THIRD TECHNICAL SESSSION

Chairman: P. Garrett

GENETIC CONTROL OF SEX RATIO, EARLINESS AND FREQUENCY OF FLOWERING IN POPULUS TREMULOIDES

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The major objective of the forest genetics program which I initiated in 1959 was to elucidate the role of the genotype and the mode of genetic control of traits in a forest tree species that are of importance to society. Trembling aspen (*Populus tremuloides* Michx.) was selected as the experimental tree, and trees in 26 natural stands scattered throughout the Adirondack Mountains and adjoining regions of New York State were selected using random procedures to serve as parents in controlled crosses. Since most economically important traits are quantitative in nature, experimental designs have been chosen that permit the estimation of the heritability of traits on the basis of the covariance between half-sibling families. The results of seven years of observations on flowering in three of the oldest experimental plantings will be reported in this paper.

An understanding of the genetic control of traits such as sex determination, sex ratio, and earliness and frequency of flowering is of more general importance than to merely satisfy the esoteric interest of aspen and poplar breeders. It is conceivable that one day we may want to plant only one of the two sexes because of some important sex-related or sex-linked trait. As an example, Gullion and Svoboda (1972) reported that the floral buds of staminate trees are a major food source for ruffed grouse (*Bonasa umbellus* L.) in northern Minnesota. They found that the more rapidly developing, hence larger staminate buds provide a greater amount of food per bud than vegetative of pistillate floral buds. This permits the bird to "fill up" quickly, thus reducing the time when it is exposed to predation and the energy expended to maintain body temperature during cold winter evening feeding periods. They reported other field observations, such as the high incidence of staminate-flowered trees within sight of drumming logs, which support their conclusion that the staminate aspen tree is an integral component in the ecology of the ruffed grouse in northern forests. Other sex-related traits that could be important in breeding aspen to serve as a component in a multiple-purpose management area include earliness and frequency of flowering.

Most studies of sex ratios in *Populus* spp. report an excess of males to females in natural stands, provenances and families from controlled crosses. Pauley (1949) found an excess of staminate-flowered clones among superior clones of several native *Populus* spp. selected by Helge Johnsson

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and him throughout United States and Canada. The results of studies since that time, summarized in Table 1, clearly indicate an excess of staminate-flowered trees in three Populus species, but in only two cases are the deviations from a 1:1 sex ratio statistically significant.

The results herein reported are based upon the sex expression in 36 families produced by controlled crosses of trees from 15 natural stands in this northern New York State population. A strong genetic control of sex ratio and earliness of flowering has been found. The numbers of years of observations of flowering, however, are inadequate to clearly elucidate genetic control of the flowering frequency. Similarities among half-sibling families and differences between unrelated groups, though, clearly suggest a predictable genetic control for this trait that can be determined in a few more years.

MATERIALS AND METHODS

Thirty-six F_1 families, each composed of 30 full siblings were planted at an 8' x 8' spacing in replicated plots at the College's Genetic Field Station, Tully, New York. The twelve families in Experiment 1 were produced in 1961 by crossing three pistillate-flowered trees, Nos. OS105, FR301, and CL307, to each of four staminate flowered trees, Nos. FU101, FR308, CL306, and ES203. One-year-old seedlings were field planted in 1962 according to a randomized complete block design with three blocks of twelve families each, and 10 trees per family per block. The stems of these trees were cut back to about one foot above ground in the spring of 1965 because of a severe infestation of Saperda beetles, and a single basal sprout was selected that next summer to serve as the new stem. Thereafter a combination of sanitary practices and spraying with insecticides controlled this insect. The effect of this cutting back of the stems, however, has resulted in trees whose root systems are four years older than the stems. The remaining 24 families were field planted in 1964 as Experiments 4-5 in a randomized complete block design with two blocks and 15 trees per family in each block. One-year-old seedlings were lined out for one year, and the two-year-old seedlings were field planted. These families represent the remnant of a large crossing program in 1962 in which 10 pistillate- and 10 staminate-flowered trees were crossed in all combinations. Failure of certain crosses and a high winter mortality of 1-year-old seedlings resulted in this "salvage" planting. Twelve of these families are from three female parents (LE203, ES110, and HE107), each crossed to four male parents (FU101, ES208, OA102, and LE108). The remaining families can be grouped in several ways for analyses, e.g., five "females" each crossed to two males from a group of five, etc. In both plantings trees that died in the first 2 or 3 years were replaced. Later, a basal or root sprout was selected for trees that died or were cut back, and the year was recorded when this occurred so tvole damage in 1967.

Beginning in the spring of 1968 and each year since, each tree has been scored for the occurrence of floral buds. The sex was determined using only one or two buds but many trees have been independently "sexed" two or more years. If an inconsistency was found in different years,

Table 1.--Sex ratios for Populus spp. in natural stands and in families from controlled crosses.

