Inheritance in Cochliobolus sativus

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ABSTRACT

Cochliobolus sativus was single-spored from asci by a feather needle technique and analyzed for genetic systems using tetrad analysis and randomly isolated ascospores. Segregation for mating type was regular and controlled by one gene. In the tetrad analysis of eight asci, colony color, colony size, and conidial production were each additive and controlled by at least two genes. Additional modifiers of the above traits were detected. Virulence to spring wheat, durum, and barley was controlled by at least two genes which were associated with dark colony color. Genes for virulence separated from genes for dark color by apparent crossing over, and were epistatic over genes for avirulence. In concurrent studies, using 200 randomly isolated ascospores, two genes were associated with virulence to spring wheat and durum, and three to four genes with virulence to barley. Sexual reproduction was prevented in progeny from one cross by a pre-crozier block (controlled by three genes), a post-crozier block, and a preascospore block. White and red strains of the fungus developed from sectors in black colonies from single ascospores. Red strains did not reproduce sexually. Varying sexual fertility, virulence and avirulence, and both mating types were found in several parts of North America.

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Cochliobolus sativus (Ito & Kurib.) Drechs. ex Dastur is a worldwide fungal pathogen of wheat, barley, and other grasses (2). It has been crossed with at least two related fungi (8). The analysis of its genetics has been hindered by multinucleate, heterokaryotic conidia and hyphal cells, and filamentous intertwining ascospores (1, 4, 10). Nuclei of individual ascospores develop from one nucleus (9), and six to eight chromosomes are evident during meiosis and mitosis in the ascus (5, 6). Tinline (11, 12) reported that sexual recombination produced new virulent strains, and that mutation in monoascosporic isolates produced new strains that differed from the original in conidial color, conidial production, and virulence. Inheritance was complicated by parasexuality and the occasional occurrence of diploid mycelium and conidia. Sexual reproduction was undetected in nature. White conidial color was recessive to tan (12). Conidial color was unrelated to pathogenicity (13).

Using randomly isolated ascospores, Tinline and Dickson (14) found mating type, "A" and "a", and conidial color, white and olivaceous, to be controlled by single allelic genes and not linked. However, among 29 analyzed tetrads segregating for white and olivaceous color and mating type they obtained "23 tetratypes, two parental ditypes and four nonparental ditypes" and postulated a complex inheritance. Some monoascosporic isolates mated with both "A" and "a" mating types. Tinline et al. (15) examined tetrads from cross of weakly virulent, poorly sporulating, white-spored strains and highly virulent, densely sporulating, dark-spored strains. The progeny divided into four groups on the basis of spore color and sporulation: (i) white conidia, abundant sporulation, (ii) white conidia, sparse sporulation, (iii) dark conidia, abundant sporulation, and (iv) dark conidia, sparse sporulation. Since all progeny were highly virulent, they concluded that virulence is quantitatively inherited.

Using cultures derived from 63 randomly isolated ascospores of a pathogenic × non-pathogenic cross, Kline and Nelson (7) found that single genes controlled pathogenicity to five grass species, and that two genes controlled pathogenicity to a sixth species. The seven different genes were not independent.

The above studies indicated both simple and complex inheritance of some traits in *C. sativus*. The objective of this study was to use tetrad analysis to clarify inheritances.

MATERIALS AND METHODS.—Isolates of *C. sativus* were obtained from diseased plants from different localities in North Dakota and from colleagues in Canada, Mexico, and several states of the United States. Monoconidial cultures of these isolates were maintained on potato-dextrose agar (PDA). Mating type or compatibility group was designated "A" or "a" by mating an isolate with either of two original isolates 18(a) and 20(A) received from R. D. Tinline. The sexual stage of the fungus was produced by the method of Tinline and Dickson (14).

