COMPATIBILITY REACTIONS ON SOLID MEDIUM AND INTERSTRAIN INHIBITION IN *USTILAGO MAYDIS*

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Received April 11, 1968

THE fungus *Ustilago maydis* is the cause of boil smut disease of corn. Although the complete sexual cycle of the fungus requires the host, rapid vegetative growth on artificial medium is easily obtained. With the work of PERKINS (1949) and the extensive investigations of HOLLIDAY (1961a) *U. maydis* has been established as a useful tool for genetical research. Since it is also a plant pathogen it may aid in the investigation of the genetics of pathogenicity.

The symptoms of the corn smut disease, gall formation and brandspore formation, are the result of the mating of two haploid strains within the host. Mating is under the control of two gene loci, *a* and *b*. There are only two *a* alleles but perhaps as many as 25 *b* alleles. Only strains with different alleles at both loci will mate; such combinations are termed "compatible", and the *a* and *b* loci are called incompatibility loci.

As early as 1932, SLEUMER and later BOWMAN (1946) and ROWELL (1955) described what they consider a mating reaction on artificial medium. They observed fusion of compatible haploid cells under a microscope; the resultant fusion product, however, developed no further. Also in 1932, BAUCH described a macroscopic indication of mating. Two compatible strains, when spotted one upon the other on malt agar, became covered with a heavy mycelial growth. Incompatible combinations and individual haploids remained yeast-like, the usual growth pattern on artificial medium.

In the present paper the reaction BAUCH described is called plate mating. Some of the internal and external restrictions on plate mating are defined. A second interaction on agar called interstrain inhibition is also described; the inheritance of this inhibition is considered in detail. It is the first reported instance of extrachromosomal inheritance in *U. maydis*. Both plate mating and interstrain inhibition provide information on the development of the fungus in corn.

METHODS AND MATERIALS

Strains: Four wild-type haploid cultures of *Ustilago maydis*, the original auxotrophic mutants, and one diploid stock DH were kindly supplied by DR. ROBIN HOLLIDAY. Following DR. HOLLIDAY's designations, the *b* alleles of the haploid strains are *b1* and *b2*. Other wild-type strains of mating types *a2b8*, *a1b9*, *a1b10* and *a2b11* were obtained from DR. PETER R. DAY. The remainder of the cultures are monosporidial isolates from naturally occurring smut galls collected and sent to the author by various scientists throughout the United States and Canada.

Media: The minimal and complete media are those of HOLLIDAY (1961a,b). In all cases solid media contain 2% agar. Double strength complete medium is complete medium in which all ingredients except agar and distilled water have been doubled. Supplements to the minimal medium were added before sterilization for 10 min. in an autoclave at 15 lbs pressure. All media were adjusted to pH 7.0 before autoclaving.

Maintenance of Cultures: All cultures were incubated at 25°C or 30°C. Stock cultures were stored at 3°C on plates of complete agar and transferred every month. Cultures were also preserved on silica gel by the method of PERKINS (1962). Sporidial suspensions in sterile skim milk added to the gel and kept at 3°C for over a year are still viable.

Crosses: Two-to-three-week-old, soil grown plants of the corn variety Golden Bantam were used for inoculation. All operations were carried out in the greenhouse. Sporidia from fresh streaks of the strains to be crossed were mixed in equal amounts in water to a total concentration of about 10^7 cells/ml. Approximately one ml of the suspension was then injected into a plant one inch above soil level with a sterile hypodermic needle. Gall formation was scored after 12 days; brandspores were harvested from the galls after an additional 10 days. Brandspores to be germinated were first suspended overnight in 1.5% $CuSO_4$ solution to kill vegetative cells, filtered through glass wool, washed twice in sterile distilled water and then plated onto complete medium.

Plate mating: Plate mating was carried out in plastic Petri dishes containing at least 20 ml of double strength complete agar medium. Only fresh, day-old cultures on complete agar were used. They were applied to the mating agar directly with a sterile toothpick or suspended in water and dropped onto the agar with a loop or micropipet. The two strains to be mated were placed no more than one mm apart. The plates were then sealed with tape and incubated at 25°C. Incidence of mating was scored after two days using a 7 to 30 × binocular dissecting microscope.

Inhibition tests: Either complete agar or minimal agar with 2% sodium glutamate was used. Tests for the inhibition of the growth of strain B by strain A were made as follows: strain A was applied to the agar with a toothpick or as a drop of sporidial suspension and incubated at 30°C for one day. Strain B was then applied as a spot of sporidial suspension within one mm of strain A. After an additional incubation at 30°C for one day the pairing was scored for inhibition.

