SEX EXPRESSION IN *POPULUS TRICHOCARPA* (T. & G. ex HOOK.) AND IN ITS HYBRIDS

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Summary

Sex expression was studied in 1964 and 1965, in two female and four male trees of P. *trichocarpa*, and in two female P. *trichocarpa* hybrids. Numbers of catkins collected ranged from 54 to 647 per tree and were taken from permanently designated sample branches. One female tree contained hermaphroditic flowers in 1964, the two female hybrids contained hermaphroditic and male flowers in both years. Sex expression varied markedly among branches of a single tree but independently of their position in the crown. The majority of deviant female catkins were curved, the hermaphroditic and male flowers occurring on the inside of the curve in the area of maximum curvature. The results support the hypothesis that low auxin concentrations are associated with male sex expression.

Introduction

All species in the genus *Populus*, except for P. *lasiocarpa* Oliv., are basically dioecious, that is, male (staminate) and female (pistillate) catkins (aments) are carried on different trees. However, deviations from this fundamental pattern toward hermaphroditism have been reported for at least seven different species and several interspecific hybrids (reviewed in Lester 1963). Furthermore, Seitz (1954) has demonstrated that hermaphroditic individuals are capable of effective selfing. No published data have been found on sex expression in P. *trichocarpa* T & G. ex Hook., the native black cottonwood.

Information on breeding mechanisms is essential for our understanding of the genetic structure of natural populations. Furthermore, the study of breeding mechanisms frequently leads to the discovery of sex-deviant individuals which may become excellent experimental material for research on sex controlling mechanisms. Both aspects are of significance to our present studies concerning the production of homozygous material in black cottonwood (Stettler et al. 1965).

Before doing an extensive survey on sex expression in black cottonwood, it seemed reasonable to first investigate (a) whether there are patterns of sex expression within a crown that must be considered in a

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large-scale sampling scheme; and (b) whether sex expression in a given tree is consistent enough from year to year that comparisons made among different populations, sampled in different years, will be valid. For this purpose, it was decided to make an intensive investigation of sex expression in a small number of trees over an initial period of five years.

This paper presents the results from the first two years of this study.

Materials and Methods

During the winter of 1963/64, four female and four male sample trees were selected. Six of them are located on the University of Washington Campus, two in the White River Forest at the Mud Mountain Dam near Enumclaw, Washington. The sample trees had to be healthy individuals with well developed crowns carrying abundant flower buds.

Since the trees were selected in the winter condition it was not noticed until the spring of 1964 that two (FG#2 and #5) were not pure P. trichocarpa but contained also germ plasm from P. deltoides. On the basis of several vegetative and floral traits these two trees were classified as putative F_1 hybrids; however, they were retained as sample trees.

On each tree (except #6), four to six branches were chosen as permanent sample branches representing several positions along the vertical axis of the crown. At the peak of flowering time, catkins were collected individually from the designated branches and then stored in polyethylene bags at -20°C. On most branches, a sample of 40-100 catkins was taken more or less at random, on a few branches the entire crop. To minimize damage to the tree, resulting from climbing, most of the collection was done with the aid of a ladder truck.

In 1964, collecting began on March 31 and lasted until April 17. The majority of catkins were found at the stage of dehiscence (or peak receptivity in the case of females), some of them at earlier stages of emergence. Because of mild weather, flowering in 1965 occurred about three weeks earlier. Although the collecting was done correspondingly earlier (March 9 – April 14) some catkin abscission had occurred on trees #2 and #3 by the time of sampling.

During the summer and fall, catkins were taken out of the freezer in small lots and classified. In the beginning, a trial series was conducted in which each catkin was inspected, first by the naked eye and then under the dissection scope. As a result of the good agreement between the two methods, we decided to routinely classify four out of five catkins by the naked eye, but the fifth on the basis of a careful dissection under the microscope. Furthermore, each apparently **deviant** catkin was dissected under the microscope, and the sex expression of each flower was recorded.

Results

a) The types of deviations found

All four male sample trees showed completely normal sex expression in both years. In contrast, deviations from normal sex expression were found in three of the four females sampled. Deviant catkins contained either (a) one or several hermaphrodite flowers, or (b) one or several male flowers, or (c) both. No entirely male catkins were found on these deviant females.

Hermaphroditic flowers contained from one to seven stamens which were typically carried inside the "perianth" cup and attached to the base of the pistil. Occasional stamens had broad, flat filaments and only one to two microsporangia instead of four. However, all stamens appeared functional and those that were dissected contained pollen. The pistils of hermaphroditic flowers were indistinguishable from those of normal female flowers.