Ascospores for tetrad analysis were isolated by removing pseudothecia from culture and rolling them on 3% water agar to clean them of conidia and other debris. They were then rinsed three times in sterile distilled water, and each pseudothecium was transferred to a small drop of water on a glass microscope slide where it was crushed

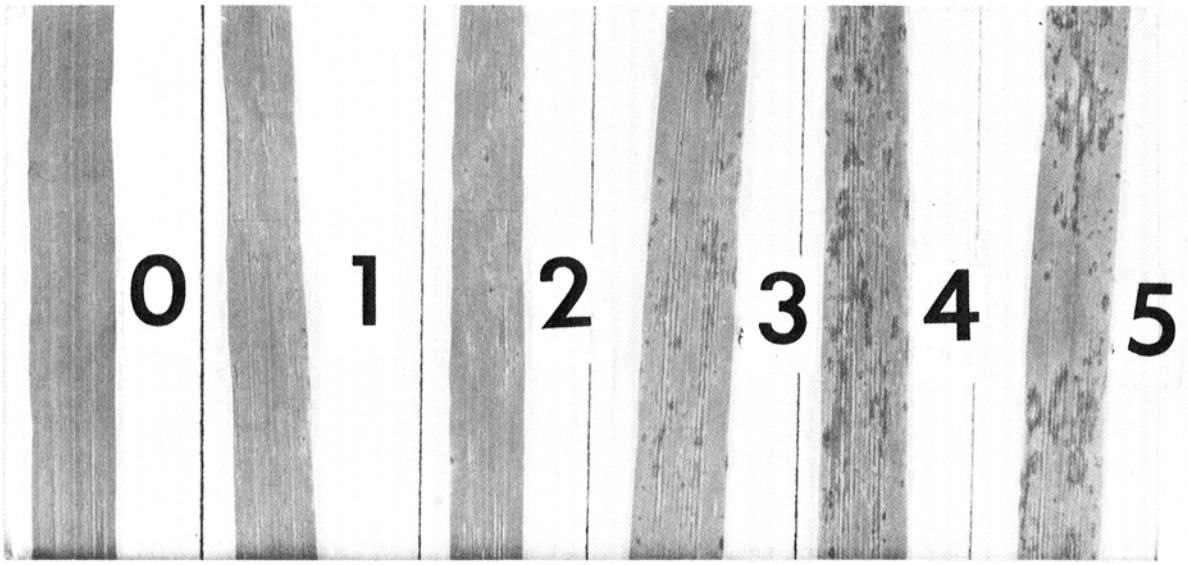

Fig. 1. Wheat leaves inoculated with conidia from single ascospore cultures of *Cochliobolus sativus*, showing virulence types of the fungus. Rating system: (0-2) no lesions to a few small lesions—*avirulent* (v), (3-5) large necrotic spots with chlorotic halos to larger blotches covering considerable surface area and having chlorotic halos-virulent (V). Lesions on barley are similar.

gently with a fine steel needle. Individual asci were separated from the crushed pseudothecium and transferred to a small drop of water on a separate slide, using a needle made from a longitudinal section of the central rib of a large turkey feather (Gerald Thorne, *personal communication*). Ascospores were extruded from the individual asci by pressing and/or puncturing the ascus with the fine steel needle. Using the feather needle, spores were separated and each transferred to a petri dish of PDA (0.5% agar).

For the inheritance studies, cultures 1 and 6 were each obtained from single conidia in colonies from leaf spots on wheat in North Dakota. Culture 1 was dark, virulent on all tested cereals, and of the "A" mating type. Culture 6 was dark, virulent on all tested cereals, and of the "a" mating type. These cultures were mated, and from this mating single-ascospore cultures 38 and 40 were obtained. When crossed, cultures 38 × 40 produced single-ascospore cultures 16, 47, 177-184, 241-248, and 281-288. Cultures 183 × 184 produced single-ascospore cultures 301-308 and 309-316. Dark culture 16 produced a white true breeding sector, 16M. Cultures 16M × 47 produced single-ascospore cultures 401-408, 409-416, and 417-424 and eight colonies from each of two more asci. Eight consecutive numbers represent the eight ascospores in an ascus. The cultures are described as needed in the Results and Discussion section. All cultures were grown on PDA.