Formation of diploids: The methods of HOLLIDAY (1961b) were used to recover diploids heterozygous at both the *a* and *b* loci and diploids homozygous only at the *a* locus. Diploids homozygous only at the *b* locus were formed by the following method: Two haploid strains carrying complementary biochemical markers and different *a* genes but identical *b* genes were streaked side by side on a plate of complete medium. A segment of an actively growing corn leaf was placed over the streak. The leaf segment had been surface sterilized by immersing it in 0.1% $HgCl_2$ for three minutes and washing it once in 2% chlorox and twice in sterile water. After four days incubation at 30°C the leaf was stripped off the complete agar and transferred unwashed to a plate of minimal medium. Substantial sporidial growth would appear around the leaf after a week at 30°C. If this growth was sampled, suspended in water and plated on minimal agar, most colonies which arose were diploid.

Other procedures: Other techniques such as tetrad analysis, random progeny analysis, induction of mitotic recombination in diploids and detection of biochemical requirements are essentially the same as those described by HOLLIDAY (1961a,b). Determination of mating type of strains was usually made by plate mating against testers of known mating type. More rarely, mating type was determined by crossing the unknown with standard testers in the corn plant.

RESULTS

Plate mating: Haploid strains of *Ustilago maydis* normally produce yeast-like growth on agar medium. Sometimes, however, when colonies touch and coalesce, hyphae up to a millimeter in length form along the line of contact. Strains which show this reaction are invariably compatible, that is they form galls in corn. Pairings between strains with the same *a* allele and/or the same *b* allele do not

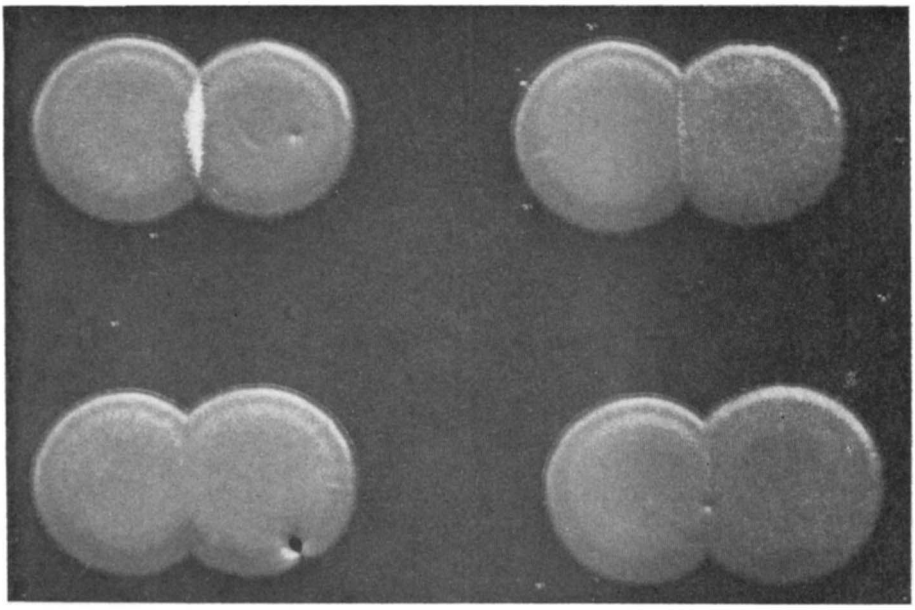

FIGURE 1.—Plate mating (upper left pairing) which occurs between two strains carrying different alleles at both the *a* and *b* loci. Upper right pair carry the same *b* allele. Lower right, the same *a* allele. Lower left pair carry identical *a* and *b* alleles. (5 × magnification)

form the hyphae (Figure 1). An intimate contact between the two strains is necessary. If a millipore filter (pore diam 0.22 μ) is interposed between the two strains, no line of hyphae forms. Such a filter permits the passage of large molecules and even virus particles but not fungal cells. This need for cell contact and the strict dependence on the incompatibility loci suggest that the formation of hyphae is a mating reaction; therefore, it is called plate mating.

Although pairings of two strains carrying different *a* alleles but identical *b* alleles do not form plate mating hyphae, they sometimes do show a characteristic reaction. This is visible in the upper right pairing of Figure 1. The interaction can best be described as an area of rough growth along the line of contact. Often there is a broad depression or ridge in the contact area. The interaction is difficult to see unless the two strains have a very smooth colony surface. A pairing of two strains carrying the same *a* allele, on the other hand, shows a smooth continuous confluent growth at the line of contact.

Cultural conditions affect the expression of plate mating. Most striking is its dependence on medium. Plate mating occurs most reliably on double strength complete agar, sporadically on complete agar and not at all on minimal. The required additive in the former two is casein hydrolysate, and consistent plate mating requires a high concentration (0.5% to 1.0%). An amino acid mixture of about the same total concentration is equally effective. A more specific determination of the nutrient requirement for plate mating has not been made. Tem-

perature is less critical. The range 25°C to 30°C permits plate mating, although at 25°C it is maximal.

