Effect of Inbreeding on Pathogenicity in Race 8 of *Ustilago hordei*

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ABSTRACT

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Race 8 of *Ustilago hordei*, is pathogenic on barley cultivar Odessa, but not on the other seven covered smut differential cultivars. Selfing of an ordered tetrad (isolate K, race 8 of *U. hordei*) produced a dikaryon (K-1, 1 × 4) pathogenic on the barley cultivars Odessa and Hannchen. The four sporidial lines also were testcrossed with virulent lines and these matings were pathogenic only on Odessa. The sporidial matings from a selfed teliospore of isolate K-1, 1 × 4 from Hannchen all were pathogenic on cultivars Odessa, Hannchen, Lion, and Trebi. The second generation selfs of isolates K-1, 1 × 4 also were pathogenic on the same four cultivars. By analysis of F_1, F_2, and testcross data, three single recessive gene pairs were identified in isolate K-2-8 that condition pathogenicity on cultivars Hannchen, Lion, and Trebi. The genes for Lion and Trebi were linked with 13.1% recombination between the loci. A hypothesis involving a regulator gene is proposed for the increase in pathogenicity associated with the inbreeding of race 8 of *U. hordei*.

Ustilago hordei (Pers.) Lagerh. causes covered smut of barley. Teliospores of *U. hordei* germinated on potato dextrose agar (Difco) form four haploid sporidia (8), which may be segregated sequentially with a micromanipulator for tetrad analysis. Mating type is bipolar (1,8) and when the four sporidia from one tetrad were mated in all combinations, only four of the matings formed the dikaryotic infection hyphae that are required to infect barley seedlings (8,11,27).

The term physiologic race was used first to describe

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pathogenically distinct dikaryotic uredial rust clones. These rust clones can be maintained indefinitely without passing through meiosis; thus, they remain relatively stable even though they are heterozygous at one or more pathogenicity loci. However, germinating smut teliospores must pass through a meiotic phase. If both nuclei do not contain identical genes for pathogenicity, teliospore isolates may not be regarded as stable physiologic races.

Within the eight smut differential barley cultivars, race 8 of *U. hordei* is pathogenic only on cultivar Odessa, which is susceptible to all known races of the fungus (24). Jensen (10) reported that repeated inbreeding of race 8 increased its pathogenicity on the other seven differential cultivars in a stepwise progression. One mating from a tetrad of race 8 produced smut sori on plants of resistant cultivar Hannchen. Teliospores obtained from that infected Hannchen plant were selfed, and the matings were pathogenic on four additional cultivars: Nepal, Lion, Pannier, and Trebi. Subsequent selfing of the teliospores from these four cultivars produced dikaryons that were pathogenic on all eight differential cultivars. Jensen (10) attributed this increase in pathogenicity to parental heterozygosity at several loci.

The original cultures were lost and this hypothesis could not be tested. This study was undertaken to test the hypothesis that the pathogenicity of *U. hordei* on barley could be increased by selective inbreeding of race 8 of *U. hordei*, and to investigate the mechanisms involved.

TABLE 1. The origins and differential pathogenicities of the isolates of *Ustilago hordei* and *Ustilago nigra* used in this study

Isolate	Origin	Pathogenicity on differential cultivars
1279[a]	Second self of race 8, *U. hordei*	All except Jet
1727[a]	Second self of race 8, *U. hordei* (10)	Odessa only
1874[b]	Second self of race 6, *U. nigra* (5)	Hannchen, Himalaya, Lion, Odessa, Trebi, and Jet
K	Teliospore from race 8, *U. hordei*	Odessa only
K-1	First self of race 8, *U. hordei* mating 1 × 2	Odessa and Nepal
K-2	First self of race 8, *U. hordei* mating 1 × 4	Odessa and Hannchen
K-2-8	Second self of race 8, *U. hordei*	Odessa, Hannchen, Lion, and Trebi

[a]Teliospore cultures developed by L. L. Jensen.
[b]Teliospore culture developed by L. Darlington.

