GENETICS AND BREEDING OF AMERICAN ELM[1]

Donald Lester[2]

BACKGROUND

Since its discovery in Ohio in 1930, Dutch elm disease, *Ceratocystis* ulmi (Buisman) C. Moreau, has been a stimulus for much concern and research in the United States. Radial spread of the disease to a present distribution throughout most of the natural range of American elm (*Ulmus americana* L.) has led to accelerated study of the pathogen, of insect vectors, and of pathogenicity. Relatively little effort, however, has been applied to study of the host, particularly in relation to genetics and breeding. A few studies of mass selection for resistance to Dutch elm disease and some attempts to produce interspecific hybrids represent the major part of research on genetics and breeding of American elm to date. Although American elm is of economic significance, primarily as an ornamental, the techniques and long-term outlook of forest tree breeders seems to be needed to initiate genetics and breeding research. At the University of Wisconsin this research is coordinated with major research on the pathogen and on insect vectors of the disease.

From a genetic point of view, the predominate feature of American elm is polyploidy. All other species in the genus have a gametic chromosome number of 14 while American elm is a tetraploid with gametes containing 28 chromosomes. Polyploidy is not a common feature of the forest species on which most genetics and breeding research has been conducted although black cherry and yellow birch are among the important hardwood species which are polyploids. It may be well, therefore, to note the genetic consequences of tetraploidy. Tetraploids may be of three types: autotetraploids, allotetraploids, or a combination of both. Autotetraploids are characterized by multiples of one genome and multivalent chromosome association at meiosis. Allotetraploids arise from hybridization between diploid species and thus contain two pairs of different genomes with bivalent pairing at meiosis.

The significance of the type of tetraploidy in American elm lies in the differences of expected genetic ratios for different types of tetraploids. For example, a selfed autotetraploid of genotype AAaa would be expected to segregate 35A:la if quadrivalents form at meiosis and if the locus is linked to the centromere (random chromosome assortment). By comparison, selling of an allotetraploid AAa'a' in which bivalents form only within the different pairs of genomes would have an expected segregation of 3A:la'.

Determinations of the type of tetraploid are generally made by two complimentary methods, cytological observations of chromosome association at meiosis and genetic studies using marker genes. Quadrivalent chromosome associations indicative of autotetraploidy in American elm have been reported by Sax (1933) and Miller (1964). Identification of two genotypes containing alleles for chlorophyll deficient seedlings has provided the opportunity for preliminary genetic analysis in progenies produced by selfing in 1967 and 1968. Table 1 summarizes observed segregation ratios and analyses of goodness-of-fit to ratios expected under different assumptions on genetic control of the chlorophyll deficiency. The table chiefly serves to illustrate the complexity

---

[1] Approved for publication by Director, Wisconsin Agricultural Experiment Station. Work was supported by funds granted under the federal McIntire-Stennis appropriation for forestry research.

[2] Associate Professor, Dept. of Forestry, University of Wisconsin, Madison, Wisc.

introduced by polyploidy in genetic analysis. A segregation ratio of 47:1 is suggested, but not established, by the data. The only ratios which can be rejected are those for which the probability of a larger Chi-square value is .05 or less. The problem of identifying the correct theoretical ratio is illustrated by the need for data from 5000 individuals if a fit to the 35:1 or 47:1 ratios is to be distinguished at the .05 level of probability (Jones, 1967).

Table 1. -- Analysis of segregation ratios for chlorophyll deficiency observed in selfed progenies.

Year	Tree number	No. of seedlings		Assumed loci	Type of inheritance	Expected ratio 1/	Fit 2/	Range of ratios 3/
		Green	Yellow					
1967	21	43	1	1	Tetrasomic	35:1	.80-.90	--
				2	Tetrasomic or Tetradisomic	47:1	.90-.95	15:1 to 143:1
				3	Disomic	63:1	.70-.80	--
	25	48	3	1	Tetrasomic	35:1	.10-.20	--
			(16:1)	2	Tetrasomic or Tetradisomic	15:1	.90-.95	43:1 to 143:1
				3	Disomic	63:1	.01-.02	--
1968	25	1487	31	1	Tetrasomic	35:1	.05-.10	--
			(48:1)	2	Tetrasomic or Tetradisomic	47:1	.90-.95	32.3:1 to 143:1
				3	Disomic	63:1	.10-.20	--
1967 & 1968	25	1535	34	1	Tetrasomic	35:1	.10-.20	--
			(45:1)	2	Tetrasomic or Tetradisomic	47:1	.80-.90	32.3:1 to 143:1
				3	Disomic	63:1	.05-.10	--

1/ Expected ratio most similar to observed ratio.
2/ Probability of a larger value of Chi-square.
3/ Expected ratios at which the probability of a larger value of Chi-square is less than 5%.