Mycelial, ropy, and flaky growth forms which arise in culture through mutation or under stress of environment can mask the plate mating reaction or even actively suppress it. Some of these variants, however, can be induced to show yeast-like growth. A temperature of 25°–30°C, media with high concentrations of salts or sugars, constant illumination, and the sealing of culture plates with tape all enhance yeast-like growth. Unfortunately not all morphological variants respond to this treatment; they must be avoided in plate mating analyses.

The intensity of the plate mating reaction varies with the two compatible strains paired. A strain which plate mates strongly with one compatible strain may show an almost undetectable reaction with another. Some pairings which form galls in corn show no plate mating at all. Except for interstrain inhibition, another plate phenomenon considered in the next section, the causes of this variability have not been investigated.

All diploids heterozygous at the *a* and *b* loci form a heavy, white mycelial growth on double strength complete agar (Figure 2). This mycelial growth is reduced on complete and does not occur on minimal agar. Furthermore these diploids yield no mycelial growth types among meiotic products. Therefore, the mycelial over-growth seems analogous to the hyphae formed in plate mating. Diploids homozygous at the *a* locus but heterozygous at the *b* locus also become

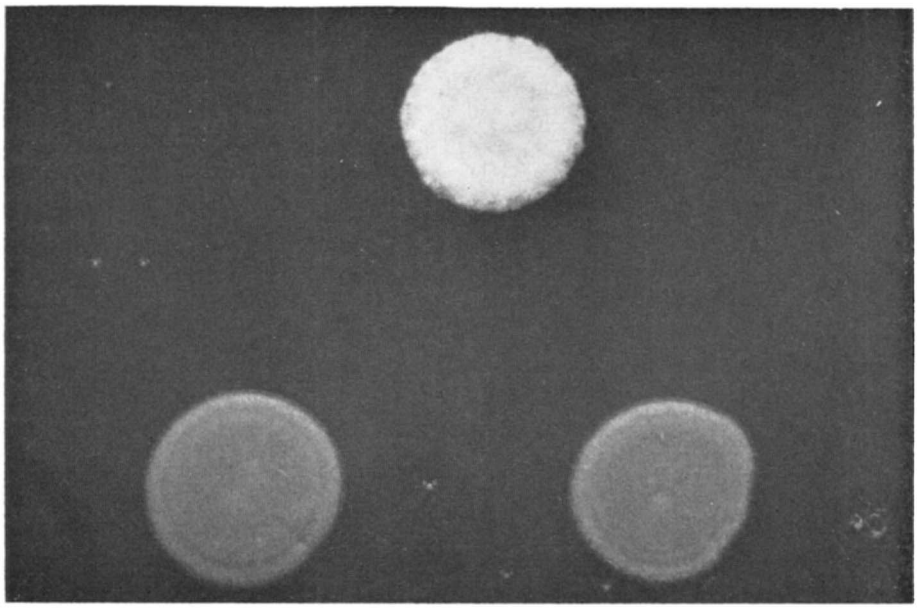

FIGURE 2.—Morphology of a diploid and haploids on double strength complete agar. The upper colony is a diploid heterozygous at both the *a* and *b* loci. The two lower colonies are the two haploids used to synthesize this diploid by the method of HOLLIDAY (1961b). (5 × magnification)

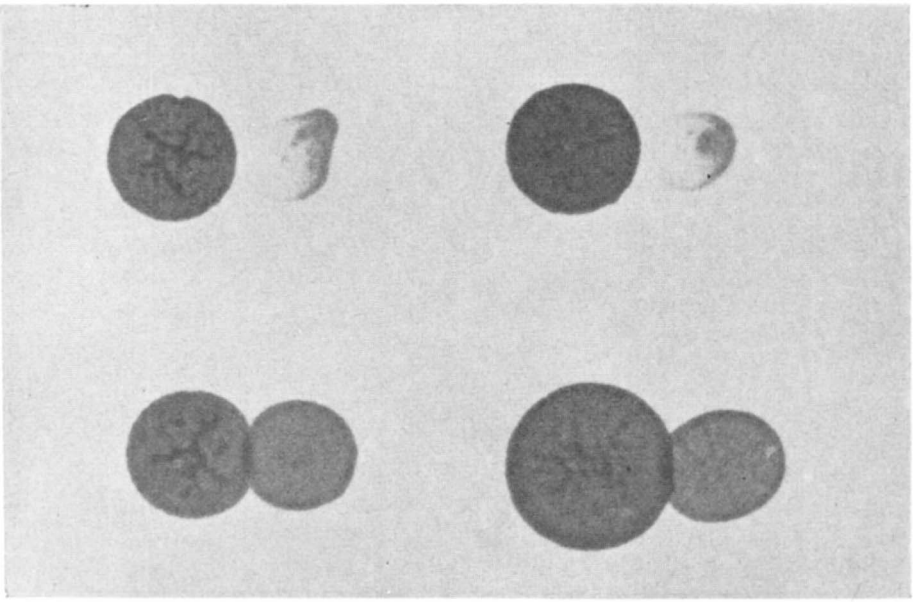

FIGURE 3.—Interstrain inhibition in the two upper pairings. Upper left, P1 + P2s (compatible); upper right P1 + P2s (incompatible). In both cases the P2s strain is on the right. Lower left, P1 + P1. Lower right, P2 + P2. (3 × magnification, illumination from behind)

mycelial. Diploids homozygous at the *b* locus remain yeast-like. All diploids which show this mycelial overgrowth also form galls when injected singly into corn plants.

