Genetics of Heterokaryosis in Thanatephorus cucumeris

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Scientific Journal Series Paper No. 7798, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul 55101.

The photographic work of Brian Palk of the Waite Institute is gratefully acknowledged.

Accepted for publication 30 March 1972.

ABSTRACT

Heterokaryon formation in *Thanatephorus cucumeris* ('Praticola-type' AG4) is controlled by two closely linked genes which together are referred to as the H factor. Homokaryons carrying H factors different at either or both genes, when paired, produced tufts of heterokaryotic hyphae. Thiamin and nitrate reductase auxotrophs were used with wild-type monokaryons to establish heterokaryosis. Nonparental H factors were detected at rates of 1.6, 1.7, and 2.2% of the progenies of three isolates. Heterokaryons formed between single-basidiospore cultures obtained from various countries. Fifteen different H factors were obtained. A similar H factor was found in isolates from South Australia, California, and Minnesota, and another factor was common to isolates from England, Canada, and Nebraska. The H factor promotes outbreeding in isolates shown to be primary homothallic.

Phytopathology 62:1057-1065

Additional key words: Rhizoctonia solani, incompatibility.

Host plant resistance to the various diseases caused by *Rhizoctonia solani* Kuehn has been difficult to obtain. One of the reasons for this is now becoming clear. Recent work by Parmeter et al. (11) showed that *Thanatephorus cucumeris,* or *Rhizoctonia solani,* as it has historically been known, is not a single species but is composed of at least four groups which can be separated on ability of field isolates to anastomose; anastomosis occurs between isolates from the same group but not between isolates from different groups. Cultural characteristics of a representative isolate from each anastomosing group (AG) are shown in Fig. 1. Based on morphological characteristics of the fruiting structure, Talbot (15) considers the four anastomosing groups to be a single collective species, *Thanatephorus cucumeris* (Frank) Donk, even though these groups comprise four non-interbreeding populations. Isolates in AG1 and AG4 infect many different host plants; those in AG2 are pathogens mainly of crucifers, and the isolates in AG3 infect potatoes.

Heterokaryon formation in AG1 (7, 16, 18) and also in AG4 (8, 9) was shown to be associated with tufts of hyphae which form at the junction of paired homokaryons. Progenies from AG1 and AG4 field isolates segregate as if a single heterokaryon incompatibility factor (H factor) controls tuft-heterokaryon formation. Heterokaryosis occurs between paired homokaryons having different H factors. Field isolates contain two H factors (HX and HY), and a number of different H factors have been described in each anastomosing group. At the time we started this work, heterokaryosis in both the AG1 and AG4 groups was thought to be controlled by a single factor with multiple alleles.

Our purpose was to investigate in more detail the genetic control of heterokaryosis in *T. cucumeris* 'Praticola-type' AG4 using isolates from various countries. This group has been known as *T. praticola* (Kotila) Flentje, and is composed of important plant pathogens.

MATERIALS AND METHODS.—Isolates of *T. cucumeris* 'Praticola-type' AG4 and AG1 used in these studies are listed in Table 1, with their hosts and geographic origins. Hyphal tips of all field isolates were removed and grown on Potato Vegemite-dextrose agar (10), then transferred to soil-extract agar (3) for basidiospore production (Fig. 1). For heterokaryosis studies, single-basidiospore cultures were opposed on Migration Complete (MC) agar (13) to observe both tuft formation and barrage reactions. Optimum temperature for tuft formation was 20-22 C. From each field isolate, 12-15 single-spore cultures were obtained and paired in all combinations. Based on these pairings, two tester H factor homokaryons (referred to as HX and HY) were selected. Selections were based on ability to produce large tufts of fast-growing hyphae at the junction of paired homokaryons. Other progeny were then paired against these testers. The two testers were opposed in the center of the agar plate with different homokaryons above and below the testers, and a third homokaryon on either side of the tester pair (Fig. 2). Naturally occurring thiamin and nitrate-reductase auxotrophs were used in tests to determine whether heterokaryosis and hybridization had occurred.

