Genetic Studies with the Chestnut Blight Fungus, *Cryphonectria parasitica*

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ABSTRACT. The chestnut blight fungus is an Ascomycete that preferentially outcrosses, but is capable of selling. Frequency of selling has been tested in American chestnut trees in the field, and in petri dishes in the laboratory. Selfing produces progeny that have different mating types but the same vegetative compatibility type. Vegetative compatibility (v-c) is controlled by several genes and studies have continued to elucidate the genetic relationships between several common v-c types.

The chestnut blight fungus is an Ascomycete that reproduces sexually primarily by crossing with sexually compatible strains. When we did our first studies (5) with *Cryphonectria parasitica* (Murr.) Barr, one of the perithecia examined (from a chestnut tree in the field) appeared to be the result of "selling," that is, the progeny were all like the female (mycelium) parent and not the result of sexual crossing. Rarely have laboratory matings resulted in selfed perithecia, but tests with mixtures of strains indicate that there are at least two (female) nuclei in the protoperithecia (2). The ascospores in perithecia can be the result of: 1) both nuclei crossed with one male parent, 2) one nucleus crossed with one male parent and one crossed with another male parent, 3) one nucleus crossed with one male parent and one nucleus "selfed," 4) or both nuclei "selfed." This leads to progeny segregation data that is hard to analyze.

I tested frequency of selling in American chestnut trees in the field, and in petri dishes in the laboratory. I used strain 389 (ATCC #38980) of *C. parasitica* with genetic markers Crel (cream colored mycelium and conidia), Tsl (temperature sensitive), and in vegetative compatibility type 5, which has mating type allele Mat1-1. Vegetative compatibility (v-c) tests were done on Potato Dextrose Agar (PDA, Difco, Detroit, Mich.) by pairing isolates with strain 389 (1, 3). Vegetative compatibility is controlled by several genes in the nucleus (1, 3) and strains are compatible if they have the same alleles at all of the *vic* loci. Matings in the laboratory were done on autoclaved chestnut stems supported by 2% water agar in deep petri dishes. Isolates were mated with strain 389 (Mat1-1) and "wild type" strains 67 (Mat1-2) ATCC #38753, and 155 (Matl-1) ATCC #38755 (3).

LABORATORY MATINGS

Strain 389 was mated with strain 67 in the laboratory. The twenty-five perithecia isolated were all taken from the cream colored mycelium of strain 389 (i.e., were 389 female x 67 male, and not 67 x 389). They yielded 1,122 single ascospore isolates that included 544 cream colored and 578 orange cultures. A completely selfed perithecium would have produced progeny that all had the pigment phenotype of the female parent (that is, all cream colored). None of these were found (Table 1).

FIELD TESTS

Few perithecia were formed after strain 389 had grown for 2 mo in chestnut sprouts in the field, but all eleven removed were the product of crossing. The 1,312 single ascospore isolates included 640 cream colored and 672 orange (Table 1).

Abundant perithecia were found on the stems harvested after 21 mo. A total of 77 perithecia and 1,616 ascospore isolates were examined, and the results are summarized in Table 1. There were 10 perithecia that yielded ascospore isolates that included all of the phenotypes expected, and must have resulted from crossing.

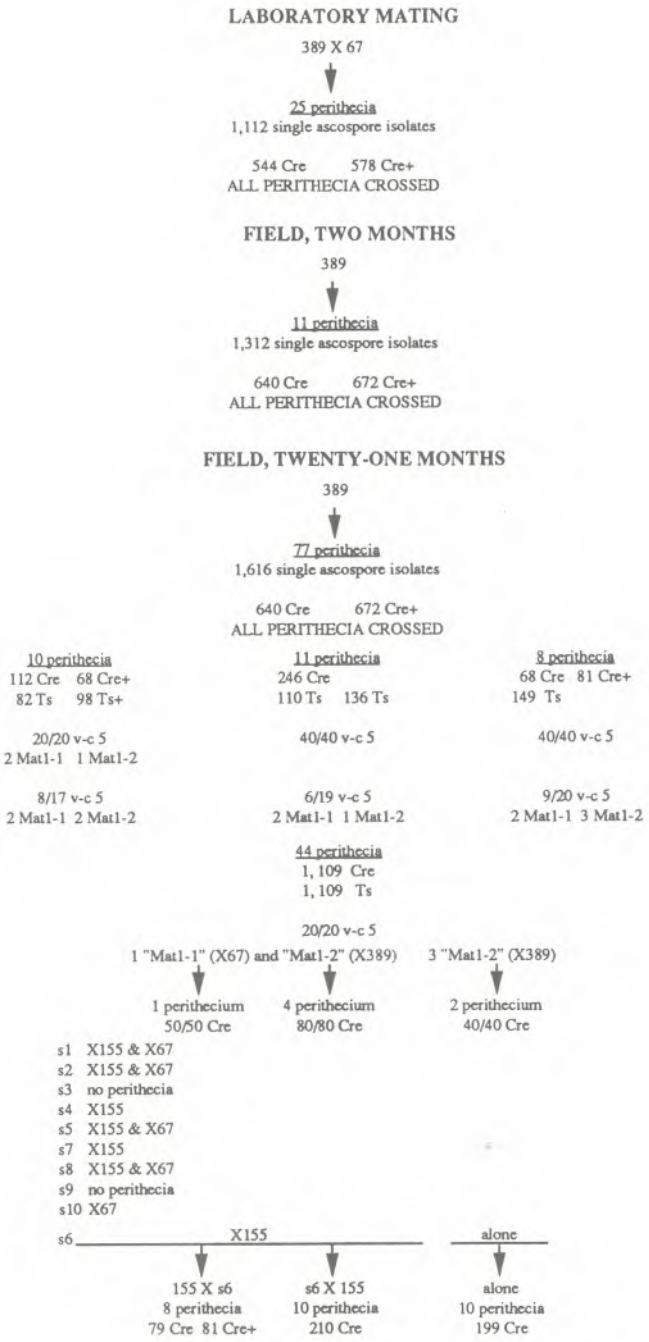
There were 23 perithecia that yielded ascospore isolates that did not include all of the expected phenotypes, but still were not all the same phenotype as 389. Missing from 15 perithecia were orange isolates, and missing from 8 perithecia were isolates that were not temperature sensitive.