Interstrain inhibition: Some strains of *U. maydis* inhibit the growth of strains which are near them on agar medium. The inhibition is caused by a substance which diffuses out of the cells into the surrounding medium (Figure 3). Strains which produce this inhibitory substance are called P1 strains; those which do not are P2 strains. The P2 strains can be divided into P2s types, sensitive to the inhibitor, and P2r, not affected by the inhibitor. P1 strains are not inhibited.

A group of 35 monosporidial isolates representing a sample of naturally occurring strains from across the United States and Canada was tested for P type. Only four produced inhibitor and were thus P1. Of the remaining 31 isolates, 19 were P2s and 12 were P2r. It is not clear whether all four P1 strains produce the same inhibitor because the effectiveness of inhibition varies from one P1 strain to another. Moreover, there is considerable variation in the sensitivity of the P2s strains.

Inhibitor production occurs on complete or double strength complete agar but not on minimal agar. As in plate mating, the active component in the former two is casein hydrolysate, although lower concentrations (0.25 to 0.50%) are needed. Individual amino acids have also been tested for activity; several are effective (Table 1).

Cultures of haploid P1, P2r and P2s strains have been through more than a

TABLE 1

Ability of compounds to support inhibitor production by P1 strains

Additive	Response*
Hydrolyzed nucleic acids (1% vol/vol)	—
Vitamin solution (5% vol/vol)	—
Yeast extract (0.2% wt/vol)	+
Casein hydrolysate (1% wt/vol)	+
Sodium glutamate (0.5% wt/vol)	+
Asparagine (0.5% wt/vol)	+
Methionine (0.5% wt/vol)	—
Arginine (0.5% wt/vol)	+
Histidine (0.5% wt/vol)	+
Phenylalanine (0.5% wt/vol)	—
Tyrosine (saturated soln)	—

Tests made on minimal medium with specified additive. See HOLLIDAY (1961a) for composition of hydrolyzed nucleic acids and vitamin solutions.
* + = inhibitor was produced, — = no inhibitor detected.

dozen consecutive mass transfers on complete agar without a change in P type. Large numbers of single cell clones from these strains are all of the parental P type. P2 strains are also stable in crosses. Only P2 progeny are recovered from crosses between two P2 strains (Table 2,A). On the other hand, all crosses so far

TABLE 2

Analysis of random progeny from crosses between various P types

	P types crossed	Number and P type* of progeny	Percent P1 progeny
A.	*P2 × P2*		
	(1) *a1b1 × a2b2*	164 P2	0
	(2) *a1b9 × a2b2*	270 P2	0
	(3) *a1b13 × a2b1*	100 P2	0
	(4) *a1b14*(P2r) × *a2b1*(P2r)	100 P2r	0
	(5) *a1b14*(P2s) × *a2b1*(P2s)	100 P2s	0
	(6) *a2b1*(P2s) × *a1b14*(P2r)	52 P2s, 48 P2r	0
B.	*P1 × P1*		
	(7) *a1b14 × a2b15*	278 P1, 12 P2	96
	(8) *a2b15 × a1b40*	197 P1, 4 P2	98
	(9) *a1b14 × a2b41*	192 P1, 8 P2	96
	(10) *a1b40 × a2b41*	178 P1, 22 P2	89
C.	*P1 × P2*		
	(11) *a1b14 × a2b1*	331 P1, 21 P2	94
	(12) *a2b15 × a1b1*	95 P1, 5 P2	95
	(13) *a2b14 × a1b2*	101 P1, 9 P2	92
	(14) *a1b14 × a2b2*	94, P1, 6 P2	94
	(15) *a2b14 × a1b1*	97 P1, 3 P2	97

* Unless stated specifically no distinction is made between P2r and P2s types.

made between two P1 strains yield a small percentage of P2 progeny (Table 2,B). This will be considered in more detail below.

Crosses between two P2r strains or between two P2s strains produce either all P2r or all P2s progeny, respectively. In crosses between a P2r strain and a P2s strain, however, one half the progeny are P2s and one half are P2r (Table 2,A). This same one-to-one segregation occurs within individual tetrads. This is the typical behavior of a pair of nuclear alleles. Therefore, P2s strains are considered to carry a gene *s* which confers sensitivity to P1 inhibition; P2r strains carry its allele s^+ and are not sensitive.

