Perkins, D.D. Evidence confirming location of het-d in linkage group IIR.

Laura Garnjobst (1953) identified two heterokaryon-incompatibility genes designated het-c and het-d, and mapped them by conventional crosses, employing heterokaryon tests to score the progeny. Similarly, het-e was mapped by Wilson and Garnjobst (1966). The map locations assigned to het-c (IIL) and het-e (VIIL) have since been amply confirmed, using an independent method (Perkins 1968, 1975). In contrast, there has been no opportunity until now to verify the position assigned by Garnjobst to het-d, far distal in IIR. The original assignment was based on tetrad data, with 21 PD : 0 NPD : 21 T for het-d and the distal IIR marker fluffy. Linkage with fl is indicated by the excess of parental over nonparental ditypes. However, linkage in I was also suggested, because the PD : NPD numbers of het-d with respect to mating type (IL) were 21 : 7 (combined data, 1953, 1955), a statistically significant departure from 1 : 1. Additional evidence of a different sort has now been obtained which confirms Garnjobst's conclusion that het-d is located in IIR.

Confirmation was made possible by the discovery of a translocation, T(II → V)ALS176, having one break point just right of centromere in linkage group II and a second break point at one end of linkage group V. When ALS176 is crossed by normal sequence strains carrying appropriate markers, about one third of the viable progeny carry the entire right arm of II in duplicated condition. IIR markers from the two parents are heterozygous in a majority of the duplication progeny.

Strains of known het-d genotype were identified by Garnjobst and Wilson and are available from FGSC. When any one T(II → V)ALS176 translocation strain was crossed to known het-d and het-D testers, the duplication progeny from one of the test crosses were phenotypically abnormal, but those from the other test cross were not. On germination from ascospores, the abnormal progeny showed inhibited growth, abnormal morphology, and brown pigmentation on complete medium. Eventually, after 5 or 6 days at 25° C, sectors of normal growth appeared and overgrew the cultures. This behavior resembles that of other duplications known to be heterozygous for other heterokaryon incompatibility genes (Perkins 1975; Mylyk 1975). The

abnormalities of growth, morphology, and pigment are not seen in the progeny when both parents, translocation and normal, carry the same het allele. ALS176 duplications thus provide confirmation that het-d is located within the limits of the duplicated segment, that is, in IIR.

T(II · V)ALS176 het-D and het-d strains of both mating types have been obtained for use in testing strains of unknown het constitution to determine which d allele is present, and these have been deposited in FGSC (Nos. 2414, het-D A; 3014, het-D a; 3013, het-d A; 2415, het-d a).

A note of caution is necessary regarding use of these ALS176 het-d testers. A complication arises because crosses of T(II · V)ALS176 X Normal produce a second, distinct class of duplications that probably result from nondisjunction of centromeres resulting in so-called 3 : 1 chromosome segregation. This minority class is duplicated for the left arm of linkage group II, where het-c is located. If the two parents differ in het-c alleles, then a second class of inhibited duplications result that are het-C / het-c.

Fortunately, het-c heterozygotes are phenotypically distinguishable from het-d heterozygotes. het-C / het-c are "brown-flat" with vigorous spreading surface growth, and intense browning, especially concentrated in coarse trunk hyphae. Growth and pigmentation of het-D/het-d duplications is slower, surface growth is smoother, and the hyphae are less coarse.

The four ALS176 het-d testers deposited in FGSC are apparently all het-C. Most of our laboratory stocks are also het-C (as are the Oak Ridge and Lindegren wild types). Consequently, most tests involving laboratory strains are expected to be homozygous het-C, and any inhibited duplications can be ascribed to heterozygosity of het-d. (Assistance of Monika Bjorkman is gratefully acknowledged). - - - Department of Biological Sciences, Stanford University, Stanford, CA 94305.