Sex Ratio	Number of trees				
♂:♀ = 1	Total	Male	Female	Origin	Reference
Populus tremuloides					
1.39:1	67	39	28	Natural stands	Stantamour 1956
1.18:1	131	71	60	Control poll.	---- 1956
1.15:1	456	244	212	" "	---- 1956
2.55:1**	206	148	58	Natural stands	Pauley and Mennel 1957
1.12:1	138	73	65	Natural stands	Lester 1963
1.17:1	91	49	42	Natural stands	Einspahr 1962
1.61:1**	1198[a]	739	459	Open poll. seed	Avanzo 1970
P. deltoides					
1.17:1	551	297	254	Natural stands	Farmer 1964
X*P. tremula-tremuloides*					
1.34:1	131	75	56	Control poll.	Muhle-Larsen 1954
P. tremula					
4.5:1	---		---	Natural stands	Carev 1969[b]
1:1	---		---	Natural stands	Dolgoseev 1968[b]

** The deviations from a 1:1 sex ratio are statistically significant at the 1% level of probability.

[a] An additional 74 hermaphrodites or intersexes were also reported.

[b] Original article not seen.

multiple samples from several crown locations were taken. These inconsistencies, however, were rare and proved to be due to hermaphroditism occurring in parts of the crowns rather than classification or recording errors. The sex was not determined for a few trees (17 in Experiment 1 and 20 in Experiment 4-5) due to the presence of only a few floral buds high in the crown that couldn't be reached with a pole pruner. Tree ladders weren't used because of damage due to the excessive pruning of branches required for their use.

RESULTS

Sex Ratio.--The ratio of males to females and the total number of flowering trees for the years 1970 through 1974 for Experiment 1 and 4-5 are given in Table 2. The data for 1968 and 1969 are not included as the numbers are small. Only 13 trees, all pistillate, flowered in 1968, and in 1969, eight trees, 5 staminate- and 3 pistillate-flowered were counted. Thereafter the numbers are sufficiently large to give reasonably reliable estimates of the sex ratios. Note that the number of males exceeds females for all experiments and the pooled data for the years 1970-1973 except for Experiment 4-5 in 1970 when the numbers of each sex were equal. In 1974, however, which proved to be one of the best "flowering years" for aspen in this area in many years, the sex ratios are close to 1:1 for each of the separate experimental groups and for the pooled data. A similar result is obtained if all data for all years are pooled. The results of the Chi-square tests for Goodness of Fit to an expected sex ratio of 1:1 clearly support the conclusion that in the first years of flowering the proportion of males exceeds that of females, but as the trees become older, a larger proportion of the trees flowering for the first time are female so that the overall sex ratio approaches the expected 1:1 ratio. This was attained in these experimental groups in 1974.

A small number of trees in each experiment flowered, but the sex was not determined, This group includes 17 trees in Experiment I, or 7% of the flowering trees, and 20 trees or 5% of the flowering trees in Experiment 4-5. These could be predominantly male as some trees that were originally not sexed, were later identified as female when the catkins were observed to persist after pollen shed ceased in other trees, and later seed was shed. If all of the trees of unknown sex were male, the sex ratios for each of the experimental groups and for the pooled data would not differ from the 1:1 ratio, but in all cases the number of males would slightly exceed that of females.

It was noted in the early years of classifying these trees that families in which tree FU101 served as the male parent were composed largely, if not wholly, of pistillate-flowered trees. Quite by chance this tree was used in both experimental groups considered here. Of the 122 progeny of this tree that flowered in or before 1974, 116 are female, four are male, and two are unknown, as the sex was not determined. When these families are excluded from the data as shown in the last lines of Table 2, the males greatly outnumber the females, and the results of the Chi-square tests for Goodness of Fit to a 1:1 sex ratio are statistically significant at the 1% level of probability for Experiment 1, Experiment 4-5 and for the pooled data.

Table 2.--Sex ratios of flowering trees for 1970 through 1974 for Experiments 1 and 4-5.

Year		Ratio of ♂:♀ = 1 Experiments		Total
		1	4-5	
1970	Sex ratio	2.00:1*	1.00:1	1.28:1
	Number of trees	57	100	157
1971	Sex ratio	2.25:1**	1.16:1	1.56:1**
	Number of trees	104	119	223
1972	Sex ratio	2.92:1**	1.49:1**	1.79:1**
	Number of trees	98	242	340
1973	Sex ratio	1.59:1	1.38:1	1.44:1*
	Number of trees	57	133	190
1974	Sex ratio	1.11:1	0.95:1	1.01:1
	Number of trees	207	377	584
All data	Sex ratio	1.08:1	0.95:1	0.99:1
	Number of trees	212	388	600
All data minus FU101 progeny	Sex ratio	2.33:1**	1.41:1**	1.65:1**
	Number of trees	153	321	474

* Chi-square Test for Goodness of Fit to a 1:1 ratio, statistically significant at the 5% level of probability.