MATERIALS AND METHODS

Teliospores of race 8 of *U. hordei* and seed stocks of the eight differential barley cultivars originally were obtained from V. F. Tapke and are maintained at North Dakota State University, Fargo. An additional cultivar, Jet (CI 967), was added to the list of differential cultivars (18). Seed of this cultivar was obtained from the World Barley Collection of the United States Department of Agriculture. These nine cultivars will be referred to as the differential cultivars throughout this paper. The cultures of *U. hordei* and *U. nigra* used in this study are listed in Table 1.

The differential cultivars were inoculated with teliospores of race 8, *U. hordei* by employing the following procedure:

(i) Seeds of all hulled cultivars were hand dehulled, soaked in water for 2 hr and placed in petri dishes lined with moist filter paper. The petri dishes were wrapped in aluminum foil and incubated at 20 C for 24 hr.
(ii) Seeds of all hulless cultivars were soaked and incubated under the same conditions as the hulled cultivars, except after 24 hr the pericarp was removed with a sharp forceps.
(iii) Following the 24-hr germination period, teliospores were placed on the exposed coleoptiles of nine seedlings per cultivar with a sterile artist's paint brush.
(iv) Petri dishes containing the seedlings were rewrapped in aluminum foil and incubated at 20 C for an additional 48 hr.

The incubated seedlings were transplanted into 15-cm diameter clay pots filled with an autoclaved mixture of sand, soil, and peat moss (1:1:1, v/v). The plants were maintained in a greenhouse at 22 C until maturity. At harvest, any plant having at least one smutted head was considered susceptible. The percentage of diseased plants was obtained by dividing the number of infected plants by the total number of inoculated plants.

Teliospores of race 8 also were germinated on 3% potato dextrose agar and the four primary sporidia were sequentially isolated from each teliospore with a Leitz micromanipulator. Twenty-one ordered tetrads were obtained and designated A through U. After the four sporidia were separated from the promycelium, they were allowed to form haploid colonies. These sporidical colonies were transferred to V-8 juice agar (16). This medium was used for all sporidial crossings and for the storage of sporidial cultures. The cultures were transferred to new agar plates every 3 mo. Both sporidial and teliospore cultures were stored at 5 C.

Selfing is the mating of the four sporidial cultures derived from one ordered tetrad in the four compatible combinations. These four sporidial cultures will be referred to as an ordered tetrad throughout this paper. Mating type was determined for each sporidial culture via the modified Bauch test (16). Barley seedlings

TABLE 2. The percentage of diseased plants of five differential barley cultivars following inoculation with selfed ordered tetrads from isolates of race 8, *Ustilago hordei*

Isolate designation	Sporidial mating	Diseased plants of indicated differential cultivars (%)[a]					Isolate accession number
		Hannchen	Nepal	Lion	Odessa	Trebi	
K	1 × 2	0	8	0	66	0	K-1
	1 × 4	8	0	0	71	0	K-2
	3 × 2	0	0	0	66	0	K-3
	3 × 4	0	0	0	87	0	K-4
K-2 (Odessa)	1 × 2	0	0	0	89	0	K-2-1
	1 × 3	0	0	0	87	0	K-2-2
	4 × 2	0	0	0	78	0	K-2-3
	4 × 3	0	0	0	56	0	K-2-4
K-2 (Hannchen)	1 × 3	100	0	66	80	89	K-2-5
	1 × 4	100	0	44	100	89	K-2-6
	2 × 3	100	0	89	77	89	K-2-7
	2 × 4	100	0	77	100	100	K-2-8

[a]The differential cultivars, Jet, Excelsior, Himalaya, and Pannier, were resistant to the isolates of *U. hordei*.

were inoculated with sporidial cultures by means of the paint-brush technique (10). The inoculated seedlings, also nine per cultivar, were handled as previously described for teliospore inoculations.