TABLE 1. Ratios of virulence-avirulence and colony color in progeny of three crosses within *Cochliobolus sativus*

Cross	Ascus no.	Progeny characteristics[a] [Ratios of phenotypes in each ascus (8 spores per ascus)] VB	VD	VW	vD	vW
38 × 40 (VD) (VD)	1,2,3	2	4	...	...	2
183 × 184 (vW) (VB)	4	2	4	...	...	2
	5	1	5	...	...	2
16M × 47 (vW) (VD)	6	2	2	...	...	4
	7,8	...	2	2	2	2

[a] V = virulent, v = avirulent, B = very dark black, D = dark, W = white. Virulence vs. avirulence was tested on barley cultivar Larker, barley selection B112, spring wheat cultivars Chris and Justin, and durum wheat cultivar Wells.

Pathogenicity in the fungus was studied in the glasshouse. Conidial concentration, temperature, relative humidity, and incubation period were selected from ranges of these factors to produce severe spotting on susceptible hosts, and extremely light spotting on resistant hosts. Inoculum was obtained by growing isolates of the fungus in petri plates on PDA containing 2% agar. After 7 days of growth at 25 C, conidia and mycelium were scraped from the PDA in water suspension and filtered through cheesecloth. The concentration of conidia was adjusted to approximately 5,000 conidia per ml using a colorimeter and hemacytometer. One drop of Tween 20 was added to 100 ml of spore suspension, and eight 10-day-old seedlings of wheat or barley in each of 10 pots were sprayed with the spore suspension, using a sprayer with a fine nozzle. Control plants were inoculated with water only. Barley cultivar, Larker; barley selection, B112; spring wheat cultivars, Chris and Justin; and the durum cultivar, Wells, were used throughout the studies. These selections were chosen because B112 had displayed resistance and Larker susceptibility to *C. sativus* in the field (A. E. Foster, *personal communication*) and Chris, Justin, and Wells were major commercial cultivars in North Dakota. They were grown in an autoclaved soil mixture (2 parts Fargo clay, 1 part sand, and 1 part peat moss, v/v) in 15.2-cm (6-inch) diameter clay pots. The inoculated plants were