Assuming that 47:1 is the correct theoretical ratio for segregation in these progenies, the parental genotypes under tetrasomic inheritance could be AaaaBBbb or AAaaBbbb and under tetradisomic inheritance could be AaaaBb or AaBbbb (Jones, 1967). The 47:1 ratio is based on the assumption that the mutant type appears only when the dominant allele of one gene and all recessive alleles of the other gene are combined in the same genotype. A genetic system of this type for chlorophyll mutants would be unusual, if not unique.

Presuming that American elm is an autotetraploid, additional consequences of tetraploidy are illustrated in table 2. The progeny sizes necessary to insure the recovery of certain genotypes reach relatively unmanageable proportions as the number of genes involved increases and even in the most simple genetic system progeny sizes are a limiting consideration unless evaluation can be accomplished at an early age.

Table 2. -- Potential genotypes in diploids and tetraploids.

One gene		Two genes		Three genes	
Diploid	Tetraploid	Diploid	Tetraploid	Diploid	Tetraploid
AA, Aa, aa (3)	AAAA, AAAa, AAaa, Aaaa, aaaa (5)	(9)	(25)	(27)	(625)

Consequences of tetraploidy in a two-gene model

Diploid crossing - AAbb × aaBB
↓
AaBb × AaBb
↓
aabb (1 seedling in 16)

Tetraploid crossing - AAAAbbbb × aaaaBBBB
↓
AAaaBBbb × AAaaBBbb
↓
aaaabbbb (1 seedling in 1296)

AN ATTEMPT TO INDUCE POLYHAPLOIDY

A reduction in the ploidy level thus has substantial appeal from the viewpoint of simplified inheritance patterns and the possibility of gene exchange with diploid species. Reduction of chromosome numbers in somatic tissues to the gametic level has been accomplished in several crop species and on a large scale particularly in the cultivated potato (Solanum tuberosum L.). The success in potato is an especially encouraging example because the induction of seedlings with one-half the typical chromosome number (polyhaploids) has been accomplished with high frequency and subsequent crossing of polyhaploids among themselves and with diploid species has been successful (Hougas, et al., 1964; Perez-Ugalde, et al., 1964). With this in mind, experiments on induction of polyhaploids in American elm were initiated in 1967.

The technique chosen was to stimulate the female gametophyte through the use of pollens believed to be incapable of producing a zygote. Pollen was collected from branches forced in a greenhouse about one week before the pollinations. Branches for maternal parents were cut about 10 days before natural anthesis and were placed in a culture solution of sucrose, with a fungicide, and a bacteriacide. Six pollen mixes were used: non-irradiated American elm (control), red elm (U. rubra rock elm (U. thomasii Sarg.), irradiated American elm (100,000r from Ce 137), and a 1:1 mixture of red or rock elm with irradiated American elm pollen. Pollen from at least three trees was represented in each pollen mixture. Flower buds were pollinated as stigmas projected above the unopened anthers. Some flower buds to be used as checks on isolation were enclosed in translucent glassine bags prior to pollination. Artificial pollinations were completed about 1 day before dehiscence of anthers in the pollinated flowers.

Table 3A summarizes total fruit yield. The abundant fruit development on three of four intraspecific crosses suggests that cultural conditions were adequate. The sparse fruit development following interspecific crosses was in accord with reported failures of interspecific pollinations on American elm. The stimulatory effect of the irradiated American elm pollen on fruit development was apparent. Finally, the presence of fruits in control bags raised questions about experimental technique; questions which will be deferred until the reported self-sterility of American elm is discussed.

In counting the yield of these crosses on a light table, a wide range in embryo size was noted. In view of the possibility that polyhaploid embryos would be rather small it was necessary to sow all seeds and yet the viability of very small embryos seemed questionable. Therefore, embryos were visually classified as either filling more or less than one-half of the embryo sac. Overall germination was 51% for the larger embryos and 5% for the smaller ones. Either a more detailed determination of the relationship between embryo size and viability or possibly a more precise means of determining relative embryo size thus is required if any fruits are to be discarded.