** Chi-square Test, statistically significant at the 1% level of probability.

The variability in sex ratios appears to be related to parentage. This can be seen in Table 3 in which sex ratios are given by groups of maternal and paternal half-sibling families for both Experiments 1 and 4-5. The total number of living trees in each group in 1974 and the percentage of the living trees that have flowered are also given in the table. In general, higher proportions of males occur in the Experiment 1 families than in Experiment 4-5, but one of the paternal half-sibling groups in Experiment 4-5, WA111, exhibits the most disproportionate sex ratio, namely 3.43:1. The number of flowering trees, however, is small so that this could represent chance variation. The group with the largest number of males relative to females is the ES203 paternal half-sibling group in Experiment 1 which has a ratio of 3.17:1. The excess of males to females, however, occurs in all groups except the OS105 maternal half-sibling and the FU101 paternal half-sibling groups in Experiment 1. In contrast, only two of the five maternal half-sibling groups in Experiment 4-5 have more males than females, but in all of the paternal half-sibling groups except FU101 males exceed females, which is in accord with the observations for the Experiment 1 paternal half-sibling groups.

The effect of FU101 on the maternal half-sibling groups is to greatly distort their sex ratios. If families with FU101 as the male parent are excluded from the maternal half-sibling groups, the sex ratios in Experiment I become 1.79:1 for OS105, 1.95:1 for FR301, and 3.75:1 for CL307 and in Experiment 405, 2.31:1 for LE209, 1.32:1 for LE203, 1.94:1 for ES110, 1.08:1 for HE107, and 1.35:1 for CL108. It is obvious that this one staminate-flowered tree has an enormous effect on the sex ratios in the maternal half-sibling groups as well as on the overall sex ratios for each of the experimental groups and the pooled data (see Table 2).

<u>Earliness of</u> Flowering.--Summaries of the data for earliness of flowering are presented in Table 4 for Experiment 1. The results are given as the mean age and the range in age when floral buds were first initiated, i.e., the year prior to flowering. These values are given for each sex and all trees in each family, in each maternal and paternal half-sibling group of families, and for all trees in this experiment. A bar graph showing the number of trees of each sex and of unknown sex that first formed floral buds at each year of age is given for Experiment 1 in Figure 1.

In Experiment 1 the mean age of floral bud formation, based on all trees, is 5.8 years, with the average for the staminate-flowered trees almost one year less than for the pistillate-flowered trees, namely 5.3 and 6.2 years, respectively. This difference between sexes is found within all families except two, OS105 x FR308 and OS105 x ES203. The numbers of flowering trees in these two families are small, so that this non-conformity to what is observed in the other ten families could represent chance variation. The sex difference is also obtained for each of the seven half-sibling groups, with the average age of first flowering for staminate-flowered trees less than that for pistillate-flowered trees. The actual basis for these differences in mean values can be clearly seen in Figure 1, with the majority of the males flowering at ages 4 and 5 and females at 8.

Table 3.--Sex ratios of maternal and paternal half-sibling family groups for Experiments 1 and 4-5.

		Flowering Trees			Living Trees	
Exper. No.	Common Parent	Sex Ratio ♂:♀ = 1	No.	Sex Unknown	No.	Percent Flowered
1	Maternal half-sibling family groups.					
	OS105	0.76:1	58	7	113	58
	FR301	1.03:1	74	6	114	70
	CL307	1.50:1	80	4	110	76
	Paternal half-sibling family groups.					
	FU101	0.05:1**	59	2	85	72
	FR308	2.38:1**	44	7	85	60
	CL306	1.95:1*	59	5	84	76
	ES203	3.17:1*	50	3	83	64
All data		1.08:1	212	17	337	68
4-5	Maternal half-sibling family groups.					
	LE209	1.24:1	56	3	109	54
	LE203	0.90:1	95	2	131	74
	ES110	1.43:1	56	8	132	48
	HE107	0.81:1	121	3	154	81
	CL108	0.79:1	61	4	107	61
	Paternal half-sibling family groups.					
	ES208	1.17:1	76	10	138	62
	FU101	0.02:1**	67	--	121	55
	OA102	1.07:1	60	1	81	75
	FR211	(1.45:1)[a]	27	1	53	53
	LE108	1.64:1	58	3	79	77
	HA311	(1.27:1)	34	3	58	64
	WA111	(3.43:1)	31	1	54	59
	HA211	(1.57:1)	36	1	49	76
All data		0.95:1	388	20	632	65
Pooled data for Exper. 1 & 4-5		0.99:1	600	37	969	66

* Chi-square Test for Goodness of Fit to a 1:1 ratio, statistically significant at the 5% level of probability.

** Chi-square Test, statistically significant at the 1% level of probability.

[a] Ratios surrounded by parentheses were not tested statistically due to small numbers.

Table 4.--Summary of mean and range in age of trees when floral buds were first formed in Experiment 1.