RESULTS.—*Heterokaryon formation.*—To determine whether the tufts of hyphae which form at the junction of paired homokaryons of different H factors were heterokaryotic, thiamin and nitrate-reductase auxotrophs were paired with wild-type homokaryons. Tufts from three combinations were subcultured, hyphal-tipped, and fruited. The segregation patterns of the progenies with respect to the

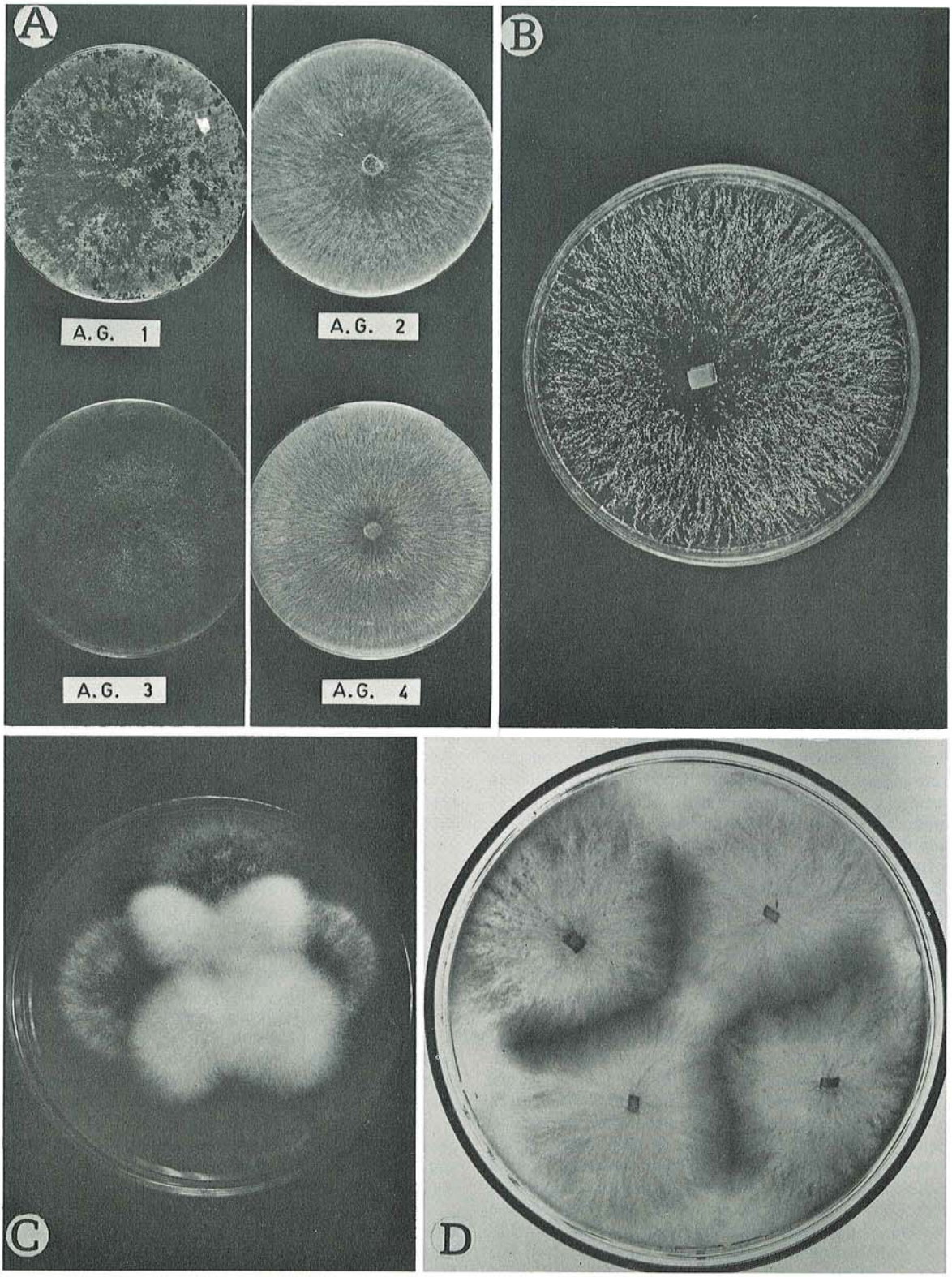

Fig. 1. A) Representatives of Anastomosis Groups 1 to 4 (*Thanatephorus cucumeris*) on Potato Vegemite agar showing cultural characteristics. **B)** Fructifications of field isolate 127 of *T. cucumeris* 'Praticola-type' AG4 on soil-extract agar. **C)** Large tufts from pairings of single-spore cultures of field isolate 146 which have recombinant H factors. **D)** Tufts at junction of single-spore cultures 42-10 (H11) and 42-3 (H12), viewed from under side of petri dish showing "knots" of hyphae.

