Meiotic Products from Natural Infections of Ustilago maydis

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ABSTRACT

Most teliospores of *Ustilago maydis* from natural smut galls on field corn germinate and form whole tetrad colonies showing a mosaic pattern. This pattern results from the interaction of compatible meiotic products. Colonies in which only one or two incompatible products survive are yeastlike. At least three products were found in 87.2 and 95.6% of the colonies from two samples. Colonies made up of unreduced diploid cells are easily scored, as they are not mosaic but rather, yeastlike or entirely mycelial. One class of diploids probably arose by mitotic recombination prior to teliospore formation. Phytopathology 61:1020-1021.

Additional key words: mating type, pathogenicity.

Teliospores of *Ustilago maydis* (DC.) Cda. are diploid, and normally undergo meiosis at germination. The haploid products are yeastlike cells which, as single-cell lines, are nonpathogenic. Pathogenicity and completion of the life cycle normally require mating of compatible haploid cells. Two loci, *a* and *b*, govern mating (9). The *a* locus, with two alleles, controls cell fusion. The *b* locus, with 18 known alleles (8), controls the formation of dikaryotic infection hyphae. Only matings between cells with different *a* and *b* factors give rise to a pathogenic dikaryon. The alleles present in haploid cell lines can be identified by their mating reactions with tester strains on agar medium (7). Infection hyphae, visible as a white mycelium, form only where compatible colonies touch. Diploids heterozygous for *a* and *b* ($a\neq b\neq$) are pathogenic as single-cell lines (solopathogenic), and form colonies uniformly covered with aerial hyphae, whereas diploids homozygous for *b* ($a\neq b=$) are not solopathogenic, and form yeastlike colonies (7).

Here we report analyses of meiotic products of *U. maydis* from field infections on corn (*Zea mays* L.) based on a new and simple method of examining whole tetrads grown as colonies from single teliospores.

Materials and Methods.—Two teliospore samples, A and B, collected from natural infections on field corn in Connecticut in August 1969 and July 1968, respectively, were used. Media and methods are described in (7). To guard against contamination from vegetative cells, teliospores were soaked for 6 hr in 1.5% (w/v) copper sulfate solution prior to spreading them on complete (CM) or double-strength complete medium (DCM). The mating reaction is most easily detected on DCM in taped dishes in constant light at 25 C.

Results and Discussion.—When single teliospores germinated and formed colonies on DCM, adjacent compatible cells within a colony mated, giving rise to sectors of aerial infection hyphae on the colony surface. Teliospores transferred to DCM, after germination on CM and verification of their single-spore origin, also gave rise to similar mosaic colonies (Fig. 1). Two kinds of variants were observed, colonies which remained entirely yeastlike and colonies which were uniformly covered with hyphae. The frequencies of different colony types are shown in Table 1. The variants were isolated and their phenotypes confirmed by further tests on DCM. At least 16 single-cell isolates from each yeastlike variant were tested for mating type (7).

More than half of the yeastlike colonies in both samples were incomplete tetrads resulting from the survival of only one or two incompatible meiotic products. The mating-type test is very sensitive, since it reveals a compatible interaction even with an excess of one component of the order of 10^5 to 1. It is likely, therefore, either that the missing products were never formed or that they died early in development. Survival was not determined by mating type, as all four classes occurred with approximately equal frequencies among the incomplete tetrads in each sample. Published figures (4) suggest that the frequency of tetrads in *U. maydis* with at least three surviving products may be quite low (10-30%) from crosses carrying induced markers. Since all such tetrads will normally possess compatible cells, their frequency can be estimated in the two samples by correcting for the number of tetrads with two compatible surviving products. This is equal to 50% of pairs selected from tetrads ditype for *a* and *b* and 25% of pairs from tetratype tetrads. The frequency would certainly be no larger than the observed number of incompatible pairs plus the number of monotypic tetrads, some of which could be misclassified incompatible pairs. Such a calculation shows that more than 95.6% of sample A and 87.2% of sample B tetrads had three or four surviving products. The low rates of survival reported by Holliday (4) are most likely due to the presence of auxotrophic markers which are known to reduce viability.

