Variation in Sex Expression of Black Cottonwood and Related Hybrids

By REINHARD F. STETTLER*)

Introduction

Sexual polymorphism has been well studied in the Genus Populus, notably in the Sections *Leuce* DUBY and Aigeiros DUBY. In these sections all the species studied generally conform to the principle of dioecism, but varying proportions of individuals deviate from this principle by being bisexual (see the reviews of LESTER, 1963 and MELCHIOR, 1967). By contrast, comparatively little is known about sexual polymorphism in section Tacamahaca SPACH. in general (SAUER, 1954) or the Black cottonwood, Populus *trichocarpa* T. & G. ex HOOK, in particular.

This paper describes a study of sex expression in Black cottonwood made during the period 1964 to 68. Its object was to find out, using a small number of individuals, whether there exists variation in sex expression within an individual tree during the same or different years. This information would help in the design of sampling schemes for subsequent studies of natural populations. An additional object was to gain information that might have a bearing on the mechanisms of sex differentiation and their possible control. In our program on experimental induction of haploid parthenogenesis in Black cottonwood (STETTLER and BAWA, 1971) we anticipate the possibility that all parthenogenotes will be of the same sex. This would, of course, limit their usefulness in any breeding scheme. To this end, information on possible ways of manipulating sex differentiation would be desirable. Even for more general breeding programs with poplars, directed manipulation of sex differentiation would be a valuable tool to the breeder whenever he wants gene exchange between and within individuals or clones of the same sex.

Materials and Methods

In the winter of 1963164 a sample of four female and four male trees were selected. They were (a) apparently healthy; (b) with a well developed crown carrying ample flower buds; (c) accessible with a ladder truck; (d) located closely enough to permit periodic inspection and intensive follow-up studies if necessary; and (e) seemingly safe from man and other agents inimical to long-term investigations. Six trees were found on the University of Washington campus, two in a University Forest near Enumclaw, King County, Washington. Their age was estimated to range from 15 to 25 years. Whereas six of the eight trees were pure Populus trichocarpa T. & G. ex HOOK it became apparent in the spring of 1964 that two trees (FG 2, FG 5) contained also germ plasm of another poplar species. According to several fruit and leaf characters (capsule shape and surface, leaf shape, margin, and undersurface, petiole cross-section) they were considered as F, hybrids or advanced-generation recombinants between P. trichocarpa and P. deltoides BARTR. Because these two trees showed much deviation in sex expression they were nevertheless retained as sample trees. Unfortunately, one of them (FG 5) was cut after two years of study.

On each tree, four to six branches were chosen and designated as permanent sample branches at different positions on the vertical axis of the crown. In the five consecutive springs of 1964—68, female catkins were collected, if possible at peak receptivity, and male catkins were collected immediately prior to anther dehiscence. However, due to phenological variation within a tree and between years, many catkins had to be collected at earlier or later stages. In 1966, tree FG 101 had shed most of its catkins at collection time and was therefore excluded from that year's sampling. Catkins were carefully removed from the branch, placed in polyethylene bags and stored at —20° C. On most branches, a sample of 40—100 catkins was taken, on a few branches the entire crop was collected. To minimize damage to the tree most of the collection was done from a ladder truck.

The catkins were inspected during summer and fall, being taken from the freezer in small lots and thawed. Initially, we compared inspction by the naked eye with that under the dissection microscope. The agreement was good and we decided to examine four out of five catkins by naked eye while the fifth, as well as any catkins showing abnormalities, was inspected under the microscope. Those catkins inspected under the microscope were dissected, flower by flower, each flower classified and recorded as to its position on the axis. For the anatomical study of floral primordia in tree FG 2, we collected the buds, removed the bud scales and most bract primordia and fixed the material in Formalin-Propiono-Alcohol (JOHANSEN, 1940) for at least 24 hours. After dehydration in tertiary butyl alcohol the primordia were infiltrated with paraffin, sectioned at 10μ and stained with Safranin 0 and Fast Green according to the method of JOHANSEN (1940).

Results

Morphological *and* anatomical observations

In all five years, the four male trees produced normal staminate catkins that agreed with the descriptions of BRAYSHAW (1965) and HITCHCOCK et *al.* (1955) and conformed with the principle of dioecism in poplars. Since this material contributed little to our understanding of the factors governing sex expression in Black cottonwood, any detailed descriptions are omitted irom further discussion.