TABLE 1. List of isolates of *Thanatephorus cucumeris* showing origin, allocated H factor number, and citations to information on cultures

Isolate no.[a]	Allocated H factor no.	Origin: Host or substrate	Origin: Geographical	Citation[b]
AG4 (Praticola-type)				
127	H1, H2	*Pisum sativa*	USA: Minn.	(9)
134	H3, H4	*Beta vulgaris*	Nebr.	(9)
140	H5, H6	*Medicago sativa*	Minn.	(9)
141	H7, H8	*Amaranthus* sp.	Nebr.	(9)
145	H9, H10	*Phaseolus* sp.	Nebr.	(9)
42	H11, H12	*Beta* sp.	England: Slough	(4)
87	H13, H14	Soil	Australia: S.A.	(1) as L-7
233	H15, H16	*Pinus* sp.	USA: California	(11) as C-233
173	H17, H18	*P. banksiana*	Canada: Sask.	(11) as S, V-2235
170	H19, H20	*P. banksiana*	Sask.	(11) as C-110, V-R2041
146	H21, H22	*Medicago sativa*	USA: Minnesota	
241	H23, H24	*Picea glauca*	Canada: Quebec	(11) as C-41
154	H25, H26	Soil	Australia: S.A.	(2) as L-54
195	H27, H28	*Phaseolus* sp.	England: Slough	(11) as CMI-34886
209	H29, H30	*Pinus resinosa*	Canada: Sask.	(11) as S, V-1244
AG1				
160	H101, H102	*Linum usitatissimum*	USA: Minn.	(7) as 60
153	H101, H102	*L. usitatissimum*	Minn.	(7) as 53
158	H101, H102	*L. usitatissimum*	Minn.	(7) as 58
143	H105, H106	*Pinus* sp.	Canada	(11) as R-43
239	H107, H108	*Glycine* sp.	Canada	(11) as C-239
165	H109	*Picea glauca*	Canada	(11) as C-65

[a] Flentje culture collection number
[b] Citation numbers refer to literature cited.

TABLE 2. Parental genotypes and segregation patterns of basidiospore progenies of three heterokaryons of *Thanatephorus cucumeris* ('Praticola-type' AG4)

Heterokaryon	Parental: Monokaryon	Parental: Genotype	Segregation			
1[a]	127-23 141-7	H1, Thi^+ H8, Thi	H1, Thi^+: 28	H1, Thi^-: 28	H8, Thi^-: 31	H8, Thi^+ 24
			$x^2 = .892$, $P = .95–.5$			
2	42-2 141-7	H12, Thi^+ H8, Thi	H12, Thi^+: 19	H12, Thi^-: 20	H8, Thi^-: 11	H8, Thi^+ 18
			$x^2 = 2.941$, $P = .5–.3$			
3[b]	42-2 127-11	H12, $Nar_3{}^+$ H2, Nar_3	H2, Nar^+: 15	H2, Nar^-: 10	H12, Nar^-: 13	H12, Nar^+ 11
			$x^2 = 1.204$, $P = .95–.5$			

[a] Two additional single-spore cultures did not form a tuft with either H1 or H8 tester cultures. One culture grew in the absence of thiamin, the other did not.
[b] One H factor recombinant was detected in this cross which grew in presence of $NaNo_3$.

auxotrophy and H factors are presented in Table 2. The data indicate that the tuft hyphae were heterokaryotic and that genetic recombination occurred.

The genetic control of the formation of tuft hyphae, and thus heterokaryon formation, is evident in Table 3. The progenies of three field isolates of *T. cucumeris* 'Praticola-type' AG4 segregate as if a single factor (H factor) controlled heterokaryon formation. Each field isolate produced four classes of progeny. Most of the progeny were in two classes of approximately equal numbers. With these two groups, interclass pairings of homokaryons produced hyphal tufts