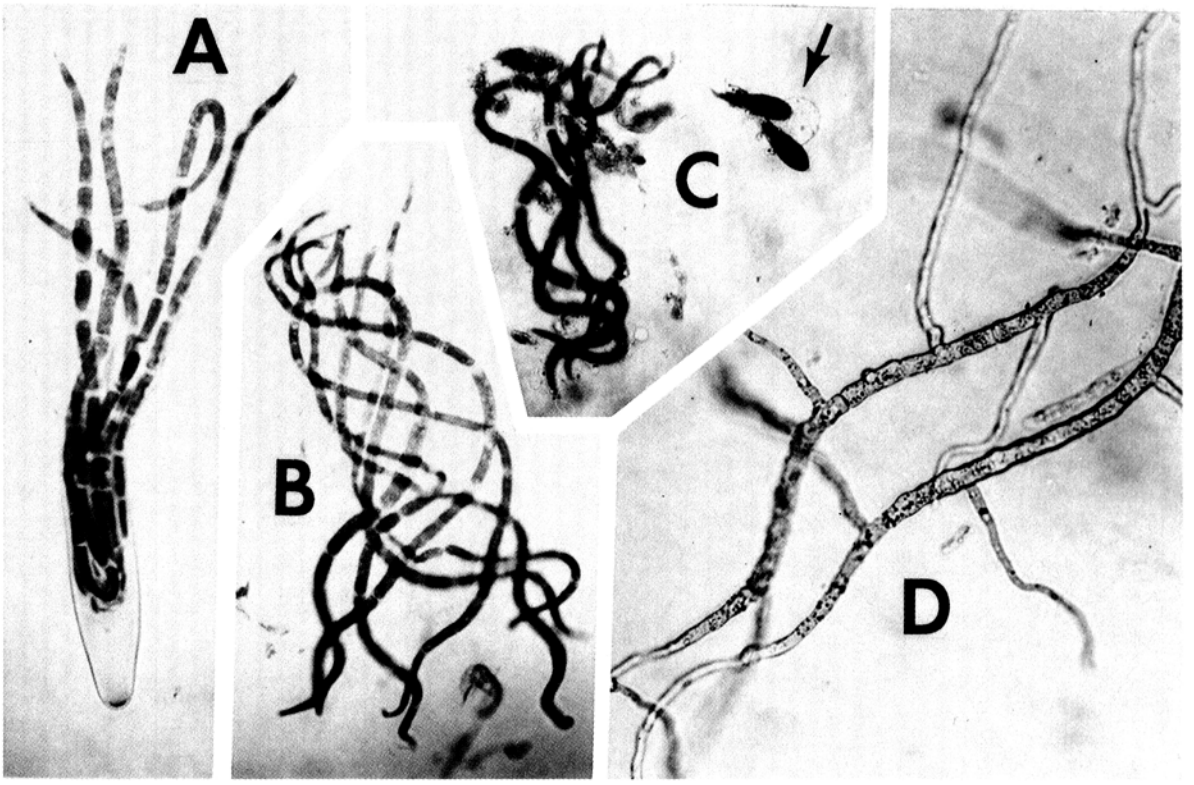

Fig. 2-(A to D). Ascospores of *Cochliobolus sativus:* **A)** three in an ascus × 255. **B)** the seven from an ascus × 255. **C)** the six from an ascus, four long and filiform, two short and thick (indicated by arrow) × 255. **D)** two germinating from all cells × 816.

TABLE 2. Pathogenicity in cultures of *Cochliobolus sativus* from 200 randomly selected single ascospores of the cross virulent, dark 38(a) × virulent, dark 40(A)

			Larker barley	B112 barley	Chris spring wheat	Justin spring wheat	Wells durum
Observed no.	(v:V)[a]		12:188	20:180	44:156	58:142	43:157
Ratio	(v:V)		1:15	1:7	1:3	1:3	1:3
$P(\chi^2)$	(v:V)	$P >$	.75	.25	.25	.10	.25

[a] v = avirulent; V = virulent.

incubated for 24 hours at 21 ± 2 C in a mist chamber kept at 100% relative humidity by maintaining free water on the leaves. The chamber was covered by a transparent plastic sheet that permitted sunlight to enter. Following incubation, the plants were placed on a glasshouse bench maintained at 21 ± 3 C. The fungal isolates were evaluated for their actions on hosts one week after inoculation using the scale illustrated in Fig. 1.

RESULTS AND DISCUSSION.—Unmated dark isolates of *C. sativus* often produced structures resembling dark protothecia; unmated white isolates always produced white structures resembling protothecia. Asci from crosses contained two-to-eight ascospores with the most common number being eight. The ascospores usually were filiform, hyaline, many septate, and pointed at both ends (Fig. 2-A to D). Each cell of an ascospore was capable of germination (Fig. 2-D). Occasionally short, thick, nongerminating, single-celled spores (see arrow, Fig. 2-C) were formed along with the filiform spores. This was similar to the findings of Tinline (10). We did not detect his postulated (12) anastomosis between ascospores in the asci.

Segregation for mating type was regular in all crosses, including back crosses of the eight progeny from an ascus of 38(a) × 40(A) and eight from an ascus of 183(A) × 184(a) with their parents. Mating was controlled by one gene pair.

Virulence and color were simply inherited in the progeny from eight tetrads (Table 1). Progeny from ascus 1 of the cross between the two virulent, dark single ascospore isolates 38(a) and 40(A) segregated in ratios of one virulent, very dark (jet black) colony to two virulent, dark (black, but differing in color intensity), colonies to one avirulent, white colony. In progeny of asci 2 and 3