The remaining 44 perithecia yielded 1,109 single ascospore isolat s that were cream colored and temperature sensitive like 389, their female parent.

VEGETATIVE COMPATIBILITY TESTS OF ASCOSPORE ISOLATES

Progeny from two of the crossed perithecia from stems cut after 21 mo were chosen for more phenotype tests, and were paired with strain 389 on PDA to see if they were of the same or a different vegetative compatibility type as their female parent (v-c 5). From one perithecium, eight ascospore isolates were the same v-c type as 389 and nine were not. The 20 ascospore isolates from the other perithecium were all vegetatively compatible with strain 389.

Table 1. **Results of experiments in tabular form.**



Progeny from three of the perithecia that had no Cre+ ascospore isolates were paired with strain 389 on PDA. Those from one perithecium included six isolates like 389 and 13 isolates that were different. Twenty from each of the other two perithecia were the same v-c type as 389.

Progeny from three of the perithecia that had no Ts+ ascospore isolates gave similar results, with those from one perithecium including nine like 389 and eleven that were different, and twenty from each of the other two perithecia the same v-c type as 389.

One perithecium whose ascospores showed no segregation for the Cre phenotype was also checked, and all 20 of the ascospore isolates had the same v-c type as 389.

MATING TYPE TESTS OF ISOLATES

Four ascospore clones from each of the two crossed perithecia characterized for v-c were mated in the laboratory to determine their mating type. Those from the perithecium that had all the same v-c type as 389 included two Mat1-1 and one Mat1-2, and one that failed to mate with either 389 or 67 (after 6 mo, two replicates). Those from the perithecium that had segregated for v-c type included two Matl-1 and two Mat1-2.

Four ascospore clones from one of the perithecia that yielded no Cre+ isolates but segregated for v-c were crossed, and three of them produced perithecia in the laboratory; two Matl-1 and one Mat1-2. Four from one of the perithecia that yielded no Ts+ isolates but segregated for v-c mated, and their phenotypes included one Mat1-1 and three Mat1-2.

Finally, four isolates from one of the perithecia that had not segregated for pigment, temperature sensitivity or v-c type also produced perithecia when mated in the laboratory. All four produced perithecia in matings with 389 and 120 ascospore isolates (6 perithecia) were cream colored (proving that there had not been contamination with wild type Mat 1-2 conidia). One also produced a single perithecium in a mating with 67, and 50 ascospore isolates were cream colored. Ten of these (sl through s10) were saved.

When these ten "selfed" ascospore isolates were crossed with 67 and 155 and placed alone on mating plates, four of them produced perithecia with both tester strains, two with only 155, and one with only 67. Only one of them, "selfed 6," produced abundant perithecia alone. When 10 perithecia from "selfed 6" X 155 were examined, all ascospore progeny (210 total) were white. On the other hand, eight perithecia from 155 X "selfed 6" all segregated both white and orange progeny (79 white and 81 orange total). Ten perithecia from "selfed 6" alone yielded only white ascospore isolates (199 total).

DISCUSSION

The lack of segregation for v-c type among progeny of some perithecia is not too surprising, since v-c 5 is a common type in Connecticut. The abnormal segregation for phenotypes Cre and Ts was not expected, but might

have resulted from mutation. Both mating types were found among these progeny, suggesting that they were the result of crossing.

Four of the isolates from "selfed" perithecia were able to mate with 389 (their female parent). It is possible that by chance, four of the same mating type were chosen for testing, but the probability of choosing four individuals that are the same from a population that has equal numbers of the two mating types is 1/8. More likely is the explanation of mutation of the mating type gene.

The clone that produced one selfed perithecium when mated with strain 67, produced many perithecia when mated with strain 389. Thus, this single perithecium may have been the result of yet another mutation event, back to type Mat1-1, or selection of a nucleus in the mycelium that still carried the original Mat1-1 allele. The fact that ten progeny from this single perithecium failed to show regular segregation for the two mating type alleles, (and one produced perithecia alone) suggests that some heritable trait is influencing determination of ability to mate or "self."

If ascospores are the main source of chestnut blight infections, and they are presumed to result from crossing, a "founder effect" with a single v-c type predominating is hard to explain (4). I have previously speculated that two *C. parasitica* cankers in the same v-c group and different mating type, survived, on stems not yet killed by their cankers, to fertilize each other and provide most of the ascospores for the next cycle of the epidemic. However, if the fungal strains in the few remaining cankers on living stems are able to undergo mating type mutation, ascospores will be produced with the v-c type of the mycelium in the cankers. The largest cankers will produce the most ascospores, and a founder effect will be seen in the population as new cankers are initiated.

Crucial to predicting the population dynamics of a pathogen, is information about the potential diversity of the propagules responsible for infection. Studies with a fungus that usually outcrosses but can also produce perithecia alone by changing the mating type gene, will be difficult. We must better define the conditions under which these two kinds of sexual reproduction occur.

ACKNOWLEDGMENTS

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