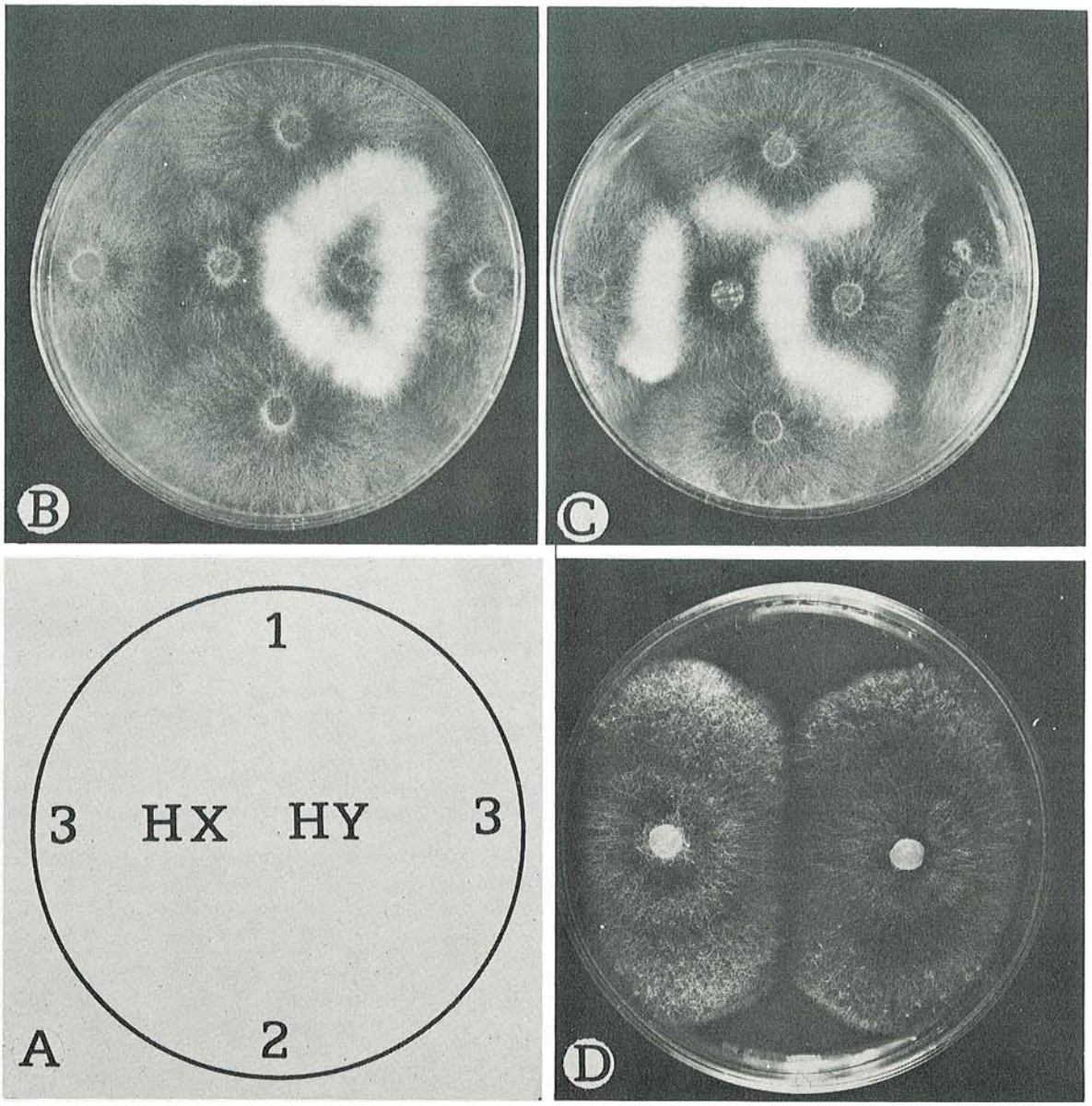

Fig. 2. A) Diagram to illustrate method used in determining the H factor of three single-spore cultures of *Thanatephorus cucumeris* per petri dish. Tester isolates (HX and HY) placed in center and homokaryons around periphery of dish. **B)** All three homokaryons formed a tuft with the HY tester. **C)** Single-spore culture in position 1 had a recombinant H factor and formed a tuft with both the HX and HY tester. The homokaryon in position 2 formed a tuft with the HY tester; the homokaryon in position 3 formed a tuft with the HX tester isolate. **D)** The barrage reaction resulting from the pairing of two different single-spore cultures of field isolate 127 which contain the same H factor.