Teliospores from the 1 × 2 mating of teliospore K were collected from infected Nepal and Odessa plants and were designated K-1 (Nepal) and K-1 (Odessa), respectively. Teliospores also were obtained from the dikaryon 1 × 4 of teliospore K from infected Hannchen and Odessa plants and were designated K-2 (Hannchen) and K-2 (Odessa). One ordered tetrad from each of the following lines, K-1 (Nepal), K-1 (Odessa), K-2 (Hannchen), and K-2 (Odessa), was selfed and pathogenicity was tested on the differential cultivars. Teliospores from Hannchen, Lion, Odessa, and Trebi plants infected with K-2 (Hannchen) were designated K-2-8 plus the cultivar name and one ordered tetrad from each cultivar was selfed. Pathogenicity again was tested on the differential cultivars. Isolates K-1 (Nepal), K-1 (Odessa), K-2 (Hannchen), and K-2 (Odessa) also were tested for pathogenicity via teliospore inoculations.

Sporidial cultures from the ordered tetrad of teliospore K were testcrossed to sporidial cultures from isolate 1279, race 8, *U. hordei* and from isolate 1874, race 6, *U. nigra* (Table 1). The pathogenicity of these matings was determined by inoculating the differential cultivars.

An ordered tetrad from isolate K-2-8, pathogenic on the cultivars Hannchen, Lion, Trebi, and Odessa, was crossed in all possible matings with two sporidia of opposite mating type from isolate 1727, an inbred line of race 8 pathogenic only on Odessa. These dikaryons were designated W, X, Y, and Z. Two ordered tetrads and 30 random sporidia from the cross K-2-8 × 1727 were testcrossed to K-2-8. F_2 and testcross segregation ratios were used to determine the number of genes conditioning pathogenicity on the cultivars Hannchen, Lion, and Trebi. Ratios of the parental genotypes and recombinants were used in the mapping of these pathogenicity genes.

RESULTS

Only the barley cultivar Odessa developed smut sori when inoculated with teliospores of race 8. Twenty-one complete ordered tetrads from teliospores A through U were selfed and only two of 84 matings were able to infect any cultivars except Odessa. The mating 1 × 2 from teliospore K was pathogenic on Nepal and Odessa, while 1 × 4, also from teliospore K, was pathogenic on Hannchen and Odessa (Table 2). Two selfed tetrads from teliospores produced by the 1 × 2 mating on Nepal and Odessa, one from each, were repeatedly pathogenic only on Odessa. The two teliospores from the cross 1 × 4, designated K-2 (Hannchen) and K-2 (Odessa), also were selfed and the four matings from K-2 (Odessa) were pathogenic only on Odessa. However, matings from K-2 (Hannchen), designated K-2-5 through K-2-8, all were pathogenic on Hannchen, Lion, Odessa, and Trebi, but repeatedly nonpathogenic on the other five differential cultivars (Table 3).

Teliospore inoculations with isolates K-1 (Odessa) and (Nepal) and isolate K-2 (Odessa) were repeatedly pathogenic only on Odessa (Table 4). Teliospores from isolate K-2 (Hannchen) were pathogenic on both Hannchen and Odessa and teliospores from K-2-8 (Odessa) were pathogenic on Hannchen, Lion, Odessa, and Trebi.

All of the testcrosses between sporidial cultures from teliospore K and sporidial cultures from isolates 1279, *U. hordei*, and 1874, *U. nigra*, were pathogenic only on Odessa.