Part C of Table 2 shows the results of crosses between P1 and P2 strains. An overwhelming percentage of the progeny are P1 even though segregation for known nuclear genes in these crosses is one-to-one. Samples of 5 to 10 whole tetrads from these crosses reveal no segregation for the P1/P2 alternative within individual tetrads. All four members of a tetrad are either P1 or P2.

P1 strains from the progeny of P1 × P2 crosses have been carried through two successive backcrosses to the parent P2 strains. In all cases the results are the same as those between parental P1 and P2 strains: most of the progeny are P1 (Table 3), and there is no segregation for the P1/P2 alternative in individual tetrads. The percentages of P1 progeny from these crosses are more variable, but no definite trend toward a lower or higher percentage is seen through the two consecutive backcrosses. Some P2 progeny from P1 × P2 were also crossed to the P2 parents. As in crosses between two parent P2 strains, only P2 progeny are recovered.

Tables 2 and 3 show that there is considerable variation in the percentage of

TABLE 3

Analysis of random progeny of crosses between derived P1 strains and stock P2 strains

Cross	P1 parent	Percent P1 progeny	Number tested
(1)	Four members of	98	100
(2)	a P1 tetrad	96	98
(3)	from cross (11)	70	100
(4)	in Table 2	91	100
(5)	Four members of	83	100
(6)	a P1 tetrad	73	100
(7)	from cross (11)	98	100
(8)	in Table 2	94	100
(9)	Four members of	96	110
(10)	a P1 tetrad	88	109
(11)	from cross (3)	86	22
(12)	above	95	110
(13)	Two members of a P1 tetrad	100	44
(14)	from cross (1) above	99	110

TABLE 4

Effect of varying the input ratio of the two members of P1 × P2 crosses

Parents	Ratio P1 : P2		P1 progeny	Number tested
	Hemocytometer count	Viable count		
a1b14 P1 × a2b1 P2	12 : 1	18 : 1	99	497
	1 : 9	1 : 6	97	460
a1b14 P1 × a2b2 P2	12 : 1	11 : 1	100	239
	1 : 8	1 : 9	95	446
a2b15 P1 × a1b1 P2	10 : 1	20 : 1	99	100
	1 : 10	1 : 5	98	100

Suspensions in water of the two parents were made separately and the numbers of cells/ml determined with a hemocytometer and by plating on complete medium. The two suspensions were then mixed to give the ratios listed above.

P1 progeny of crosses between P1 and P2. Values between 70% and 100% are found. That this variability is not a reflection of the relative amounts of the two parents in the inoculating suspension is shown in the following experiment. Several duplicate P1 × P2 crosses were prepared. In one duplicate the inoculating suspension had a majority of P1 cells; in the other the P2 cells were in the majority. The suspensions were injected into corn plants, galls harvested and the percentage of P1 progeny scored. Table 4 lists the results; even a ten-fold excess of one parent has no significant effect on the percentage of P1. There is also evidence that the variability is not under genic control. Considerable variation in the percentage of P1 progeny is found both in two replications of the same cross (91% and 100%) and in two samples of the same gall (84% and 98%).

In all the initial crosses between a P1 strain and a P2s strain, the P2 progeny tetrads show a 1:1 segregation for sensitivity *vs.* resistance to inhibition. Since the P2s parent by definition carries the *s* gene, these resistant progeny must have received the s^+ allele from the P1 parent. Segregation of the s/s^+ gene pair should also be occurring in the P1 tetrads of these crosses. However, all four members of such P1 tetrads are resistant. Nonetheless, two of the four members are carrying the *s* gene. This can be shown by crossing them to a P2s strain and examining P2 progeny tetrads. All four members of these tetrads are P2s. It appears, therefore, that the *s* allele can be present with the P1 trait and not be expressed.

In some P1 × P2s crosses about 1% of the tetrads, though P2 by the criterion of no inhibitor production, do not show expression of the *s* allele. In other words, they are wholly resistant to P1 inhibition. Phenotypically the four members of such tetrads are P2r; but when they are crossed to P2s strains, the results are quite different from the usual P2r × P2s crosses. Instead of the expected segregation for sensitivity in every tetrad, many tetrads recovered are wholly resistant. This crossing behavior characterizes a new P group called P3 and distinguishes it from P2r. Such P3 types have been recovered only from tetrads which are

TABLE 5

Progeny analysis of some P3 × P2s crosses

Cross (P3 parent first)	Percent resistant progeny in random sample	Number tested	Tetrad segregation Resistant : Sensitive (Number of tetrads scored)
(1) *a1b14* × *a2b1*	86	107	4:0 (4), 2:2 (2)
(2) *a2b14* × *a1b1*	88	100	4:0 (5), 2:2 (10)
(3) *a1b1* × *a2b14*	47	100	4:0 (5), 0:4 (5)
(4) *a2b1* × *a1b14*	59	100	4:0 (3), 0:4 (1)