(Fig. 2); intraclass pairings did not produce tufts, but the barrage reaction was frequently observed (Fig. 2). The remaining progeny were in the other two classes, the recombinant H factor class and the class derived from HX + HY basidiospores. The members in the recombinant class formed tufts when paired with single-spore cultures having either the HX or HY factor, whereas those in the latter class did not form a tuft in either pairing.

Binucleate basidiospores.—Three single-basidiospore cultures which did not form a tuft with either parental H factor homokaryon were allowed to sporulate. Behavior of their progenies is summarized in Table 4, which indicates that all three of these single-spore cultures were heterozygous for the H factor. Each of the above cultures probably arose from a binucleate spore. Besides failing to form tufts with homokaryons possessing either parental H factor,

TABLE 3. Segregation pattern of single basidiospore progeny from each of three field isolates and two synthesized heterokaryons of *Thanatephorus cucumeris* ('Praticola-type' AG4) for the H factor which controls heterokaryon formation

Isolate	Formed tuft reaction with HX[a] (or) HY	Formed tuft with both HX and HY		Did not form tuft with HX or HY		Estimate of binucleate spores, N + N
	No.	*No.*	*%*	*No.*	*%*	*%*
127	76	77	1	0.6	1	0.6	1.2
42	251	221	11	2.2	13+4[b]	3.4	6.8
146	196	188	7	1.7	27	6.4	12.8
42-47-20 + 42-43-69[c]	248	237	8	1.6	9	1.8	3.6
146-42 + 146-54[c]	226	254	0	0.0	20+1	4.2	8.4

[a] HX and HY are used to denote the two H factors in each field isolate and heterokaryons.
[b] These four cultures resemble 42-47. In some pairings they formed small tufts with both parental H factors. On sporulation these four cultures were shown to contain both the H11 and H12 factors.
[c] The heterokaryons were synthesized between recombinant H factor homokaryons from field isolates 42 and 146.

TABLE 4. Segregation for the H factor in the progeny of three single basidiospore cultures of *Thanatephorus cucumeris* ('Praticola-type' AG4) which did not form tufts

Cultures								
42-35			42-43			127-25		
	Segregation			Segregation			Segregation	
H factor no.	H11	H12	H factor no.	H11	H12	H factor no.	H1	H2
52	+	–	50	+	–	35	+	–
53	–	+	52	–	+	31	–	+
4	–	–	0	–	–	3	–	–
2	+	+	1	+	+	1	+	+

these single-spore cultures formed the "barrage" reaction when paired with their HX or HY progeny. A similar reaction occurred in pairings of field heterokaryons with their HX and HY haploid progeny. An exception to the above was noted with single-spore culture 42-47, whose single-basidiospore progeny gave the following interactions when paired with the H11 and H12 tester cultures. Twenty-five single basidiospore cultures formed the tuft reaction with the H11 tester, 15 single-spore cultures formed a tuft with the H12 tester, two single-spore cultures failed to form a tuft reaction with either tester isolate, whereas one single-spore culture formed the tuft reaction with both the H11 and H12 tester cultures. Single-spore culture 42-47 contained both the H11 and H12 factors. Pairings were made between 42-47 and its progeny, and between 42-47 and 22 other single-spore cultures from field isolate 42, some of which contained the H11 factor and some the H12 factor. The barrage reaction followed by small tufts resulted from the pairings of 42-47 with its H11, H12 progeny and in 17 of 22 pairings between 42-47 and other single-spore cultures from field isolate 42. Single basidiospore cultures which contain two different H factors can therefore be detected either by a pairing with HX and HY testers or by allowing them to sporulate and testing the progeny for the presence of both H factors. Isolates from binucleate basidiospores with HX + HX or HY + HY genotypes were not detected. An estimate has been made (Table 3) of the total number of basidiospores which were binucleate (HX + HX, HX + HY, HY + HY) in three field isolates and two synthesized heterokaryons. Percentages of basidia with 2, 3, 4, and 5 basidiospores in three field isolates of *T. cucumeris* ('Praticola-type' AG4) and one AG1 isolate are presented in Table 5.

Recombinant H factors.—Single basidiospore cultures that produced tufts in pairings with parental cultures with either H factor were detected at rates of 0.6, 1.7, and 2.2% among the progenies of three field isolates. These cultures probably contained H factors that were recombinants of the original parent H factors. A heterokaryon was synthesized between recombinant H factor monokaryons 42-47-20 and 42-43-69 from field isolate 42, and allowed to sporulate. Nonparental H factors with respect to this pairing were detected in 1.6% of the progeny of this heterokaryon (Table 3).