All F_1 matings between isolates K-2-8 and 1727, *U. hordei*, were also pathogenic only on Odessa. When tetrads from teliospores W-2 and Z-2, progeny from the cross K-2-8 × 1727, were selfed, the matings segregation was in 1:3 ratios for pathogenicity on

TABLE 3. The percentage of diseased plants of four differential barley cultivars inoculated with selfed, ordered tetrads from teliospores of isolate K-2-8, race 8, *Ustilago hordei*

Isolate designation	Sporidial mating	Diseased plants of indicated differential cultivars (%)[a]			
		Hannchen 532	Lion 923	Odessa 934	Trebi 936
K-2-8 (Hannchen)	1 × 2	66	50	82	50
	1 × 3	75	17	67	75
	4 × 2	75	50	82	45
	4 × 3	88	33	89	33
K-2-8 (Lion)	1 × 2	100	33	100	89
	1 × 3	100	22	77	89
	4 × 2	89	22	100	89
	4 × 3	100	22	100	100
K-2-8 (Trebi)	1 × 2	100	22	100	66
	1 × 3	77	44	100	77
	4 × 2	100	55	100	100
	4 × 3	100	33	89	100
K-2-8 (Odessa)	1 × 2	100	33	77	77
	2 × 4	89	22	100	77

[a]The other differential cultivars, Nepal, Jet, Excelsior, Himalaya, and Pannier, were resistant to the isolates of *U. hordei*.

TABLE 4. The percentage of diseased plants of four differential barley cultivars inoculated with teliospores from isolates K-1, K-2, and K-2-8 race 8, *Ustilago hordei*

Isolate designation	Source[a]	Diseased plants of indicated differential cultivars (%)[b]			
		Hannchen	Lion	Odessa	Trebi
K-1	Odessa	0	0	60	0
K-1	Nepal	0	0	30	0
K-2	Odessa	0	0	33	0
K-2	Hannchen	20	0	30	0
K-2-8	Odessa	77	44	78	42

[a]The teliospores were obtained from the infected plants of the differential cultivar indicated.

[b]The differential cultivars, Nepal, Jet, Excelsior, Himalaya, and Pannier, were resistant to the isolates of *U. hordei*.

TABLE 5. The percentage of diseased plants of four differential barley cultivars inoculated with four F_1 matings of *Ustilago hordei* K-2-8 × 1727 and the selfed tetrads from teliospores produced by these matings

Isolates	Sporidial matings	Diseased plants of indicated differential cultivars (%)[a]				Isolate accession number
		Hannchen	Lion	Odessa	Trebi	
K-2-8 × 1727	1 × 2	0	0	80	0	W
	2 × 1	0	0	88	0	X
	3 × 1	0	0	75	0	Y
	4 × 2	0	0	80	0	Z
W-1	1 × 2	0	0	60	0	
	1 × 4	33	17	100	17	
	3 × 2	0	0	83	0	
	3 × 4	0	0	33	0	
W-2	1 × 2	0	0	100	0	
	1 × 3	20	0	66	0	
	4 × 2	0	0	83	0	
	4 × 3	0	17	100	33	
Z-1	1 × 2	33	17	100	80	
	1 × 3	0	0	60	0	
	4 × 2	0	0	100	0	
	4 × 3	0	0	50	0	
Z-2	1 × 3	0	0	100	0	
	1 × 4	50	0	50	50	
	2 × 3	0	0	100	0	
	2 × 4	0	17	100	0	

[a]The differential cultivars, Nepal, Jet, Excelsior, Himalaya, and Pannier, were resistant to the isolates of *U. hordei*.

Hannchen, Lion, and Trebi (Table 5). Two ordered tetrads and 30 random sporidia from isolates W-2 and Z-2 segregated in 1:1 ratios for pathogenicity on Hannchen, Lion, and Trebi when testcrossed to sporidial cultures from isolate K-2-8 (Tables 6 and 7).

Assuming three single and independent gene pairs conditioning pathogenicity on Hannchen, Lion, and Trebi, distances between the genes were determined using a three-point cross (Table 8). Data from tests for randomness of the three genes indicate that the genes conditioning pathogenicity on Lion and Trebi are linked with 13.1% recombination, but the gene for pathogenicity on Hannchen segregated independently.