Table 3B summarizes seed germination three weeks after sowing. A total of 74 seedlings, excluding seedlings from crossing with non-irradiated American elm pollen, were produced. These presumably could have been polyhaploids with a chromosome number of 28, triploid interspecific hybrids with 42 chromosomes, or tetraploid American elms produced by selfing. Fifty-nine seedlings survived transplanting and insecticide damage. Root tips from each seedling were used for chromosome counts. All counts were tetraploid. All of the seedlings thus were apparently produced by self-pollination.

Table 3, A and B. -- Results of crosses to induce polyhaploidy in U. americana (numbers of fruits or seedlings per pollination bag of 3 to 4 fascicles).

Maternal tree	Pollen						
	Non-irradiated			Irradiated	Irradiated American plus		Unpollinated controls
	American	Red	Rock	American	Red	Rock	
A. Total fruits							
5	70.6	1.7	1.2	42.9	47.8	50.5	4.0
9	2.7	0	0.1	9.6	10.1	6.5	0.4
13	59.4	1.3	1.9	46.1	52.9	39.8	3.4
32	61.6	0.2	1.1	6.2	7.2	7.5	--
B. Seedlings							
5	16.0	0.2	0.7	0.1	0.4	0.9	1.5
9	0	0	0	0.1	0.1	0.1	0
13	34.1	0.3	0.4	0.1	0.3	0.3	0.2
32	42.5	0	0.7	0	0.2	0.3	--

SELF-STERILITY

In view of these results, the question of self-sterility in American elm needs clarification. In most reports of crossing experiments with elms the assumption has been made that elms are self-sterile as a consequence of protogyny, that is, the precocious development of a stigma before anthers in the same flower dehisce. To test for variation in self-sterility in the absence of protogyny, branches from 34 elms were collected, flower buds were forced in the greenhouse, and self-pollinations were made on 12-15 fascicles for each tree as the stigmas reflexed above the anthers. Selfing on 32 trees produced seed with a range of up to 19 seeds per fascicle and a mean of 1.4 ± 0.6 seeds. Germination ranged from 0 to 100% with a mean of 34 ± 16%, in contrast to an average germination of 1.5% for seeds produced by natural selfing in American elm as recorded by Johnson (1946). Self-compatibility thus represents an obstacle in the interpretation of crossing studies with American elm. In a new attempt to induce polyhaploidy, maternal parents with apparent high self-sterility have been chosen. Based on the absence of seeds in check bags on some maternal parents in crosses for 1968, variation in self-sterility may be of some value in research on genetics and breeding of American elms. As yet we have not started the necessary chromosome counts to determine whether apparent self-sterility has been maintained in our crosses.

SUMMARY

The consequences of tetraploidy for genetic analysis and for breeding of qualitative traits was discussed. An unsuccessful attempt to produce polyhaploid seedlings was described. Self-fertility was shown to be an obstacle in interpretation of crossing studies although the range of variation in self-fertility suggests that self-sterile individuals may exist.

REFERENCES

Hougas, R. W., S. J. Peloquin, and A. C. Gabert. 1964. Effect of the seed parent and pollination on the frequency of hybrids in *Solanum tuberosum.* Crop Sci. 4:593-595.

Johnson, L. P. V. 1946. Fertilization in *Ulmus* with special reference to hybridization procedure. Can. Jour. Res. 24C:1-3.

Jones, A. 1967. Theoretical segregation ratios of qualitatively inherited characters for hexaploid sweetpotato *(Ipomoea batatas* L.). U.S.D.A. Tech. Bul. 1368. 43 pp.

Miller, R. G. 1964. Hybridization and reproductive cytology in *Ulmus.* Unpublished MS thesis. Syracuse University. 80 pp.

Perez-Ugalde, G., R. W. Hougas, and S. J. Peloquin. 1964. Fertility of S. *phureja* and haploid S. *tuberosum* hybrids. Am. Potato Jour. 41:256-262.

Sax. K. 1933. Chromosome numbers in *Ulmus* and related genera. Jour. Arnold Arb. 14:82-84.