The four P3 strains above are all from a single tetrad of cross *a1b14* P1 × *a2b1* P2 (cross No. 11 of Table 2).

wholly P3. The P3 tetrad listed in Table 5 was recovered from a P1 × P2s cross in which the P1 parent carried the s^+ allele. The recovery of wholly P2s tetrads from crosses (3) and (4) in Table 5 confirms that indeed these P3 parents are carrying the *s* allele. Clearly the *s* allele is not expressed with the P3 trait. It follows, therefore, that the wholly resistant progeny tetrads of these same crosses are also P3. Only two tetrad types are recovered from these crosses: wholly P2 and wholly P3. There is no segregation for the P3/P2 alternative within individual tetrads. This seems to be the case in crosses (1) and (2) as well. The wholly resistant tetrads of these crosses are P3; the tetrads segregating for sensitivity are P2. Since each of the latter tetrads contains two sensitive types and two resistant types, the P3 parent must be carrying the s^+ allele.

Haploid P3 strains are stable in vegetative subculture. No variation is found among numerous single cell subclones. Preliminary data from crosses between P3(*s*) and P3(s^+) strains, however, show that as many as 25% of the random progeny are P2s. As noted earlier P2 strains also arise in crosses between two P1 strains. The P2 progeny of these crosses are all resistant to inhibition and are found only in tetrads which are wholly P2. Other experiments show that both P1 parents used in these crosses carried the s^+ gene. Therefore, these resistant P2 progeny could be P2r or P3. In order to make the distinction five such P2 strains, four from a P2 tetrad of cross P1(a1b14) × P1(a2b15) and one from a P2 tetrad of cross P1(*a1b40*) × P1(*a2b41*), were crossed to standard P2s strains. As the results in Table 6 show, all five are true P2r.

The discovery of P3 types raises the question of the exact nature of the twelve resistant P2 strains found among the 35 natural isolates of smut. Are they P2r or P3? As in the case of the P2 progeny of P1 × P1 crosses, this distinction can be made by crossing these natural resistant P2 strains with standard P2s types. This has been done and in all twelve cases the isolates are true P2r.

The P type of only a few diploids has been determined. Five diploids from matings between two P2s strains are all P2s. Five diploids, two from crosses between two P1 strains and three from crosses between a P1 and P2 strain, are all P1. As yet diploids between a P2s and a true P2r strain have not been made so the dominance relation of the s/s^+ gene pair is not known.

TABLE 6

Analysis of progeny from crosses between stock P2s strains and P2 strains derived from P1 × P1 crosses

Cross (derived P2 parent first)	Percent resistant progeny in random sample	Number tested	Tetrad segregation Resistant : Sensitive (Number of tetrads scored)
(1) *a2b14* × *a1b1*	62	60	2:2 (10)
(2) *a1b15* × *a2b1*	46	114	2:2 (6)
(3) *a1b14* × *a2b1*	50	100	2:2 (9)
(4) *a2b14* × *a1b1*	50	100	2:2 (5)
(5) *a1b41* × *a2b1*	60	100	2:2 (8)

DISCUSSION

The relationship of plate mating to mating in corn: Plate mating and gall formation show the same dependence on the incompatibility loci. There has never been an exception to the rule that a pair of strains which plate mates also forms galls in corn. This has been the strongest evidence for considering plate mating a true mating reaction. Yet at best it is only an incomplete duplication of the sexual cycle in corn. Plate mating hyphae have been allowed to incubate on mating agar for several months with no sign of brandspore formation. No studies of the reaction hyphae themselves have been made. Whether they are dikaryotic or even heterokaryotic is unknown. Apparently they are at least the result of cell fusion since actual physical contact between paired strains is essential.

Some pairings of haploids which incite gall formation show only a slight plate mating reaction or none at all. This discrepancy may signify a basic difference between plate mating and mating in corn or simply reflect the nutritional differences between agar medium and corn tissue. The latter possibility is more likely; the limitations of agar medium are evident, for example, in the lack of brandspore formation on agar by genetically compatible combinations. Future studies of plate mating directly on corn tissue or on corn tissue extracts may clarify some of these problems.

Plate mating does not occur on minimal medium, yet BOWMAN (1946) noted that compatible sporidia fuse even in water. In other words, the requirement for casein hydrolysate is not related to fusion of haploids but rather to the growth of the fusion product. It is the *b* locus which apparently controls this growth (ROWELL 1955; HOLLIDAY 1961b). Further investigation of the medium requirement for plate mating may shed more light on the function of the *b* locus itself.

Even though the relationship between plate mating and true mating is uncertain, the use of the plate reaction as a genetic tool is obvious. It is a time and labor saving method of scoring mating type. Consequently it will be of inestimable value in genetic studies of the incompatibility loci. Some of these studies are now underway.