DISCUSSION

Pathogenicity of *U. hordei* on barley is controlled by recessive genes (4,6,7,10,15,17,22,25). For a sporidial mating to be pathogenic on a barley cultivar having a gene(s) for resistance, both sporidia must possess the gene(s) for pathogenicity for the cultivar being tested. Initially it was assumed, therefore, that sporidia 1 and 4 from teliospore K both must possess the pathogenicity gene for barley cultivar Hannchen at the time of infection (Table 2). However, testcrosses performed with cultures of the four sporidia from teliospore K and isolates 1279 and 1874, both pathogenic on Hannchen, Lion, and Trebi, indicated the four sporidia of teliospore K were not carrying pathogenicity genes for the three cultivars. These testcrosses were performed on the original sporidial cultures of teliospore K after pathogenicity had been detected on Hannchen. No cross pathogenic on Hannchen, Lion, or Trebi was obtained from selfing tetrads of teliospores from K-2 (Odessa). The results of the testcrosses and selfings of tetrads from K-2 (Odessa) are evidence that the increase in pathogenicity on Hannchen did not occur during meiosis of teliospore K or in the culturing of sporidia from teliospore K. Therefore, this increased pathogenicity must have occurred within Hannchen between the time of inoculation and teliospore formation.

During the early phases of this study, attempts to repeat Jensen's work by selfing ordered tetrads of his line 447 (Odessa) always resulted in pathogenicity on Odessa only. After we discovered the difference between the tetrads from K-2 (Odessa) and K-2 (Hannchen), we found that selfing ordered tetrads from 447 (Hannchen) produced isolates which were pathogenic on Nepal, Lion, Trebi, and Pannier as well as Odessa and Hannchen. These results are very similar to Jensen's (10). Therefore, it apparently is very important to consider the role of the host when selecting for increased pathogenicity.

More than five inoculations of the barley differentials with teliospores of race 8 over the past 10 yr have resulted in infections of Odessa only. Hannchen has been used repeatedly as a check cultivar in all tests involving race 8 teliospore inoculations without once becoming infected.

Precautions purposely were taken to eliminate any possibility of contamination in this study; eg, sterilization of pots and soil; use of clean greenhouse seed; seed germination and inoculation under aseptic conditions; the use of aseptic techniques during single sporing of sporidia, transferring of sporidial cultures, and inoculation of seedlings; and the absence of any other cultures of *U. hordei* and *U. nigra* being used in the laboratory or the greenhouse during the time of single sporing, culturing, inoculating, or planting the seedlings used in these tests. Matings and inoculations with sporidial cultures were done with moist fungal material which would prevent windborne contamination. No "volunteer" infections have been recorded on seed produced for inoculation studies. No unmated cultures produced infections. All greenhouse seed was hand harvested and threshed.

Pathogenicity on Hannchen is recessive (10,20) and controlled by a single gene pair. *U. hordei* requires the dikaryotic stage for host infection (8). If this increase in pathogenicity was attributed to simple mutations, it would require two mutations, one for each nucleus at the same locus or a single mutation in one nucleus followed by mitotic recombination.

Kozar (14) and Megginson and Person (17) demonstrated that somatic recombination readily occurs in *U. hordei*. Kozar also was able to isolate an apparent diploid sporidium from infected tissue that was capable of reinfecting a susceptible host.

In both Jensen's (10) and this study the initial mutation to greater pathogenicity, which could be continued in further selfings, was found on the cultivar Hannchen. Hannchen is a two-rowed barley which produces many more tillers for a longer period of growth under greenhouse conditions than the other eight differentials (9). Since the plants of Hannchen under greenhouse conditions continue to tiller even after main tillers are mature, meristematic tissue continues to be produced in the crown. This would provide a suitable substrate which would allow a mutation to pathogenicity in Hannchen followed by mitotic recombination and teliospore production in the heads of late tillers to sporulate.