The male flowers on deviant female catkins were different from normal male flowers by having only few stamens, often contained in an irregularly shaped "perianth" cup. Sometimes, two to three flower positions were occupied by a single, extended, cup structure carrying sparsely distributed stamens. All stamens had normal filaments and four-lobed anthers containing pollen. No trace of a gynoecium was noticed at the gross morphological level in these deviant "female" flowers.

In addition to hermaphroditic and male flowers, catkins were found which contained abnormal vegetative structures near or at the apex, or occasional twin pistils in a single "perianth" cup. For the simplicity of this presentation, these deviants were classified among the "normal" category.

b) The quantity of deviations found

Table 1 summarizes for each sample tree the frequency of deviant catkins in the two consecutive years. Whereas none of the male trees showed any detectable abnormality in sex expression, one female tree showed deviations in the first year, two in both years. The first of these female trees, FG#6, was sampled in a systematic fashion in 1964, that is, morphologically abnormal catkins were preferentially included in the collection. In 1965, however, the same tree was sampled at random. Thus, we can state that deviant catkins were found on this tree in 1964, but we cannot make any quantitative comparisons between years or between this tree and the others.

In contrast, such comparisons can be made for FG#2 and #5, both hybrids, because they were subject to random sampling in both years. A chi-square test performed on the data in Table 1 suggests that FG#5 had significantly more deviant catkins than FG#2 in 1964 while the opposite was true in 1965. Furthermore, both trees had significantly less deviant catkins in 1965 than in 1964.

Table 1. Percentage of deviant catkins for each sample tree, in two consecutive years. [1]

SAMPLE TREES	SPECIES [2]	1964		1965	
		Sample Size	% Deviant	Sample Size	% Deviant
MALES					
FG# 1	T	371	None	617	None
FG# 4	T	496	None	523	None
FG#101	T	327	None	647	None
FG#102	T	469	None	384	None
FEMALES					
FG# 2	TxD	479	10.2	428	5.8
FG# 3	T	239	None	280	None
FG# 5	TxD	338	19.8	533	0.9
FG# 6	T	54*	18.5	173	None

1 Deviant = catkins containing at least one hermaphroditic or opposite-sex flower.

2 T = _P. trichocarpa_; TxD = _P. trichocarpa_ x _deltoides_ hybrid.

* non-random.

Table 2. Percentage of deviant catkins on each sample branch of trees FG#2 and 5.

Tree FG#	Branch # (1)	1964 Sample size	1964 % normal	1964 % Herm-aph.(2)	1964 % Male (3)	1965 Sample size	1965 % normal	1965 % Herm-aph.(2)	1965 % Male (3)
2	1	107*	82.3	11.2	6.5	108*	96.3	2.8	0.9
	2	122	78.7	18.0	3.3	101	85.2	8.9	5.9
	3	106	98.2	0.9	0.9	89	94.4	5.6	---
	4	99	98.0	1.0	1.0	89	100.0	---	---
	5	45*	100.0	---	---	41*	97.6	2.4	---
5	1	65	83.1	7.7	9.2	102	99.0	1.0	---
	2	43	86.1	11.6	2.3	219*	99.1	0.9	---
	3	100	77.0	11.0	12.0	104	98.1	---	1.9
	4	106	76.4	14.2	9.1	99	100.0	---	---
	5	22	90.9	9.1	---	9*	100.0	---	---

(1) Branches are numbered in acropetal sequence

(2) Hermaph. = catkins containing at least one hermaphroditic but no male flower

(3) Male = catkins containing at least one male flower

* Complete collection.

Table 3. Average number of deviant flowers for three different types of catkins.[1]

Type	Number of catkins analyzed	Number of flowers				Av. number of deviant flowers per catkin	
		normal	hermaph.	male	Total	hermaph.	male
Straight	122	4146	63	4	4213	0.51	0.03
Curved[2]	58	1810	95	11	1916	1.64	0.19
Sharply curved[3]	43	1156	119	58	1333	2.76	1.35

[1] pooled data from trees FG# 2 and 5, for 1964 and 1965.

[2] 45-90-degree angle.

[3] 90-degree angle and more.

Table 4. Number of catkins in each of three different categories of deviant sex expression.[1]

Categories	1964	1965
Catkins with hermaph. but no male flowers	93	20
Catkins with hermaph. and male flowers	53	13
Catkins with male but no hermaph. flowers	5	1
Total	151	34

[1] Pooled data from trees FG# 2 and 5, including non-random samples from tree #5 of 36 inflorescence, in 1964, and 4 in 1965.