The genetic control of interstrain inhibition: The vegetative stability of strains which produce inhibitor and the transmission of the trait through crosses leave

little doubt that inhibitor production is under genetic control. Lack of segregation for inhibitor production within tetrads from P1 × P2 crosses suggests extrachromosomal control. The lack of segregation for the P3/P2 alternative also seems likely in tetrads of P3 × P2 crosses. It is tempting to consider that the determinants for the three P types, P1, P2, and P3, are in the cytoplasm, although there is no direct evidence for this. Under such an assumption P1, P2, and P3 would represent three different cytoplasmic types, or states.

Crossing data clearly indicate that P2s and P2r types differ essentially by a single nuclear gene. P2s strains carry the *s* allele, which confers sensitivity to inhibitor produced by P1 strains. P2r strains carry the s^+ allele and are resistant to inhibition. The dominance relationship between the two alleles is still unknown. No linkage between the *s* gene and the incompatibility loci has been found in numerous crosses. Other linkage determinations have not been made. The *s* allele is expressed only with the P2 trait. If P2 is truly a cytoplasmic state, this is a clear case of nucleo-cytoplasmic interdependence.

True P2 progeny have been found in P1 × P1 crosses. They apparently occur only in tetrads which are wholly P2. The percentage of the progeny of P1 × P1 crosses which are P2 is about the same as that of P1 × P2 crosses. The P2 progeny may arise by the same mechanism in both cases. If so, the P2 progeny of P1 × P2 crosses do not represent P2 cytoplasm which has "come through" from the P2 parent. Rather they spring directly from the P1 parent. At present, data are too limited to state whether P1 × P1 crosses also produce P3 progeny. Crosses between two P3 strains also yield true P2 progeny at about the same frequency as crosses between P3 and P2 strains. As in the case of P1 strains, it is tempting to consider that in both P3 × P3 and P3 × P2 crosses the P2 progeny are the result of the instability of the P3 parent.

Although P1 strains can give rise to P2 and P3 types and P3 strains can give rise to P2, no other interconversions have been found. In crosses between two P3 strains or between a P2 and P3 strain, P1 progeny do not arise. Furthermore P2 strains are completely stable and never yield P3 or P1 derivatives. An understanding of this pattern of instability may now be gained by defining the P1, P2 and P3 types. Two criteria are needed to distinguish the three; these are inhibitor production [I] and suppression of *s* gene expression [S] P1 strains produce inhibitor and suppress the *s* allele. Symbolically they are $[I^+S^+]$. P3 strains do not produce inhibitor but still suppress the *s* allele. They are $[I^-S^+]$. P2 strains are $[I^-S^-]$. A fourth combination $[I^+S^-]$ has not been found. It may exist, but the present methods of analysis can not detect it. The I and S functions may be related to actual physical components of the cytoplasm. P1 strains possess both components, P3 strains possess only the [S] component and P2 strains possess neither. If loss of either of these components is considered irreversible then the pattern of instability among the three P types can be easily explained. For example, the P2 progeny of P1 × P2 crosses arise from the P1 parent through a loss of both the [S] and [I] components.

When a P1 strain fuses with a P2 strain in corn, the fusion product probably

is a mixture of the cytoplasms of both strains. There is no evidence that only one of the parents contributes the cytoplasm to the progeny as is the case in Neurospora (MITCHELL and MITCHELL 1952) or in the green alga Chlamydomonas (SAGER 1954). The cytoplasm of the fusion product should contain the [I] and [S] components of the P1 parent and, therefore, be P1. Consequently all the progeny will be P1 except for the few derivative types. In crosses between P3 and P2 the fusion product should be P3, having received the [S] component from the P3 parent. Crosses between P1 and P3 should yield a P1 fusion product and the progeny should be the same as those from P1 × P2 crosses. Such P1 × P3 crosses have yet to be analyzed.

The instability of P1 and P3 in crosses is really an instability of P1 and P3 in corn tissue. On agar medium these strains are quite stable. *Ustilago maydis* is normally yeast-like on agar but changes to a mycelial form in corn. In the mycelial form the [I] and [S] components of the cytoplasm may not be distributed evenly throughout. There may be areas which lack one or both components. Within a developing gall in corn there is an extensive network of mycelium. Brandspores form *in situ* when this mycelium breaks up into short cells and these cells develop a heavy wall. If brandspores form in areas of the mycelium lacking [I] or both [I] and [S], they will be P3 or P2, respectively. This heterogeneity within the gall is supported by variations in the percentage of P2 progeny which have been found between two samples of the same gall.

The low incidence of P1 types among the 35 natural isolates is unexpected. If P1 is at such an advantage over P2 in crosses, P1 types should be in the majority in the population. Perhaps the P1 trait imposes disadvantages in nature which are not evident in the laboratory. Moreover the sample may not be truly random.