from the same cross, the results were the same, except that the moderately dark colonies did not differ in color intensity. These segregations indicated that pathogenicity was controlled by at least two genes, and that virulent genes were epistatic over avirulent genes. Colony color was additive, and controlled by at least two genes. Dark colony color was linked to virulence. One exception occurred on Chris wheat where the eight isolates from ascus 1 segregated in a ratio of one very dark and one dark virulent colony to one dark and one white avirulent colony. Since this did not happen with asci 2 and 3, this suggested the loss of a gene for virulence. When avirulent, white 183(A) and virulent, very dark, 184(a), from the cross 38(a) × 40(A), were crossed, their offspring from ascus 4 segregated in a ratio of one virulent, very dark colony to two virulent, dark colonies to one avirulent, white colony. Their offspring from ascus 5 segregated in a ratio of one virulent, very dark colony to five virulent, dark colonies to two avirulent, white colonies. These segregations further supported the inference of two-gene inheritances indicated by the colonies from asci 1, 2 and 3. The ratio of one very dark to five dark colonies from ascus 5 suggested an inherited modifier of color in addition to the two proposed genes. When two other colonies of 38(a) × 40(A), avirulent white 16M(A) and virulent dark 47(a) were crossed, their offspring from one ascus (number 6) segregated in ratios of one virulent, very dark colony t one virulent, dark colony to two avirulent, white colonies. Their offspring from two other asci (numbers 7 and 8) segregated in ratios of one virulent, dark to one avirulent, dark to one virulent, white to one avirulent, white. These segregations also support the concept of two-gene inheritances of pathogenicity and color, and indicated in the progeny of asci 7 and 8 that virulence could unlink from dark colony color. Among the progeny from ascus 7, for some unexplained reason, culture 411 was virulent on Wells durum and culture 413 was avirulent, a reversal of their pathogenicity on the four other tested cereals.

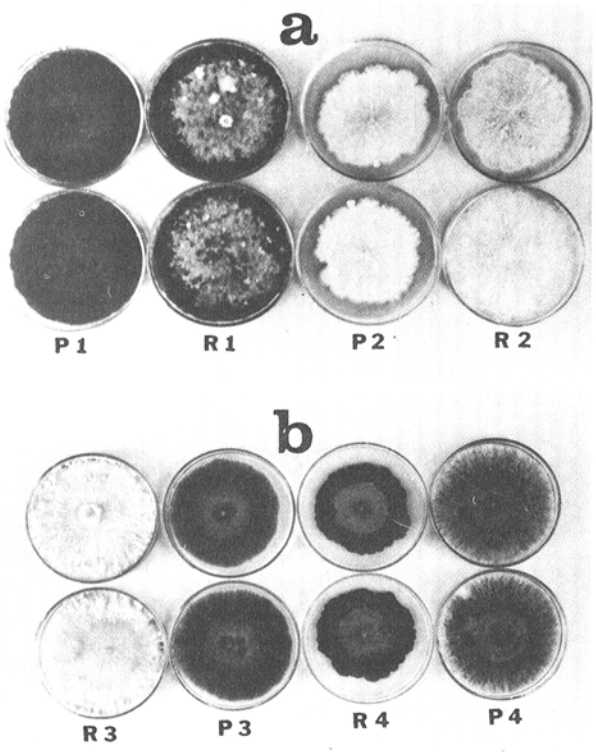

Fig. 3-(A, B). Colony characteristics among the eight single ascospore isolates of asci from two crosses within *Cochliobolus sativus*. "P" is parental type. "R" is recombinant type. **A)** from cross 16M(A) × 47(a): Pl-dark colony, prostrate mycelium, many conidia. Rl-dark colony, white aerial mycelium, several conidia. P2-white colony, prostrate mycelium, many conidia. R2-white colony, upright mycelium, several conidia. **B)** from cross 38(a) × 40(A): R3-white colony, fluffy upright mycelium, very few conidia. P3-dark colony, scant upright mycelium, many conidia. R4-very dark colony, prostrate mycelium, very many conidia. P4-dark colony, sparse upright mycelium, several conidia.

Concurrent pathogenicity studies with 200 randomly isolated ascospores from the cross 38(a) × 40(A) resulted in a ratio of one avirulent to three virulent on Chris and Justin spring wheats and Wells durum, indicating control by two genes (Table 2). On B112 barley there was a ratio of one avirulent to seven virulent, indicating involvement of three genes. On Larker barley, there was a ratio of one avirulent to 15 virulent, indicating involvement of four genes (Table 2). This suggested that two-to-four genes control pathogenicity to cereals. Our tetrad sample consisted of 64 isolates and our random sample of 200 isolates derived from the same progenitors. It is puzzling that they should both indicate the presence of two genes for pathogenicity to wheats, but differ with regard to the barleys. Perhaps, our tetrad sample was too small, or our random sample unknowingly selective, or something else was happening that our hypothesis did not encompass.

