GENETIC ANALYSES OF *ENDOTHIA PARASITICA*: LINKAGE DATA FOR FOUR SINGLE GENES AND THREE VEGETATIVE COMPATIBILITY TYPES

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ABSTRACT

The loci *cre*, *met* and *ts* segregate independently in *Endothia parasitica*. The phenotype brown (br) seems to be determined by an allele at or very near the *cre* locus. The vegetative compatibility types (v-c) 5 and 39 are determined by different alleles at a locus that is not linked to *cre*, *met* or *ts*. Analysis of two crosses of v-c 5 strains by v-c 10 strains provides evidence that these two v-c groups are different at 5 or more v-c loci.

THE chestnut blight fungus, an ascomycete, *Endothia parasitica* (Murr.) And., can be crossed in the laboratory, allowing genetic analysis of this important plant pathogen. Mutants have been characterized and a system of vegetative incompatibility described (PUHALLA and ANAGNOSTAKIS 1971; ANAGNOSTAKIS 1977; and ANAGNOSTAKIS 1980). This report describes tests for linkage and tests to determine the number of v-c loci segregating in crosses between strains in v-c groups 5 and 10, which are strongly vegetatively incompatible (ANAGNOSTAKIS and WAGGONER 1981), and between strains in v-c groups 5 and 39, which are not strongly vegetatively incompatible (ANAGNOSTAKIS, unpublished).

MATERIALS AND METHODS

Mutants were described previously (PUHALLA and ANAGNOSTAKIS 1971; ANAGNOSTAKIS 1980). Those used in this report were: *cre-1*, cream colored mycelium and conidia; *met-1*, mycelium requires methionine for growth; *ts-1*, temperature sensitive (osmotic) with no growth at 35°; *A/a*, the two alleles at the mating type locus; *br*, brown mycelium and conidia.

Sexual incompatibility is homogenic; strains are incompatible if they have the same alleles at the mating type locus (*A/a*). Crosses were made as described in ANAGNOSTAKIS, 1979, perithecia were isolated (PUHALLA and ANAGNOSTAKIS 1971), and ascospores were spread and germinated (ANAGNOSTAKIS 1982). Single ascospore clones were transferred to potato dextrose agar (Difco) with methionine (100 mg/l) and biotin (1 mg/l) (PDAmb). Pigment formation was assessed at 25° on PDAmb in white fluorescent light (16 hr/day). Auxotrophy was assessed on Endothia minimal medium made with noble agar (PUHALLA and ANAGNOSTAKIS 1971), and temperature sensitivity was assessed on PDAmb at 35° in the dark.

Vegetative incompatibility is heterogenic; strains are incompatible if they have different alleles at any of the v-c loci. The mating type locus (alleles *A/a*) is not a v-c locus. Small pieces of mycelium in agar (cubes about 2 mm on a side) were paired on PDAmb with strains of known v-c (ANAGNOSTAKIS 1977). Strains to be paired were placed not more than 2 mm part to improve detection of barrage. Plates were incubated at 25° in the dark and then transferred to light (16 hr/day, 25°) for 2 or more days before scoring for v-c.

The following tentative genotypes were used for v-c types to make linkage discussion easier:

v-c 5 *B, C, D, E, F*
v-c 39 *b, C, D, E, F*
v-c 10 *b, c, d, e, f*

Strains used were:

329 *cre ts met*$^+$, *a*, v-c 5
338 *cre ts met*$^+$, *a*, v-c 5
290 *cre*$^+$ *ts*$^+$ *met*, *A*, v-c 39
364 *br ts*$^+$ *met A*, v-c 39
42 wild type, *A*, v-c 5 (ATCC #38751)
110 wild type, *A*, v-c 5
67 wild type, *a*, v-c 10 (ATCC #38753)
501 wild type, *a*, v-c 10

It was possible to tell which was the female parent in crosses involving pigment mutants (perithecia were isolated from cream-colored stroma). Crosses made were 388♀ × 290♂, and 329♀ × 364♂. In crosses of wild types, the barrage zone between strains could sometimes be used to differentiate between stroma types but this information was not recorded. These crosses were 42 × 67 and 110 × 501.

RESULTS

The distribution of random single ascospore progeny from six perithecia of cross 338 × 290 is given in Table 1. Seventy-six of the 288 progeny were tested for v-c with the parental types, v-c 5 and v-c 39. All 76 strains merged with one or the other of the testers. Assuming these two types to be different from each

TABLE 1

Randomly isolated progeny from crosses of v-c 5 (338 and 329) and v-c 39 (290 and 364) genotypes