By contrast, three of the four female trees showed various degrees of departure from dioecism. In Black cottonwood, the normal pistillate catkin carries an average of 30 to 60 and occasionally more than 100 pistillate flowers, the mean number varying significantly among different trees (STETTLER and BAWA, 1971). The flowers are spirally arranged on the catkin axis, each subtended by a laciniate bract and each carrying a subglobose to broadly ovate pistil inside a perianth cup (FISHER, 1928; BRAYSHAW, 1965). The abnormal catkins found on four female sample trees had one to several hermaphroditic and/or staminate flowers among a majority of pistillate flowers. No entirely staminate catkins were found on these three female trees. The various types of abnormal flowers are illustrated in Figs. 1 *and* 2.

Hermaphroditic flowers appeared in several forms. Some had antheroid structures attached to the inside of the cup (Figs. 1a, *2c)*, or to the carpel wall, termed "Rucksack" anthers (SEITZ, 1953) (Fig. *1*b). Others had a single antheroid subtended by a broad, laminar structure emerging from between the carpel base and the cup (Fig. *1c)*. These antheroids had one to three poorly developed, often twisted,

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*) Associate Professor, College of Forest Resources, University of Washington, Seattle, Washington 98105.

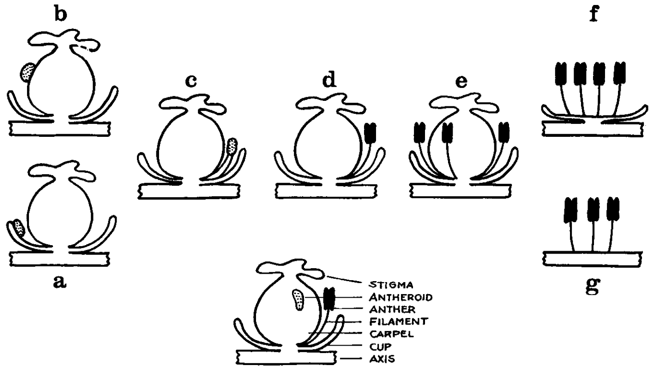

Fig. 1. — Semi-diagrammatic representation of abnormal flower types found, with the degree of maleness increasing from left to right.

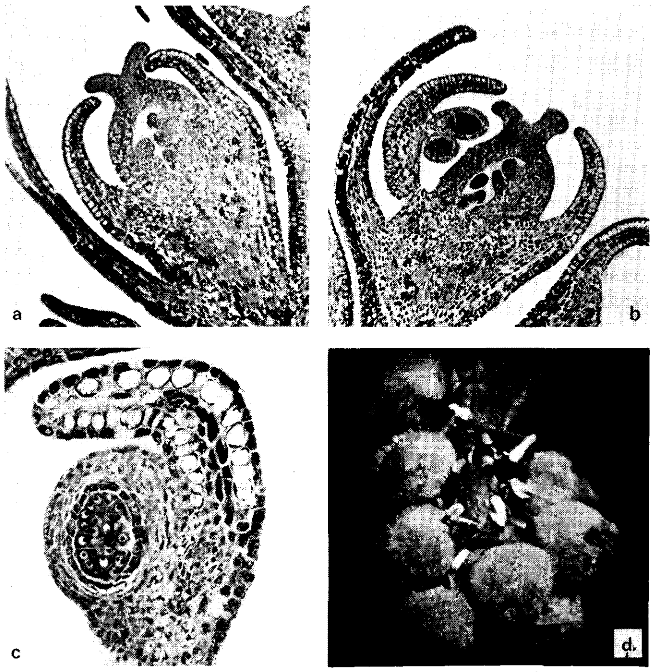

Fig. 2. — Flowers from abnormal catkins; *a*—*c*, longitudinal sections through developing floral primordia, early November; *a*, normal pistil with subtending bract, perianth cup and developing ovules, 140×; *b*, hermaphroditic flower with developing ovules and bilobed anther containing sporogenous tissue, 140×; *c*, developing perianth cup from an hermaphroditic flower with attached antheroid containing sporogenous tissue, 225×; *d*, male and hermaphroditic flowers borne on the inside of a curved catkin, April, 6×.