Male Parent	Sex	Age in Years											
		Female Parents									Paternal half-sib group		
		OS105			FR301			CL307					
		Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range	N
FU101	Female	6.4	2-8	19	6.9	4-8	17	6.4	4-8	20	6.6	2-8	56
	Male			--			--	4.3	4-5	3	4.3	4-5	3
	Unknown			--	8.0	8	1	8.0	8	1	8.0	8	2
	Total	6.4	2-8	19	7.1	4-8	18	6.2	4-8	24	6.5	2-8	61
FR308	Female	3.8	2-5	5	8.0	8	6	6.5	5-8	2	6.2	2-8	13
	Male	4.2	4-5	6	5.8	4-8	10	5.0	4-8	15	5.1	4-8	31
	Unknown	7.3	6-8	3	6.0	6	3	8.0	8	1	6.9	6-8	7
	Total	4.7	2-8	14	6.5	4-8	19	5.3	4-8	18	5.6	2-8	51
CL306	Female	6.3	4-8	6	6.6	5-8	8	5.5	4-8	6	6.2	4-8	20
	Male	5.4	3-8	11	5.5	2-8	13	5.3	4-8	15	5.4	2-8	39
	Unknown	5.0	4-6	2	3.0	3	1	4.5	3-6	2	4.4	3-6	5
	Total	5.6	3-8	19	5.8	2-8	22	5.3	3-8	23	5.6	2-8	64
ES203	Female	4.0	2-8	3	6.6	4-8	5	5.5	4-8	4	5.6	2-8	12
	Male	5.4	3-8	8	5.9	4-8	15	4.9	4-8	15	5.4	3-8	38
	Unknown	7.0	6-8	2	8.0	8	1			--	7.3	6-8	3
	Total	5.3	2-8	13	6.2	4-8	21	5.0	4-8	19	5.6	2-8	53
											Grand Total		
Maternal half-sib group	Female	5.8	2-8	33	7.0	4-8	36	6.1	4-8	32	6.2	2-8	101
	Male	5.1	3-8	25	5.8	2-8	38	5.0	4-8	48	5.3	2-8	111
	Unknown	6.6	4-8	7	6.2	3-8	6	6.2	3-8	4	6.4	3-8	17
	Total	5.6	2-8	65	6.4	2-8	80	5.5	3-8	84	5.8	2-8	229

Age in years	Sex	Number of trees 0 5 10 15 20 25 30 35 40 45 50
1	Female	
	Male	
	Unknown	
2	Female	XXXX
	Male	X
	Unknown	
3	Female	X
	Male	XXX
	Unknown	XX
4	Female	XXXXXXXXXXXXXXX
	Male	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
	Unknown	
5	Female	XXXXXXXXXXXXXX
	Male	XXXXXXXXXXXXXXXXXXXXXXXXXXXX
	Unknown	
6	Female	XXXXXXXX
	Male	XXXXXXXXXXXXXXX
	Unknown	XXXXXXX
7	Female	XXX
	Male	
	Unknown	
8	Female	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
	Male	XXXXXXXXXXXXXXXXX
	Unknown	XXXXXXX

Figure 1.--Age of first floral bud formation of pistillate- and staminate-flowered trees in Experiment 1.

Earliness of flowering also varies considerably among families and among the half-sibling groups. Family means range from 4,7 years for family OS105 x FR308 to 7.1 for FR301 x FU101. The large value in this second family, however, is largely due to the occurrence of only pistillate-flowered trees except for one whose sex was not determined, and, as just discussed, to the older average age of first flowering in females than in males. The disproportionate sex ratio due to male parent FU101 accounts for the older age of flowering for its three half-sibling families (6.4, 7.1, and 6.2 years) and for the group mean (6.5 years) compared with the other families and half-sibling groups. Only one other family, FR301 x FR308, has a mean value in this range, and the large value is primarily due to the old age for the first flowering of the female trees. Only six female trees have been identified in this family, and all six flowered for the first time in 1974. Comparisons of the three families comprising each of the four paternal half-sibling groups show that in each case, the family with FR301 as the female parent has the largest mean values for age of flowering for both pistillate- and the staminate-flowered trees and, as would be expected, also for all trees that have flowered. This suggests that the cause is genetic, with FR301, the common parent, contributing genes for late flowering. This is further supported by the fact that the values for the FR301 maternal half-sibling group means, i.e., 7.0 for females, 5.8 for the males and 6.4 for all 80 flowering trees, are greater than the corresponding values for the other two maternal half-sibling group means given at the bottom of Table 4.

Only one other family appears to differ markedly from the rest of the families in earliness of flowering in Experiment 1, namely OS105 x FR308. The mean age of first flowering in this family is the youngest for each of the sexes and for all trees compared with the other eleven families. It doesn't appear, however, that this is due to genetic factors for earliness since only fourteen of the 28 trees (2 died before flowering) had flowered by 1974. The family means, therefore, will increase considerably when all trees have flowered. The distribution of ages of first flowering in most families is somewhat bimodal with the first peak at age 4 and the second at 8. These peaks are also obvious for each sex in Figure 1. In family OS105 x FR308 the first peak is pronounced, with 6 of the 14 trees first flowering at 4, but the second peak doesn't really exist since only two trees flowered at 8 years of age. Perhaps it will occur in the next few years. In any event, the smaller number of older trees compared with the earlier flowering trees in this family relative to the other eleven families is responsible for the smallest mean values occurring for this group. The family may, in fact, have genetic factors for lateness rather than earliness since half of the trees have yet to flower!