The nature of interstrain inhibition: No chemical analyses of the inhibitor produced by P1 strains have been made. Since the production of inhibitor requires certain amino acids, it may be proteinaceous. The action of the inhibitor is also unknown. It may only prevent the growth of P2s cells or it may kill them.

Phenomena similar to interstrain inhibition have been reported in other microorganisms, e.g., the killer trait in Paramecium (BEALE 1954), colicin production in enteric bacteria (IVANOVICS 1962), barrage in Podospora (RIZET and SCHÉCROUN 1959), and killer character in *Saccharomyces cerevisiae* (MAKOWER and BEVAN 1963). In each of these cases the inhibitor was only effective against strains in the same genus or species as the inhibitor producing form. Moreover, all are controlled by definite cytoplasmic systems.

The interaction of plate mating and interstrain inhibition: In many instances where two compatible strains do not plate mate, the lack can be traced to interference from interstrain inhibition in which one strain is P1, and the other is P2s. Since media which support plate mating also support inhibitor production, the failure to plate mate is due to the inability of P2s to grow in the vicinity of P1. As yet no medium has been devised which supports plate mating but does not support inhibitor production. Yet if plate mating is indeed a part of the sexual cycle in corn, the corn tissue must be such a medium. In a cross between P1 and

P2s, fusion of the two strains implies no inhibitor production by P1. On the other hand, growth of the fusion product requires a milieu like the one that supports plate mating. Whether corn tissue is indeed such a medium is not known. Such a determination would help clarify the relationship between mating on agar and mating in corn.

I wish to acknowledge the technical assistance of Mrs. DOROTHY CLOW, Miss CAROL CHERKIS and especially that of Mr. JAY TISCHFIELD, whose keen eye first observed plate mating.

SUMMARY

Haploid strains of *Ustilago maydis* usually form yeast-like colonies on agar. Sometimes when these colonies touch, however, hyphae form along the line of contact. The hyphae form only when the two colonies are compatible. They represent the result of true cell fusion. Consequently the reaction is called plate mating. It occurs only on a medium with at least 0.5% casein hydrolysate or an equivalent amino acid mixture. The relationship between plate mating and mating in corn is unclear.—Some strains of *U. maydis* produce an extracellular substance which inhibits other strains. Sensitivity to the inhibitor is imparted by a nuclear allele *s*, but not all strains which carry the *s* allele express it. Three classes are defined: P1 strains which produce inhibitor but do not express the *s* allele, P2 which do not produce inhibitor but do express the *s* allele, and P3 which produce no inhibitor and fail to express the *s* allele. Crossing data show these three classes are heritable and under extranuclear control. They may be viewed as three different cytoplasmic states. Both P1 and P3 strains are unstable in corn and give rise to P2 strains; P2 strains are completely stable. P1 and P3, therefore, may carry cytoplasmic components which P2 lacks.—The interaction between this interstrain inhibition and plate mating is described.

LITERATURE CITED

BAUCH, R., 1932 Die Sexualität von *Ustilago scorzonereae* and *Ustilago zeae*. Phytopathol. Z. **5**: 315–321.

BEALE, G. H., 1954 *The Genetics of* Paramecium aurelia. Cambridge University Press.

BOWMAN, D. H., 1946 Sporidial fusion in *Ustilago maydis*. J. Agr. Res. **72**: 233–243.

HOLLIDAY, R., 1961a The genetics of *Ustilago maydis*. Genet. Res. **2**: 204–230. —— 1961b Induced mitotic crossing-over in *Ustilago maydis*. Genet. Res. **2**: 231–248.

IVANOVICS, G., 1962 Bacteriocins and bacteriocin-like substances. Bacteriol. Rev. **26**: 108–118.

MAKOWER, M., and E. A. BEVAN, 1963 The inheritance of a killer character in yeast (*Saccharomyces cerevisiae*). Proc. 11th Intern. Congr. Genet. **1**: 202.

MITCHELL, M. B., and H. K. MITCHELL, 1952 A case of "maternal inheritance" in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **38**: 442–449.

PERKINS, D. D., 1949 Biochemical mutants in the smut fungus, *Ustilago maydis*. Genetics **34**: 607–626. —— 1962 Preservation of Neurospora stock cultures with anhydrous silica gel. Can. J. Microbiol. **8**: 591–594.

RIZET, G., and J. SCHÉCROUN, 1959 Sur les facteurs cytoplasmiques associés au couple de genes *S-s* chez le *Podospora anserina.* Compt. Rend. Acad. Sci. **249**: 2392–2394.

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.

SAGER, R., 1954 Mendelian and non-Mendelian inheritance of streptomycin resistance in *Chlamydomonas reinhardi.* Proc. Natl. Acad. Sci. U.S. **40**: 356–363.

SLEUMER, H. O., 1932 Uber Sexualität and Zytologie von *Ustilago zeae* (Bechm.) Ungar. Z. Botan. **25**: 209–263.