lobes containing small amounts of pollen. A third group of hermaphroditic flowers had from one to seven normal, four-lobed, anthers that contained pollen and were carried on a regular filament attached at the carpel base *(Fig. 1d—e)*. The gynoecia of hermaphroditic flowers were comparable to those of normal female flowers, both during development *(Figs. 2a—b)* and after emergence, and contained apparently functional ovules. The male flowers on abnormal female catkins were different from normal staminate flowers by having 1—22 stamens (rather than the normal 40—60) contained in an irregularily shaped cup *(Fig. 1f)*. Sometimes, two to three flower positions were occupied by a single, extended, cup carrying sparsely distributed stamens *(Fig. 2d)*. In a few cases, no trace was found of a cup, the stamens being directly attached to the axis *(Fig. 1g)*. All stamens had normal filaments and four-lobed anthers containing pollen.

Table 1. — Number of normal and abnormal catkins in the eight sample trees.

Predominant sex	Tree Number	1964			1965			1966			1967			1968		
		Normal	Abnormal		Normal	Abnormal		Normal	Abnormal		Normal	Abnormal		Normal	Abnormal	
		Number	Number	%	Number	Number	%	Number	Number	%	Number	Number	%	Number	Number	%
Male	FG 1	371	—	—	617	—	—	497	—	—	513	—	—	492	—	—
	FG 4	496	—	—	523	—	—	472	—	—	398	—	—	420	—	—
	FG 101	327	—	—	647	—	—	Not Sampled			272	—	—	313	—	—
	FG 102	469	—	—	384	—	—	393	—	—	382	—	—	394	—	—
Female	FG 6	54	—	—	173	—	—	247	—	—	259	—	—	225	—	—
	FG 3	239	—	—	280	—	—	268	1	0.4	363	—	—	322	—	—
	FG 2*)	430	49	10.2	403	25	5.8	260	70	21.2	390	2	0.5	408	—	—
	FG 5*)	271	67	19.8	528	5	0.9	—	—	—	—	discontinued		—	—	—

*) Hybrids

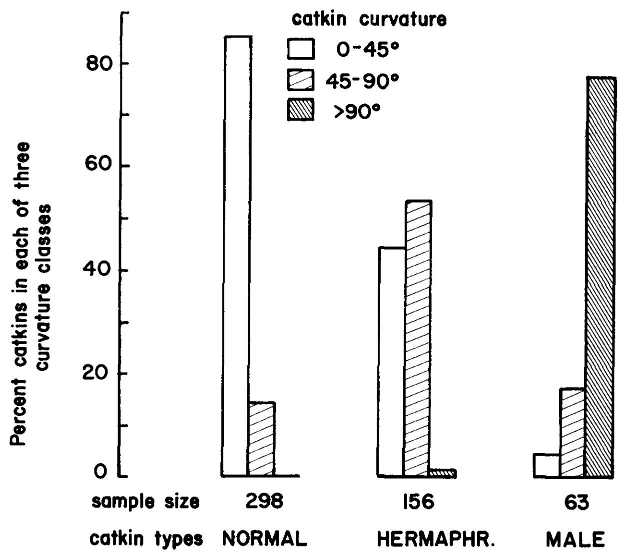

Fig. 3. — Relationship between catkin curvature and degree of maleness.
Normal = catkins with only pistillate flowers;
Hermaphr. = catkins with one or more hermaphroditic, but no male, flower(s);
Male = catkins with one or more male flower(s).

Althogether, the abnormal flower types fitted a quantitative scale ranging from almost normal femaleness to almost normal maleness, with various bisexual forms occupying an intermediate position *(Fig. 1a—g).*

On most abnormal catkins having more than one abnormal flower the latter were aggregated in "hot spots" rather than distributed randomly. Fifty eight out of 63 abnormal catkins having male flowers also contained hermaphroditic flowers, apart from the normal female flowers.

Whereas the bulk of normal catkins had a straight axis, those containing abnormal flowers typically were curved, often looped. A contingency test showed the association between the two traits to be significant at the 0.1 percent level. Furthermore, the degree of curvature was associated with the degree of maleness: the proportion of sharply curved catkins was much higher among "male catkins" (that is catkins containing at least one male flower) than among "hermaphroditic catkins" (that is catkins with at least one hermaphroditic, but no male, flower), (see *Fig. 3*).