The marked difference in the mean age of floral bud initiation for the trees in Experiment 4 (Table 5), 7.2 years, and in Experiment 1, 5.8 years, no doubt has a developmental cause rather than genetic. Recall, the trees in Experiment 1 were cut back in 1965 and a new basal or root sprout was selected to serve as the new stem. In the spring of 1974, these trees had 9-year-old stems and 13-year-old root systems. The trees in Experiment 4, however, developed from the original seedlings so that they were 12 years of age in 1974. In both experiments, however, trees died, were cut back due to Saperda galls, or were girdled by voles so that all trees are not the same age in 1974. The number of younger trees in Experiment 1 is only 37, which is 11.0% of the 336 trees still living in 1974. Of these, 15

Table 5.--Mean and range in age of trees in Experiment 4 when floral buds were first formed.

Male Parent	Sex	Age in Years											
		Female Parents									Paternal half-sib group		
		LE203			ES110			HE107					
		Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range	N
FU101	Female	7.2	2-11	15	8.0	5-11	6	7.8	3-11	17	7.6	2-11	38
	Male			--			--			--			--
	Unknown			--			--			--			--
	Total	7.2	2-11	15	8.0	5-11	6	7.8	3-11	17	7.6	2-11	38
ES208	Female	7.5	6-11	10	8.8	6-11	5	7.4	5-11	11	7.7	5-11	26
	Male	7.3	7-8	6	8.5	7-11	6	5.5	2-7	11	6.7	2-11	23
	Unknown	9.0	9	1	7.0	7	1	8.5	6-11	2	8.2	6-11	4
	Total	7.5	6-11	17	8.5	6-11	12	6.6	2-11	24	7.3	2-11	53
OA102	Female	7.7	3-11	12	8.0	7-9	4	6.4	3-9	13	7.1	3-11	29
	Male	7.5	5-11	12	8.1	2-11	8	6.4	3-9	11	7.3	2-11	31
	Unknown			--	4.0	4	1			--	4.0	4	1
	Total	7.6	3-11	24	7.8	2-11	13	6.4	3-9	24	7.2	2-11	61
LE108	Female	6.9	5-7	10	7.0	5-9	3	7.2	2-11	9	7.1	2-11	22
	Male	5.8	2-8	14	7.9	6-11	11	6.5	3-11	11	6.6	2-11	36
	Unknown			--	6.0	4-9	3			--	6.0	4-9	3
	Total	6.2	2-8	24	7.4	4-11	17	6.8	2-11	20	6.8	2-11	61
											Grant Total		
Maternal half-sib group	Female	7.3	2-11	47	8.1	5-11	18	7.2	2-11	50	7.4	2-11	115
	Male	6.7	3-11	32	8.1	2-11	25	6.1	2-11	33	6.9	2-11	90
	Unknown	9.0	9	1	5.8	4-9	5	8.5	6-11	2	6.9	4-11	8
	Total	7.1	2-11	80	7.9	2-11	48	6.8	2-11	85	7.2	2-11	213

have flowered. Three are female, eight are male, and four are of unknown sex. Their effect would be to reduce the mean age of first flowering based on all trees, as their average is 4.4 years. The effect of the younger trees on the Experiment 4 results would be much greater as 105 of the 305 living trees in 1974 arose from sprouts. This constitutes 34.4% of the trees, or three times as many as in Experiment 1. The greater damage in this experiment was primarily due to extensive girdling by voles in 1967. Forty-eight have flowered which includes 25 female, 19 male, and 4 of unknown sex. Their average age for first floral bud formation is 4.3 years. This compares with an average age of 7.99 years for first floral buds for the original trees (164 flowering trees). The net effect has been to reduce the average age to 7.2 years for all trees. It should be noted that the average age of first floral bud formation for the younger sprout trees is almost exactly the same for Experiments 1 and 4, namely 4.4 and 4.3 years, respectively. This provides additional support to the conclusion that the difference in age for first flowering in these two experiments is due to differences in their developmental histories rather than genetic. It appears that stems of sprout origin will flower, on the average, two to three years earlier than those of seedling origin.

The variation in age of floral bud initiation in Experiment 4 trees is shown in Table 5, The differences between the sexes and among the families and half-sibling groups is similar to that observed in Experiment 1. The difference between the sexes is not as large, namely 6.9 years for staminate- and 7.4 for pistillate-flowered trees. This can also be seen in Figure 2 in which there are no big differences in flowering in the two sexes in any year except perhaps 1974 with 18 females and 8 males. This difference in age of the two sexes occurs in 6 of the 9 families in which both sexes occur. The exceptions are family HE107 x OA102 in which there is no difference, ES110 x OA102 with the females' mean age 8.0 and males', 8.1, and ES110 x LE108 with female and male means of 7.0 and 7.9, respectively. The numbers of flowering trees in these last two families are not large, so that this could represent chance variation. It could, however, be in part genetic as trees ES110 and OA102 are each one of the parents in two of the three families. In addition, the mean ages for the two sexes in the two half-sibling groups is either the same (the ES110 maternal half-sibling group) or the female's mean is smaller than the male mean (7.1 and 7.3, respectively, for paternal half-sibling group OA102).