Western (28) showed that strains of *U. avenae* and *U. kolleri* were capable of penetrating the coleoptile and existing in the resistant host tissue for 7–14 days. Sampson (21) reported that in apparently resistant oat plants, the smut fungus was carried in its basal parts

TABLE 6. The percentage of diseased plants of four differential cultivars inoculated with sporidial tetrads from isolates W-2 and Z-2 testcrossed to isolate K-2-8. All isolates are derivatives of race 8, *Ustilago hordei*

Isolates testcrossed	Sporidial matings	Diseased plants of indicated differential cultivars (%)			
		Hannchen	Lion	Odessa	Trebi
W-2 × K-2-8	1 × 1	63	0	86	0
	2 × 2	0	0	38	0
	3 × 2	75	25	86	25
	4 × 1	0	13	50	13
Observed numbers[a]		2:2	2:2	4:0	2:2
Expected ratios[b]		1:1	1:1	1:0	1:1
Z-2 × K-2-8	1 × 2	25	0	71	25
	2 × 2	0	25	75	0
	3 × 1	0	0	63	0
	4 × 1	38	25	86	37
Observed numbers		2:2	2:2	4:0	2:2
Expected ratios		1:1	1:1	1:0	1:1

[a]The observed ratio of infective to noninfective sporidial matings.
[b]The expected segregation ratios of infective to noninfective sporidial matings for one gene pair.

TABLE 7. The pathogenicity of 30 random sporidial isolates from W-2 and Z-2 of *Ustilago hordei* testcrossed to sporidial isolates from K-2-8 on four differential barley cultivars

Testcrosses	Ratio of sporidial matings, infective/noninfective, on differential cultivars:			
	Hannchen	Lion	Odessa	Trebi
W-2 × K-2-8	6:4	5:5	10:0	5:5
Z-2 × K-2-8	9:11	11:9	20:0	10:10
Total observed	15:15	16:14	30:0	15:15
Expected ratios[a]	1:1	1:1	1:0	1:1
Chi-square	0.00	0.14	0.00	0.00

[a]The expected ratios for one gene pair.

TABLE 8. Calculated linkage distances among the three genes for pathogenicity in isolate K-2-8, race 8, *Ustilago hordei*

Genes[a] compared	Crossover distances (map units)	Chi-square test for linkage	*P* value
h and *l*	50.0	0.32	0.95–0.90
l and *t*	13.1	21.16	< 0.01
h and *t*	63.1	0.94	0.90–0.75

[a]Genes "*h*," "*l*," and "*t*" condition pathogenicity on Hannchen, Lion, and Trebi, respectively.

for up to 11 wk without forming teliospores. Kiesling (12) has shown that *U. hordei* will survive in resistant barley seedlings for 14 days after inoculation.

The dikaryon K-1, 1 × 2 developed sori on late tillers of the resistant cultivar Nepal. Subsequent selfings of tetrads from teliospores produced on Nepal failed to infect any cultivar except Odessa. The K-1 sorus was formed on one very late, short tiller. Brooks (2) found late tillers of apparently resistant oat plants almost completely smutted. Sampson (21) attributed this infection of late tillers to a low grade of resistance on resistant plants. In this study, no evidence of a mutational event towards increased pathogenicity was found in our K-1 (Nepal) isolate.

Although the gene for pathogenicity on Hannchen is not linked with the two genes governing pathogenicity on Trebi and Lion, selfed tetrads of teliospores of K-2 (Hannchen) showed additional genes for pathogenicity. The fact that the events in Jensen's and this study happened in the same order indicates that more than coincidence may be involved. In both of these studies, the initial change in pathogenicity on Hannchen was followed immediately in the next selfed generation by pathogenicity on Trebi and Lion. In his selfing of tetrads from teliospores from Hannchen, Jensen recovered a tetrad for which matings were all pathogenic on Trebi and Nepal and a single mating pathogenic on Trebi, Nepal, Lion, and Pannier. We also found dikaryon pathogenic on Lion and Trebi from selfed teliospores of K-2 (Hannchen). No isolates were recovered which were pathogenic on Nepal in this study. Jensen's third selfing produced two dikaryons pathogenic on six of the differential cultivars and two dikaryons pathogenic on all the differentials except Jet (10). No further changes in pathogenicity were obtained in continued selfing in this study.

