963) and *P. betulinus* (Burnett, cited in Raper, 1966) revealed no recombination within their incompatibility loci.

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REFERENCES

DAY, P. R. (1960). The structure of the *A* mating type locus in *Coprinus lagopus*. *Genetics* **45**, 64–650.

FLEXER, A. S. (1963). Bipolar incompatibility in *Polyporus palustris*. Ph.D. Thesis, Harvard University, Cambridge.

HOLLIDAY, R. (1961*a*). The genetics of *Ustilago maydis*. *Genetical Research* **2**, 204–230.

HOLLIDAY, R. (1961*b*). Induced mitotic crossing-over in *Ustilago maydis*. *Genetial Research* **2**, 231–248.

KOLTIN, Y., RAPER, J. R. & SIMCHEN, G. (1967). The genetic structure of the incompatibility factors of *Schizophyllum commune:* the *B* factor. *Proceedings of the National Academy of Sciences of the United States of America* **57**, 55–62.

PUHALLA, J. E. (1968). Compatability reactions on solid medium and interstrain inhibition in *Ustilago maydis*. *Genetics* **60**, 461–474.

RAPER, J. R. (1966). *Genetics of Sexuality in Higher Fungi*. New York: Ronald Press.

RAPER, J. R., BAXTER, M. G. & ELLINGBOE, A. H. (1960). The genetic structure of the incompatibility factors of *Schizophyllum commune:* the *A* factor. *Proceedings of the National Academy of Sciences of the United States of America* **46**, 833–842.

ROWELL, J. B. (1955). Functional role of compatibility factors and an *in vitro* test for sexual compatibility with haploid lines of *Ustilago zeae*. *Phytopathology* **45**, 370–374.

ROWELL, J. B. & DEVAY, J. F. (1954). Genetics of *Ustilago zeae* in relation to basic problems of its pathogenicity. *Phytopathology* **44**, 356–362.

STAMBERG, J. (1969). Genetic control of recombination in *Schizophyllum commune*: separation of the controlled and controlling loci. *Heredity* **24**, 306–309.