Some of the extreme mean values for earliness and for lateness in flowering appear to be associated with particular parents. Female parent ES110 seems to contribute genes for lateness to all of its half-sibling progeny families. If the means for the female, male, and the family are compared in the three families making up each of the four paternal half-sibling groups, the values for the family with ES110 as the female parent are the largest except for the female mean in the LE108 paternal half-sibling group. The HE107 x LE108 female mean value is the largest in this group. The effect of ES110 is also reflected in comparisons of the means of the maternal half-sibling groups, namely, values for the ES110 maternal half-sibling group are the largest!

Two trees appear to have genetic factors for earliness, HE107 and LE108. These factors, however, are not as consistently expressed in the half-sibling groups of the two trees when comparisons of family means are

Age in years		Number of trees 0 5 10 15 20 25 30 35 40 45 50
1	Female	
	Male	
	Unknown	
2	Female	XX
	Male	XXX
	Unknown	
3	Female	XXXX
	Male	XXXXXX
	Unknown	
4	Female	XX
	Male	XXX
	Unknown	XX
5	Female	XXXXXXX
	Male	XX
	Unknown	X
6	Female	XXXXXXXXXX
	Male	XXXX
	Unknown	X
7	Female	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
	Male	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
	Unknown	X
8	Female	XXXXXX
	Male	XXXXXXXXX
	Unknown	
9	Female	XXXXXXXXXXXX
	Male	XXXXX
	Unknown	
10	Female	X
	Male	
	Unknown	
11	Female	XXXXXXXXXXXXXX
	Male	XXXXXXX
	Unknown	X

Figure 2.--Age of first floral bud formation of pistillate- and staminate-flowered trees in Experiment 4.

made. The most unexpected result is that the family in which both of these two trees were the parents (HE107 x LE108) does not rank as the earliest flowering in either of the two parental half-sibling groups! If the two parents do have genes for earliness in flowering, they obviously are not additive in their effects. In fact, the interaction appears to have the opposite effect, resulting in lateness in flowering.

These sets of data for Experiments 1 and 4 have been analyzed using an Analysis of Variance so that estimates of the heritability for earliness of flowering could be made based upon the covariances of maternal and paternal half-sibling groups. The results of these analyses are presented in Table 6. The method of analysis using plot means for partitioning the between families sums of squares was followed for ease of calculation because of the unequal numbers of trees per family (see Kempthorne 1957). The heritability values are not especially large for either experimental group, but the estimates based on the covariances of maternal half-sibling families are larger than those based on the parternal half-sibling covariances. This could mean that there are also maternal or cytoplasmic effects present as well. The difference in size of the estimates in the two experiments probably represents chance variaiton due to the small number of parents. The real values probably occur in the range obtained here, namely 0.13 to 0.40.

<u>Frequency of Flowering.--The</u> results of the observations on the frequency of flowering for Experiments 1 and 4 are presented in Table 7. These data are based on trees that initially flowered prior to 1974, i.e., only trees that could have flowered two or more years. This has greatly limited the number of trees, especially in Experiment 1 as many trees flowered for the first time in 1974 (see Figures 1 and 2). The 1974 flowering data for trees that had previously flowered, however, are included in these results. The data are presented in two ways, the mean number of years of flowering and the mean frequency of flowering. This latter value is calculated for each tree by dividing the number of years flowering occurred by the number of years that have elapsed since flowering was initiated. If a tree flowered every year, the flowering frequency would be 100%. These data are presented for each sex and for trees whose sex was not determined in each family, each maternal and paternal half-sibling group, and the grand totals for each experiment. A statistical analysis had not been completed as the numbers of trees are small, especially in certain families.

Perhaps the most surprising result is the high frequency of flowering in both sexes. In Experiment 1, female trees flowered, on the average, approximately two out of three years (67%) once flowering had begun, and males, almost 80% of the years. The frequencies for the two sexes is even higher for Experiment 4, being 82% and 88% for females and males, respectively. This difference between the sexes is in accord with general observations that male trees flower more often than females. It amazed me, however, that the difference wasn't larger! Comparisons of the flowering frequencies of the two sexes in each family and in the pooled half-sibling groups show a general accord with this sex difference. Where exceptions occur, the numbers of trees are relatively small so that chance variation could be a factor. The higher frequencies observed for Experiment 4 compared with Experiment 1 could represent genetic differences

Table 6.--Analysis of variance and estimation of heritabilities for earliness of flowering in Experiments 1 and 4.