Cross	Progeny No.	Progeny Genotype	v-c allele of those tested B	v-c allele of those tested b
338 × 290 (*cre ts met*$^+$ *B* × *cre*$^+$ *ts*$^+$ *met b*) 6 perithecia	33	*cre ts met*$^+$	4	6
	48	*cre*$^+$ *ts*$^+$ *met*	5	5
	27	*cre ts met*	1	3
	34	*cre ts*$^+$ *met*	6	1
	32	*cre ts*$^+$ *met*$^+$	6	7
	33	*cre*$^+$ *ts met*	0	4
	44	*cre*$^+$ *ts met*$^+$	7	7
	47	*cre*$^+$ *ts*$^+$ *met*$^+$	6	8
Total	298		35	41
329 × 364 (*cre ts met*$^+$ *B* × *br ts*$^+$ *met b*) 4 perithecia	15	*cre ts met*$^+$	8	7
	12	*br ts*$^+$ *met*	7	5
	12	*cre ts met*	6	6
	13	*cre ts*$^+$ *met*	8	5
	16	*cre ts*$^+$ *met*$^+$	6	10
	14	*br ts met*	9	5
	14	*br ts met*$^+$	6	8
	17	*br ts*$^+$ *met*$^+$	6	11
Total	113		56	57

other at a single locus *B* with alleles B (v-c 5) and *b* (v-c 39), these 76 progeny were separated by genotype. Table 2 lists gene pairs with numbers of parental and recombinant progeny in a test for linkage.

Table 1 contains similar progeny data for cross 329 × 364. We tested 113 random single ascospore progeny from 4 perithecia. Gene pairs and numbers of parental and recombinant progeny are listed in Table 2.

There is no linkage between *cre*, *met* or *ts*. The phenotype "brown" seems to be determined by an allele at or very near the *cre* locus. The v-c types 5 and 39 are determined by different alleles at a single locus (*B*) which is not linked to *cre*, *met* or *ts*.

In cross 110 × 501, seven crossed perithecia yielded 263 random single ascospore clones and five tetrads that were tested for v-c type with v-c 5 and v-c 10. Subsequent pairings of 86 nonparental v-c types with each other and with previously numbered v-c types yielded a total of 59 different v-c types. Excluding parental types and tetrads, 12 v-c types were recovered more than once and 40 only once.

In cross 42 × 67, 24 crossed perithecia yielded 973 random single ascospores and eight tetrads that were tested for v-c type with v-c 5 and v-c 10. Subsequent pairings of 137 nonparental v-c types with each other and with previously numbered v-c types yielded a total of 97 different v-c types. Excluding parental types and tetrads, 21 v-c types were recovered more than once and 67 only once.

Forty new v-c types from cross 110 × 501 were paired with 58 new v-c types from cross 42 × 67 and 35/40 from 110 × 501 were the same as 35/58 from 42 × 67. There were 19 singly recovered new v-c types of cross 110 × 501 and 39 from cross 42 × 67 that were not tested with each other. However, since only 5/40 types were different between the crosses among the tested progeny, we might expect that about 3/19 would be unique in the group not tested with each other. This would make the total number of v-c groups recovered from these crosses of v-c 5 × v-c 10 approximately 106 (58 + 5 + 39 + 3). If all v-c interactions were allelic (incompatibility between strains with different alleles at the same locus) and none genic (incompatibility between strains with certain alleles at different loci), v-c groups 5 and 10 should be different at 7 v-c genes (128 total).

On the other hand, if we use the criterion suggested in ANAGNOSTAKIS (1977) of basing the estimate of genetic difference on the number of male parent type progeny we would reach a more conservative estimate. If we assume that the

TABLE 2

Linkage data for the genes segregating among the progeny from crosses 338 × 290 and 329 × 364

Gene pairs	Parental	Recombinant	% Recombinant
cre: *br*	113	0	0
cre: *met*	203	208	51
cre: *ts*	211	200	49
met: *ts*	213	198	49
cre: *B*	98	91	48
ts: *B*	93	96	51
met: *B*	83	106	56

lower number of parental v-c types in each perithecium represents the male parental type we have the numbers 11/263 (1/24) for cross 110 × 501 and 37/973 (1/26) for cross 42 × 67. This speculation would lead to a difference at 5 v-c genes between v-c groups 5 and 10 (if the incompatibility is only between strains with different alleles at the same loci).

DISCUSSION

Although the total numbers of progeny analyzed here are low, we can draw conclusions about linkage and numbers of v-c genes that will be useful in further studies. There is apparent lack of linkage between the loci *cre*, *met*, *ts* and *B*, and a close linkage between *br* and *cre* (or they are alleles at a single locus). The v-c groups 5 and 10 differ at 5 or more v-c loci.

LING and CLARK (1981) recently described three levels of vegetative isolation in a myxomycete (determined by more than 13 v-c genes). It is possible that several levels of vegetative incompatibility are operating in *E. parasitica* and that failure to distinguish them in laboratory pairings has led to the difficulty in identifying the number of v-c genes different between groups 5 and 10. These two v-c types have both been recovered several times from natural cankers in Connecticut, twice in the same area. Since vegetative isolation in *E. parasitica* does not interfere with sexual crossing, the v-c diversity possible in the field is very large. TODD and RAYNER (1980) have pointed out that "in Nature intraspecific antagonism is a vegetative mechanism operating (in fungi) to delimit individuals within freely interbreeding populations." Our concern about the magnitude of this diversity comes from our hope to use debilitating cytoplasmic agents (hypovirulence) to control chestnut blight in the forest. These agents are effectively transferred only by hyphal anastomoses (ANAGNOSTAKIS 1982). Experiments are in progress to clarify the degree of vegetative isolation between *E. parasitica* strains in all the different v-c groups using the cytoplasmic determinants for hypovirulence as markers.

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