The nonparental H factors from each of two field isolates and two heterokaryons synthesized between recombinant H factors could be grouped into two classes. These data are summarized in Table 6. Tufts

TABLE 5. The percentage of basidia with two, three, four, and five basidiospores in fields isolates 127, 134, and 140 of *Thanatephorus cucumeris* ('Praticola-type' AG4) and in AG1 field isolate 158

Isolate	Basidia with two-five basidiospores/basidium[a]				Estimated binucleate spores
	2	3	4	5	
	%	%	%	%	%
127	4	50	45	1	20.6
134	26	43	49	2	40.0
140	19	60	20	1	39.0
158	3	22	70	5	10.3

[a] Data based on 100 basidia/isolate.

TABLE 6. Classes and number of H factor recombinants obtained from two field isolates and two synthesized heterokaryons of *Thanatephorus cucumeris* ('Praticola-type' AG4)

Isolate	Class I recombinants	Class II recombinants
	no.	*no.*
42	6	5
146	5	2
42-47-20 + 42-43-69[a]	4	4
146-42 + 146-54[a]	1	0

[a] The heterokaryons were synthesized between recombinant H factor monokaryons from field isolates 42 and 146.

form in inter- but not intraclass pairings. The four Class I recombinants of heterokaryon 42-47-20 + 42-43-69 synthesized between compatible H factor recombinants from field isolate 42 had the H11 factor; the four Class II recombinants were H12. The original H11 and H12 factors from field isolate 42 had thus been reconstituted by selecting for recombinant H factors for two generations. These results indicate that the H factor is composed of at least two genes.

Number of H factors in nature.—Two compatible H-factor homokaryons were obtained from each of fifteen AG4 field isolates obtained from different countries. Isolates 87 and 154 from the same field had identical H factors. Therefore, the two H factor homokaryons from isolate 154 were eliminated from further tests. The remaining 28 homokaryons were paired in all possible combinations for determination of the number of different H factors in our sample. The results of these pairings are shown in Table 7. Pairings which did not result in tufts were repeated up to 8 times without tufts occurring. Fourteen different H factors were detected in these pairings. A number of common or repeated H factors were detected (Table 8). The H2 factor was found in isolates from Minnesota, South Australia, and California, and the H8 factor occurred in isolates from the USA, Canada, and the United Kingdom. Recombinant H factors obtained in these studies were also tested for their identity against our world sample. The two recombinant H factors obtained from isolate 42 from England appeared to be H2 and H19, respectively. However, one of the two recombinants from isolate 146 from Minnesota had not previously been reported in our world sample. Thus, we obtained a total of 15 different H factors in this study. A multiple allele system at both loci apparently controls heterokaryosis, but the distribution of these alleles at each locus of the H factor is not yet known.

Using Dobzhansky & Wright's formula (2), we estimate the total number of H factors in an infinite population based on our sample to be approximately 17. Thus, by including the recombinant H factor obtained in this study with our collection of H factors from nature, we may have isolated most of the alleles that exist.

The H factor and basidiospore formation.—Previous work with field isolate 42 (Table 1) had indicated that the fungus was homothallic (5). To determine what effect, if any, the H factor had on basidiospore production, the two H factor tester cultures derived from single spores from field isolates 127 and 140 (H1, H2, and H5, H6) were grown and then inoculated on soil extract agar. Sporulation occurred and fifty single-spore cultures were established from each of the four different H factor cultures. The H factor of each single-spore culture was determined by pairings with the two H factors from its parent field isolate. All of the cultures had the H factor of its respective parent, the H1 single-spore culture gave rise to only H1 single-spore progeny, etc. In this limited test, it appears that these single-basidiospore cultures were primary homothallic (18), and that the H factor did not affect sporulation.

Pairings between AG1 and AG4 isolates.—The tuft phenomonon is also associated with heterokaryosis in isolates of *T. cucumeris* AG1 (7, 16, 17). Seven H factors were obtained from AG1 isolates as listed in Table 1. Seven homokaryons from AG1, each containing one of these seven H factors, were paired with 15 homokaryons, each containing a different AG4 H factor. No tufts or "barrages" were observed in any of the pairings between AG1 and AG4 single-spore cultures.