To illustrate the internal variation of these two trees, a branch-by-branch summary for the two years is presented in Table 2. Considerable variation in sex expression was found between branches within a single crown. Some branches were consistently normal (2.4, 2.5) or consistently deviant (2.2) in both years. But most branches had no consistent pattern except that their percentage of deviant catkins was generally lower in the second year. The overall decrease in deviant catkins in 1965 was equally reflected in "hermaphroditic" and "male" categories. The degree of sex deviation of a particular branch seemed to be independent of its vertical position in the crown.

Many of the comparisons made are subject to sampling errors, although attempts were made to collect the catkins from the same portion of a branch in the two consecutive years. To overcome this problem in the future, we made efforts to collect all available catkins on certain sample branches (indicated in Table 2). This will help in our year-to-year comparisons of sex expression, and it will also allow to estimate the year-to-year variation in total quantity of catkins produced.

c) <u>Observations pertaining to a possible mechanism underlying sex</u> control

In contrast to the straight axis of normal catkins, the majority of deviant catkins had a curved axis, sometimes in the form of a loop or even a double loop. As soon as this trend was noticed, records were kept on the shape of each dissected catkin. The data were compiled and analyzed separately by tree and year but showed so little variation that they were pooled (Table 3). They indicate a positive correlation between the curvature of a catkin and its degree of deviation from the normal female sex expression toward maleness. The tendency toward maleness can be expressed as the number of hermaphroditic flowers per catkin, or as the number of male flowers per catkin, or as an index of both. Table 3 shows that either of the two parameters increased with the degree of curvature of a catkin.

We further observed on curved catkins that the deviant flowers typically occurred on the inside of the curve, clustered around the area of maximum curvature.

Lastly, we found that most male flowers occurred together with some hermaphroditic flowers (Table 4), the males occupying the position around maximum curvature, the hermaphrodites flanking them on either side.

Discussion and Conclusions

The intensive study of sex expression in eight trees, during two consecutive years, suggests that P. *trichocarpa*, as many other species in the genus *Populus*, is not perfectly dioecious but harbors individuals with bisexual potentialities. Whether this phenomenon is a common or a rare event cannot be determined until extensive surveys of natural populations have been conducted.

Two of the three deviant trees were hybrids. Several previous studies have shown that interspecific hybrids in the genus *Populus* have a tendency toward imbalanced sex expression (Seitz 1953, Jovanovic et al. 1960, Einspahr 1962). This imbalance in sex differentiation may be attributed to a possible imbalance in sex factors in the hybrid genome. At present, we can state that, whereas a P. *trichocarpa* female was deviant in one but not in the second year, the two hybrid females were deviant in both years. Additional years' records will be required to verify this apparent trend. Similarly, the observation that all deviant trees were females must be interpreted with caution, although it is supported by similar findings in P. *tremuloides*, based on much larger numbers (Santamour 1956, Pauley et al. 1957).

No systematic pattern was found in the distribution of deviant catkins in the crown. The same conclusion was reached by Lester (1963) in his extensive study of sex expression in P. *tremuloides*. This information may be useful in the further planning of large-scale surveys of sex expression in natural populations of black cottonwood. It suggests that random sampling of crowns may be as informative as systematic sampling. On the other hand, it may be advantageous to sample several branches of a tree at moderate intensity rather than a single branch at high intensity, since there is indication for a between-branch component of variation in sex expression (Table 2).

In our material, similar to that in aspen (Lester 1963), we found different degrees of deviations in fundamentally female catkins, ranging from an individual pistillate flower with a single, misshaped, stamen to several staminate flowers, with many intermediate forms. The fact that these different degrees of maleness were not randomly distributed over the catkin, but in an orderly sequence, strongly suggests that sex expression is responsive to gradients in the concentration of one or several substances. Auxins have been demonstrated to play a significant role in the differentiation of floral structures in several angiosperms (Heslop-Harrison 1957, Galun et al. 1963), high concentrations being associated with femaleness, low concentrations with maleness. This model would help to explain the characteristic curvature of deviant catkins in our material and particularly the phenomenon that hermaphroditic and male flowers commonly occurred on the inside of the curve and in the zone of maximum curvature.

Studies have been initiated to test the auxin hypothesis of sex control, utilizing the sex-imbalanced hybrids as initial experimental material.

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