Experiment 1

Analysis of Variance

Source of Variation	d.f.	m.s.	Value of F
Based on Individual Tree Values			
Between families	35	4.87	1.752*
Within families	193	2.78	
Based on Family Mean Values			
Replications	2	0.88	
Between females	2	4.46	1.604
Between males	3	2.67	0.960
Female x male interaction	6	0.38	
Female-male x replications	22	0.58	

*Significant at the 5% level of probability.

Estimates of Heritabilities

$h^2_{\text{females}} = 0.405$

$h^2_{\text{males}} = 0.298$

$h^2_{f+m} = 0.351$

Experiment 4

Analysis of Variance

Source of Variation	d.f.	m.s.	Value of F
Based on Individual Tree Values			
Between families	23	6.14	1.326
Within families	189	4.63	
Based on Family Mean Values			
Between replications	11	0.29	
Between females	2	2.45	0.529
Between males	3	1.38	0.298
Female x male interaction	6	0.42	
Female-male x replications	11	0.64	

Estimates of Heritabilities

$h^2_{\text{females}} = 0.207$

$h^2_{\text{males}} = 0.130$

$h^2_{f+m} = 0.169$

Table 7.--Mean number of years of flowering and frequency of trees flowering prior to 1974 in Experiments 1 and 4.

Experiment 1

		Female Parent											
		OS105			FR301			CL307			Paternal Half-sibling Group		
Male Parent	Sex	N	Mean No. Years	%	N	Mean No. Years	%	N	Mean No. Years	%	N	Mean No. Years	%
FU101	Female	9	2.44	55	5	3.00	68	10	2.80	65	24	2.71	62
	Male	--			--			3	3.67	79	3	3.67	79
	Total	9	2.44	55	5	3.00	68	13	3.00	68	27	2.81	64
FR308	Female	3	3.00	64	--			1	2.00	50	4	2.75	61
	Male	6	3.50	72	8	3.12	83	14	3.00	74	28	3.14	76
	Unknown	1	1.00	33	2	1.50	50	--			3	1.33	44
	Total	10	3.10	67	10	2.80	78	15	2.93	72	35	2.94	72
CL306	Female	4	2.75	79	5	2.80	88	4	3.50	82	13	3.00	83
	Male	9	3.22	76	7	4.00	90	10	3.40	76	26	3.50	80
	Unknown	2	1.00	33	1	1.00	50	2	1.50	50	5	1.20	43
	Total	15	2.80	72	13	3.31	88	16	3.19	75	44	3.09	78
ES203	Female	2	2.50	36	2	4.00	89	3	3.67	85	7	3.43	67
	Male	6	3.00	67	10	3.70	88	11	3.73	82	27	3.56	81
	Unknown	1	1.00	33	--			--			1	1.00	33
	Total	9	2.67	52	12	3.75	88	14	3.71	83	35	3.46	77
												Grand total	
Maternal Half-sib Group	Female	18	2.61	57	12	3.08	79	18	3.06	71	48	2.90	67
	Male	21	3.24	72	25	3.60	87	38	3.37	77	84	3.40	79
	Unknown	4	1.00	33	3	1.33	50	2	1.50	50	9	1.22	42
	Total	43	2.77	63	40	3.28	83	58	3.21	75	141	3.09	73

Experiment 4

		Female Parent											
		LE203			ES110			HE107			Paternal Half-sibling Group		
Male Parent	Sex	N	Mean No. Years	%	N	Mean No. Years	%	N	Mean No. Years	%	N	Mean No. Years	%
FU101	Female	9	3.33	80	2	2.50	40	9	3.11	68	20	3.15	72
	Male	--			--			--			--		
ES208	Female	8	3.75	91	2	3.50	88	8	3.88	82	18	3.78	86
	Male	5	4.00	83	5	3.00	75	10	3.80	95	20	3.65	87
	Unknown	1	1.00	33	1	2.00	40	--			2	1.50	38
	Total	14	3.64	85	8	3.00	73	18	3.83	88	40	3.60	84
OA102	Female	8	3.25	79	4	3.75	94	8	4.12	85	20	3.70	84
	Male	9	4.44	89	5	4.20	91	11	4.09	87	25	4.24	88
	Unknown	--			1	2.00	67	--			1	2.00	67
	Total	17	3.88	85	10	3.80	90	19	4.11	86	46	3.96	86
LE108	Female	10	4.40	86	3	3.00	90	8	3.62	78	21	3.90	84
	Male	13	4.23	96	9	3.56	84	9	3.67	85	31	3.87	90
	Unknown	--			3	1.00	38	--			3	1.00	38
	Total	23	4.30	92	15	2.93	79	17	3.65	82	55	3.73	85
												Grand total	
Maternal Half-sib Group	Female	35	3.71	84	11	3.27	86	33	3.67	78	79	3.53	82
	Male	27	4.26	91	19	3.58	84	30	3.87	89	76	3.93	88
	Unknown	1	1.00	33	5	1.50	44	--			6	1.33	42
	Total	63	3.90	87	35	3.17	80	63	3.76	83	161	3.69	84

between the parent trees used in each experiment, This is also supported by a comparison of the flowering frequencies of pistillate-flowered trees with FU101 as the common male parent in each experiment. For Experiment 4, the frequency is 72% and for Experiment 1, 62%. This presumably would be due to the different female trees in each group. It could, however, represent a difference between trees of seedling origin and of sprout origin, with those of seedling origin flowering more frequently! Another factor is chance, since the numbers of trees in these groups are quite small.