DISCUSSION.—Heterokaryon formation in *T. cucumeris* ('Praticola-type' AG4) is controlled by two linked genes. Tufts of white, rapidly growing hyphae form at the junction of paired homokaryons which differ in one or both of these genes. These tuft hyphae are heterokaryotic. Because of the fairly tight

TABLE 7. Results of pairings of H factors from 14 different field isolates of *Thanatephorus cucumeris* ('Praticola-type' AG4)[a]

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H27	H28	H29	H30
H1																												
H2	+																											
H3	+	+																										
H4	+	+	+																									
H5	+	+	+	+																								
H6	+	+	+	+	+																							
H7	+	+	+	+	+	+																						
H8	+	+	+	+	+	+	+																					
H9	+	+	+	+	+	+	+	–																				
H10	+	+	+	+	+	+	–	+	+																			
H11	+	+	+	+	+	+	–	+	+	–																		
H12	+	+	+	+	+	+	+	+	+	+	+																	
H13	+	+	+	+	+	+	+	+	+	+	+	+																
H14	+	–	+	+	+	+	+	+	+	+	+	+	+															
H15	+	+	–	+	+	+	+	+	+	+	+	+	+	+														
H16	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+													
H17	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+												
H18	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
H19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+										
H20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+									
H21	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+								
H22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+							
H23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
H24	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
H27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
H28	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+			
H29	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+		
H30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	

[a] + = tuft formed; – = no tuft.

TABLE 8. Total list of H factors showing groups of repeated H factors (across) and genetically different ones (down)

H1	(Minn., USA)	=	H18	(Sask., Canada)						
H2	(Minn., USA)	=	H14	(South Australia)	=	H16	(Calif. USA)	=	H33	(Recombinant from H11 + H12)
H3	(Nebr., USA)	=	H15	(Calif., USA)	=	H21	(Minn. USA)			
H4	(Nebr., USA)	=	H24	(Quebec, Canada)	=	H29	(Canada)	=	H32	(Recombinant from H21 + H22)
H5	(Minn., USA)									
H6	(Minn., USA)									
H7	(Nebr., USA)	=	H10	(Nebr., USA)	=	H11	(Slough, UK)			
H8	(Nebr., USA)	=	H9	(Nebr., USA)	=	H17	(Sask., Canada)	=	H28	(Slough, UK)
H12	(Slough, UK)									
H13	(South Australia)									
H19	(Sask., Canada)	=	H22	(Minn., USA)	=	H30	(Sask., Canada)	=	H34	(Recombinant from H11 + H12)
H20	(Sask., Canada)									
H23	(Quebec, Canada)									
H27	(Slough, UK)									
H31	(Recombinant from H21 + H22)									
Total	15 genetically different H factors									

linkage (1.6 to 2.2 crossover units), the two genes involved in the tuft-heterokaryon formation were originally thought to be a single factor (9). We propose that these two linked genes be termed the H factor with the two genes denoted as α and β. Similar terminology is used to describe the two-locus A factor in *Schizophyllum commune* (12), and we believe it to be a convenient way to describe the H factor in *T. cucumeris* 'Praticola-type' AG4. The function of the H factor appears to be to control heterokaryon formation and to promote outbreeding in *T. cucumeris* 'Praticola-type' AG4, which is primary homothallic (5). This outbreeding mechanism may be one reason why members of the AG4 section of this collective species have a wide host range. The AG1 group of this species has also been shown in this and previous work (7, 16, 18) to have a similar mechanism controlling heterokaryon formation, and these isolates are also nonhost-specific plant pathogens. It remains for future research to determine whether members of AG2 and AG3, which have a more restricted parasitic potential, also have an outbreeding mechanism which operates at the heterokaryon formation level, but previous work (14) with the AG2 group has given no clear indication of the operation of an H factor.

Two classes of recombinant or nonparental progeny were recovered from three field isolates and from two synthesized heterokaryons. The best evidence that the H factor consists of at least two genes comes from the experiment that reconstituted the H11 and H12 factors. From two compatible H factor recombinants (42-47-20 and 42-43-69) obtained from field isolate 42, a heterokaryon was synthesized and allowed to sporulate. Eight nonparental single basidiospore cultures were recovered from the progeny of this heterokaryon. Four cultures were found to have the H11 factor; the other four, H12. The H factor may be composed of more than two genes, but larger progeny samples than were used in this study would be necessary to detect them.