The rates of recovery of uniformly mycelial colonies from teliospores of the two samples were similar (0.43% and 0.26%). All such mycelial lines were solopathogenic in corn plants and they are considered to be unreduced diploids. The teliospores that they produced gave normal frequencies of mosaic colonies in the next generation.

Seven yeastlike teliospore colonies from sample A each contained only one cell type compatible with both *a* testers. Five of these were compatible with one of

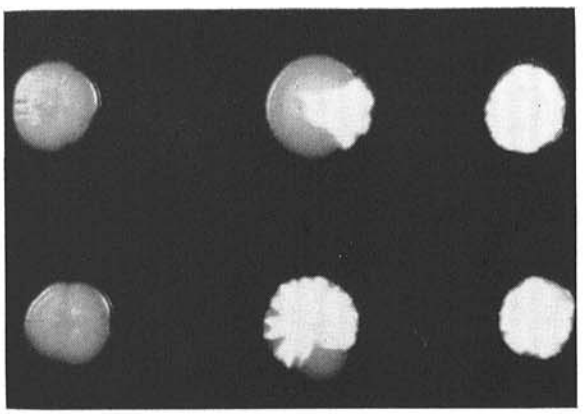

Fig. 1. Colonies of *Ustilago maydis* from single teliospores. Mosaic colonies (center) result when aerial infection hyphae are formed by mating between compatible meiotic products. Entirely yeastlike colonies (left) contain only incompatible cells. Mycelial colonies (right) are made up of unreduced diploid cells. (×2)

the *b* factor testers and two with the other. None of these cultures was solopathogenic. A DNA determination (3) made on one of them indicated a diploid DNA level (11.0 μg/10^8 cells). They are, therefore, considered to be diploid and homozygous for one or the other of the *b* factors. Two simple theories could account for their origin: (i) Crossing over between *a* and its centromere at the first division of meiosis was followed by restitution after the second division, resulting in two diploid nuclei, one of which failed to survive among the germination products; and (ii) mitotic crossing over occurred before teliospore formation, and teliospores homozygous for *b* germinated but failed to undergo meiosis.

We favor the second theory. If the first is correct, we might have expected to recover some $a{=}b{\neq}$ diploids among the mycelial class of sample A, as synthesized diploids of this constitution are generally mycelial (7). In the tests reported above, none gave rise to nonmosaic colonies alone in the next generation, the expected behaviour of $a{=}b{\neq}$ diploids. Only $a{\neq}b{=}$ diploids were found. Elsewhere (1) we report that the controlling action of *b* extends beyond gall and teliospore formation to meiosis and basidiospore formation at germination. Since one of the functions of *b* is the regulation of meiosis, we would expect that teliospores homozygous for *b* could not undergo meiosis at germination. Five of the $a{\neq}b{=}$ diploids carry one *b* factor and two the other, so that all seven could well be derived from the same mitotic event. The theory we prefer presupposes that the pathogen forms diploid nuclei which divide mitotically before teliospores are formed. Ehrlich (2) claimed to have seen diploid nuclei in developing galls. The ready recovery of diploid cells from fragments of immature gall tissue also points to their occurrence (5).

TABLE 1. Numbers of colony types from single teliospores of *Ustilago maydis*

Colony types	Samples A	Samples B
Yeastlike colonies		
One cell type	17	24
Two cell types	7	0
Diploid ($a \neq b =$)	7	0
Mycelial colonies		
Diploid ($a \neq b \neq$)	6	1
Mosaic colonies	1,357	358
Total	1,394	383

Mitotic recombination in *U. maydis* is well established from the study of diploids carrying auxotrophic markers in culture (5). Kozar (6) used similar markers and recently reported mitotic recombination in *U. hordei* which occurred in host tissue. Our evidence, although not conclusive, is unusual in that no induced markers were used. Our method of analysis does not restrict us to laboratory stocks, but provides a way of directly extrapolating from laboratory to field material.

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