The results of this study and Jensen's may be explained by the presence of recessive pathogenicity genes in the parental teliospore, contamination with pathogenic lines of the fungus, the effects of inbreeding on regulator genes, or enhanced mutation of pathogenicity genes.

Variability for pathogenicity within races of field isolates of cereal smut fungi usually has been attributed to recombination for pathogenicity characters during meiosis (3). Subculturing and inbreeding have not yielded isolates stable for pathogenic characteristics in some cases (3,5). Low percentages of infection have been explained by 'low virulence' genes (25).

The data from the testcrosses of the original sporidial cultures of the K tetrad and *U. hordei* isolate 1279 and *U. nigra* isolate 1874, established the absence or repression of pathogenicity genes in these four sporidia. This is further borne out by the repeated failure of the ordered tetrad from K-2 (Odessa) to produce matings pathogenic on any of the differential cultivars except Odessa.

Contamination is probably the most common criticism of studies which report previously undetected changes for pathogenicity in a host-parasite interaction. No amount of care can absolutely insure against contamination. However, sufficient precautions were taken to make contamination highly improbable. The fact that we and Jensen obtained similar, but not identical, results from experiments repeated over time, but widely separated from each other, also adds to the improbability of contamination. The total absence of "volunteer" infections in any of the seed production or uninoculated plants from the same seed lots being grown for crossing establishes the clean nature of the seed. The use of aseptic techniques during inoculation and planting further supports the contention that these results are not the product of contamination. Furthermore, no infections on the resistant differential cultivars were found from matings of K-2 (Odessa), K-1 (Odessa), or K-1 (Nepal).

If the first event required to initiate this sequence of mutations was the mutation for pathogenicity on Hannchen, then this indicates a relationship between this locus for pathogenicity on Hannchen and unlinked loci for pathogenicity on Lion and Trebi, since the teliospores of K-2 (Hannchen) were pathogenic on Hannchen, Trebi, and Lion. Jensen's sequence of mutations overcame the resistance of at least eight and perhaps as many as thirteen host genes (10,13). Our sequence overcame the resistance of three host genes (20). The probability of three independent natural mutations in one fungal generation is very small, but the probability of eight or thirteen independent natural mutations in two fungal generations is almost zero.

Pandey (19) proposed that regulator genes, which are frequently carried in the heterozygous condition in higher organisms are required for certain genes to function. Inbreeding tends to diminish heterozygosity and may render such regulator genes ineffective and incapable of supporting the regulated gene(s). If a single gene is necessary for the expression of one or more pathogenicity genes, then the pathogenicity genes could be carried in the recessive state in both nuclei in the dikaryon K-1, 1 × 4 without being expressed. Testcrosses to isolate 1279 and 1874 would indicate the absence of any recessive pathogenicity genes if the regulator gene also was recessive. Therefore, the sudden increase in pathogenicity associated with the inbreeding of teliospore K could have been due to a mutation followed by somatic recombination for the regulator gene. F_1, F_2, and testcross data would still reveal the presence of three recessive genes for pathogenicity in isolate K-2-8 and the regulator gene would not be detected.

Another hypothesis involves the presence of mutator genes which have been reported in other eukaryotes (26). They may be locusspecific, require more than one factor, exhibit dosage effects, or be transposable (23). The only detected mutations in this study were factors governing pathogenicity on barley. The question of why the mutation sequence in this study did not progress so far as the sequence in Jensen's initial study may be answered in part by either the number of mutator genes involved or by dosage effects.

Although we have no data to confirm any of the proposed hypotheses, it is very evident that the shift towards pathogenicity, as observed in teliospore K, could not have resulted from simple mutations. We feel that the presence of a recessive regulator gene would provide the simplest explanation for the increase in pathogenicity in race 8 of *U. hordei.*

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