Recombinant H factor progeny apparently have a selective advantage in terms of tuft formation. Field isolates having a 2% rate of recombination between the α and β genes would produce parental H factor monokaryons which would form tufts with 51% of the total progeny, whereas the recombinant H factor monokaryons would form heterokaryons with 99% of the progeny. The size and vigor of the tufts increased (Fig. 1) as recombinant H factors from field isolates 42 and 146 were sib-mated for two generations.

In nature, the rapidly growing tuft hyphae probably allow the heterokaryon to grow away from staling products. In laboratory tests, once the heterokaryon was established it appeared to be stable, and most of its subsequent basidia produced hybrid basidiospores. Although tetrad analyses have not been made, it was found that genetic recombination had occurred in the various pairings made in this study. Progeny from the field isolates segregated in a 1:1 ratio for H factors, and synthesized heterokaryons segregated for both H factors and auxotrophic markers in approximately 1:1 ratios. The fungus apparently can maintain an even nuclear balance as the four or six nuclei in its actively growing tip cells divide conjugately (6).

The geographic origin of the H factor did not appear to affect the tuft size or ability to form tufts. It appears that the AG4 section of *T. cucumeris* is a freely interbreeding population. Some differences were noted in the size of the tuft in various paired cultures, and this may be due to the interaction of other factors.

Parent field isolates, and cultures arising from binucleate spores containing both H factors, gave a barrage reaction when paired with tester homokaryons (HX + HY) + HX or (HX + HY) + HY. This is expressed as a cleared area at the junction of paired cultures. It is also commonly, but not always, observed when two homokaryons of similar H factor are paired. These areas are composed of dead cells, resulting from anastomoses of the paired cultures. A similar phenomenon is described by Flentje & Stretton (5) as a killing reaction, using AG2 isolates. In some pairings, it could also be observed accompanying tuft formation, and appears to be independent of the H factor. This occurred in pairings with single-spore culture 42-47 which apparently arose from a binucleate spore as it yielded progeny with the H11 and H12 factors. However, culture 42-47 behaved atypically, as it formed small tufts with most but not all H11 and H12 monokaryons. It is possible that 42-47 may be somewhat unstable, partly dissociating into its H11 and H12 components, and this would be a possible explanation for the barrage reaction together with the tufts. The barrage or killing reaction has been reported in AG1, AG2, and AG4, but a genetic explanation for this phenomenon has not been proposed for any of these groups.

Our estimate of the total percentage of binucleate basidiospores in isolate 42 was 6.8. This is considerably lower than the figure of 35% obtained by Flentje et al. (6) from counts of stained basidiospores of this isolate. This count refers to spores still attached to sterigmata, so that a binucleate condition arising from mitotic division in the spore would have been minimized at this stage. We offer no satisfactory explanation for the difference, but it may be due in part to age of hymenium. The age of the hymenium was observed to affect the production of binucleate spores in field isolate 127. An estimated 44.3% of the basidiospores produced in 14-day-old cultures were binucleate, whereas 20.6% were binucleate in 24-day-old cultures (8). Estimates of the percentage of binucleate spores from field isolates 134 and 140 agree quite closely with the staining work on field isolate 42. It will be important to determine the percentage of binucleate spores produced by this fungus in future genetic studies.

The H factor is part of a heterokaryon incompatibility system, and does not appear to be involved in sexual incompatibility. *T. cucumeris* 'Praticola-type' AG4 can be considered primary homothallic, at least under laboratory conditions. In nature, however, field isolates are heterokaryons. All field isolates used in this study contained two H factors. The H factor appears to limit or deter heterokaryon formation

between similar H factor homokaryons, and to promote outbreeding by allowing heterokaryons to form between single-spore cultures of different H factors via the tuft hyphae.

No tufts or barrage reactions were noted when homokaryons, each containing one of seven H factors from AG1, were paired with homokaryons each containing one of 15 different H factors from AG4. The anastomosing groups were originally delimited using field isolates (11), and our pairings between the two groups were made with single-basidiospore cultures. We believe that our work adds further evidence to the validity of the AG1, AG4 concept, and that these groups are noninterbreeding populations.

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