Colony-size and conidial production in the tetrad offspring of 16M(A) × 47(a) segregated in ratios of one dark, conidial, parental type colony, to one dark, less-conidial recombinant type colony to one white, small, dense, conidial parental type colony, to one less-white, slightly larger, less-conidial recombinant type colony (Fig. 3-a). In the progeny of 38(a) × 40(A) segregation occurred in a ratio of one large, white, more-mycelial recombinant type colony to one smaller, dark, conidial, parental type colony, to one very dark, small, dense, conidial, recombinant type colony to one dark, slightly larger parental type colony (Fig. 3-b). This indicated that colony size and production of conidia were additive and controlled by at least two genes, and that production of conidia was not linked to colony color. These findings are similar to those of Tinline et al. (15); however, they found no avirulence in their progeny (from virulent × avirulent crosses) while avirulence occurred in some of the above progeny.

The above segregations indicated that the parental ascospores of this sampling were haploid and could not be diploid. Variation in the lighter shades of dark color and colony size, and the many different variants detected in culture (1) suggested that in the population from which this sample was taken, additional genes modifying colony color and size exist.

Progeny of the cross 16M(A) × 47(a) were not sexually fertile when crossed in all possible combinations among

TABLE 3. Indications of genetic blocks to sexual development, in addition to "a" and "A" mating types in crosses within *Cochliobolus sativus*

	Ratio of structure produced to no structure produced among 32 potentially fertile matings in each column[a]			
Sexual Structures	A	B	C	D
Ascospores	0:32	0:32	0:32	32:0
Asci	2:30	4:28	4:28	32:0
Croziers	3:29	4:28	5:27	32:0
Ratio (three-gene control)	1:7	1:7	1:7	...
Probability	> 0.5	> 0.99	> 0.5	...

[a] In column A, the eight single-ascospore isolates from an ascus from the cross 16M(A) × 47(a) were mated among themselves in the 64 possible combinations. In B, the eight isolates of A were mated in the 64 possible combinations with eight isolates of a second ascus from 16M × 47. In C, the eight isolates of A were mated in the 64 possible combinations with eight single ascospore isolates from an ascus from the cross 38(a) × 40(A). In D, the eight isolates from 38 × 40 were mated among themselves in the 64 possible combinations.

themselves, or with other fertile isolates. Histological examination of these crosses revealed genetic blocks to sexual development prior to crozier formation (controlled by three genes), prior to ascus formation and prior to ascospore formation (Table 3). Developmental blocks in addition to mating type, have been detected in other fungi (3), and sterility unrelated to mating type has been observed in *C. sativus* (9, 12).

Randomly throughout the current study dark (black) colonies from single ascospores developed white (and sometimes red) true breeding sectors, similar to sectoring observed by earlier workers (9, 12). The red strains did not reproduce sexually. The white strains often attenuated in culture. This suggested mutations or deletions of chromatic material from haploid genomes and indicated high instability in the genes or replication procedures of this fungus on artificial medium.

In mating of all possible combinations of 17 isolates from Canada, eleven states of the United States and Mexico, mating types "a" and "A" were found together in local areas and present in different parts of North America. Fertility of these matings varied from a few fertile pseudothecia to many fertile pseudothecia without evident bias for place of origin. These findings were similar to those of Tinline with isolates from Canada and northern United States (10, 12). This variation in fertility and the aforementioned blocks to reproduction indicated that factors, in addition to mating type, are limiting sexual reproduction. In addition light inhibited (14) or blocked (Hosford et al. *unpublished*) sexual reproduction. The virulence or avirulence of the 17 isolates (strains) indicated that both characteristics were present in the different parts of North America. This was similar to the findings of Wood for the United States (16).

Our results show that in *C. sativus* pathogenicity to spring wheat and durum is controlled by at least two genes. Pathogenicity to barley is controlled by at least two-to-four genes. Virulence is epistatic over avirulence. Colony characteristics (color, size, and sporulation) are additive and each is controlled by at least two genes, with indications of additional modifiers. Mating type is controlled by one gene, but reproduction can be blocked by additional genes. Differing strains frequently develop from supposedly haploid single-ascospore colonies. Within North America no individual traits appeared to be limited to any geographical region.

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