Comparisons of maternal and paternal half-sibling group means do not reveal large variations in frequencies of flowering within each experiment except for female parent OS105 in Experiment 1 and FU101 in both experiments. The half-sibling group in which each of these trees is the common parent exhibits a lower frequency of flowering than the other half-sib groups. This is also generally found when families with these trees as one parent are compared to other families in the half-sibling group. For example, if the frequencies of flowering of the pistillate-flowered trees in the FR301 maternal half-sibling families are compared, the frequency for family FR301 x FU101 female trees 68%, is the lowest in the four families.

Comparisons of the data for the mean number of years of flowering in each experiment show that the value for the staminate-flowered trees is usually greater than that for the pistillate-flowered trees within families, within half-sibling groups, and in each experiment. This, no doubt, reflects two previously observed differences between the sexes, namely that males flower at a younger age (see Tables 4 and 5) hence would have more years in which to flower, and males flower more frequently. There are a few exceptions to this in the family means and also half-sibling group means, but chance could be a factor as the numbers of trees in the exceptional families are relatively small. There aren't any obvious patterns of relationship of the exceptional families that can be linked to a common parent, so it is not likely that genetic factors are involved. Larger numbers of trees and greater number of years of observation of flowering are needed to elucidate the role of genetic factors in determining the frequency of flowering in trembling aspen.

SUMMARY

Results are presented from observations of flowering in thirty-six families of Populus tremuloides over a seven-year period, 1968 through 1974. The families originated from controlled crosses of trees from 15 natural stands scattered throughout the Adirondack Mountains and adjoining regions of New York State. A system of mating was used to permit the estimation of the heritability of quantitative traits on the basis of the covariances of maternal and paternal half-sibling families. One experimental group of 12 families, produced in 1961, was cut back at four years of age because of heavy insect damage and basal or root sprouts were selected as the new stems. In 1974 these trees have 9-year-old stems on 12-year-old root systems. The other 24 families are of seedling origin and 12 years old in 1974.

The sex ratio, based on 600 flowering trees in all families does not differ from a 1:1 ratio, but if progeny of one male parent are excluded,

the deviation of the observed ratio from an expected 1:1 ratio is highly significant, Males then far outnumber females. Quite by chance this male was the only tree used in two of the experimental groups of 12 families each and so has a disproportionate effect. It produced 116 female and 4 male progeny, plus 2 whose sex wasn't determined! In most families, however, the males outnumbered females but this probably is also an age effect. The ratios vary among half-sibling groups of families, hence there appears to be a strong genetic determination for sex ratio.

Earliness of flowering also appears to be under strong genetic control, with some evidence for sexual dimorphism and a bimodality in the distribution for age at first floral initiation. Males flower earlier than females, with an average age difference of 0.9 years for trees of sprout origin and 0.5 years for those derived from seedlings. This, of course, affects the sex ratio in younger stands. The bimodality is due to two peaks that occur at ages 4 and 8 for the trees of sprout origin and at 7 and 11 for those of seedling origin. The first peak, in 1971, does not coincide with a "good seed year" for aspen so that it is felt that the distribution does not wholly reflect variations in environmental factors affecting floral initiation. There is also evidence for family differences attributable to common parentage.

Estimates of the heritability for earliness of flowering are not particularly large, being .405 and .298 based upon covariance estimates for female and male half-siblings in one group and .207 and .130, respectively, for a second group. The numbers of parent trees, however, were small so that these differences probably represent chance variation. The higher values based upon the contributions of female parents could reflect maternal effects. These values probably are underestimated and would no doubt increase if variability due to sex dimorphism and bimodality in the distribution of age at first flowering were partitioned as sources of variation. This should decrease the estimate of the environmental variance, hence increase the heritability estimates.

Information on frequency of flowering is quite limited due to the smaller number of trees available for study. Only trees flowering prior to 1974 were used. Several families had only five or fewer trees so that an analysis was not attempted. There is good evidence for sexual dimorphism for this trait, but the difference isn't large, being 12% for the trees in one experiment and 6% for the other. In both groups the frequencies are surprisingly high, with values of 73% and 84% based on all flowering trees in each of the two experiments. There is some evidence that lower flowering frequencies in certain families are due to factors contributed by a common parent. High frequencies in families, (several in both experiments were close to 90%) however, do not appear to be attributable to any particular parent. Larger numbers of trees and more years of observation are needed to elucidate the role of genetic factors in determining the frequency of flowering in aspen.

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