On these curved catkins, the abnormal flowers occurred typically on the inside of the curve clustered around the zone of maximum curvature *(Fig. 2d),* the male flowers usually flanked on either side by hermaphroditic flowers. Anatomical examination of abnormal catkin primordia showed no signs of actual or incipient curvature before bud burst. The curvature developed by differential elongation of the axis during emergence from the bud and was fully expressed by the time the pistillate flowers were receptive.

Variation between years

The frequency of abnormal catkins (those having at least one abnormal flower) varied markedly from one year to another *(Table 1).* In tree FG 2, this variation was highly significant ($\chi^2 = 163.3$, with 4 d. f.). More importantly, abnormal catkins were found in four out of five years but not in the fifth year, indicating that a tree may produce normal flowers in one year and abnormal flowers in others. Because of the small sample of trees it is impossible to tell whether the variation observed in tree FG 2 followed a unique or more widely representative pattern. However, apparent parallels were noted in two other female trees. Tree FG 5, the other hybrid, dropped from high departure in 1964 to low departure in 1965, as did tree FG 2. Tree FG 3, a normal Black cottonwood, showed its only departure from femaleness in 1966, the year when tree FG 2 had its peak departure for the entire 5-year period.

Table 3. — Catkins sampled during the 1966—68 breeding studies.

	1966	1967	1968
Number of female sample trees	9	9	9
Average sample of catkins per tree	411	432	533
Total number of catkins sampled	3699	3888	2132

Variation within a tree

The frequency of abnormal catkins also varied markedly within a tree in a given year, as shown for tree FG 2 in *Table 2.* In 1964 and 1965, this variation was significant at the 0.1 percent level ($\chi^2_{1964} = 43.3$; $\chi^2_{1965} = 22.2$; with 4 d. f.) in 1966 at the 5 percent level ($\chi^2 = 8.7$, with 3 d. f.). In three years, departures were noted in some, but not in all branches, indicating that in a given year portions of a crown may well be normal while others are abnormal. But each branch varied significantly from one year to another and none was consistently high or consistently low in abnormal catkins. Nor were there any consistent differences in sex expression between the upper and the lower crown.

Did our sampling affect the results? Conceivably, the early removal of catkins in any year may have affected sex expression in the next or subsequent years. Our data

Table 2. — Variation in sex expression in tree FG 2.

Location of Sample	1964		1965		1966		1967		1968	
	Catkins sampled	Percent abnorm.	Catkins sampled	Percent abnorm.	Catkins sampled	Percent abnorm.	Catkins sampled	Percent abnorm.	Catkins sampled	Percent abnorm.
Branch 1	107	17.8	108	3.7	31	38.8	55	—	41	—
Branch 2	122	21.4	101	14.9	47	17.0	125	1.6	47	—
Branch 3	106	1.9	89	5.6	132	23.5	105	—	100	—
Branch 4	99	2.0	89	—	120	15.8	63	—	100	—
Branch 5	45	—	41	2.4	None	—	44	—	20	—
Lower crown	—	—	—	—	—	—	—	—	100	—
Total	479	10.2	428	5.8	330	21.2	392	0.5	408	0

fail to show any systematic trends that would support this hypothesis. We found both increases and decreases in the proportion of abnormal catkins over the period of sampling, at both the tree level *(Table 1)* and the individual-branch level *(Table 2)*. Thus, if artifacts were created they oscillated in both directions.

Additional sampling

During the springs of 1966—68, nine female trees from nine geographically separate natural populations in the State of Washington were repeatedly used for breeding studies (Stettler and Bawa, 1971). For this purpose, dormant floral branches were collected and forced in the greenhouse. We were thus able to examine large numbers of female catkins *(Table 3)* when they were manipulated individually for pollination. Although this examination was far more casual than that used in the previously described study, it should have revealed departures in sex expression of any consequence.

We found no male tendencies in this material. However, we did observe occasional catkins with clusters of empty perianth cups or with incompletely developed pistils. They clearly fell outside the range of variation described in *Fig. 1* and fitted into what Lester (1963) termed "non-sexual abnormalities".

Discussion

Among the eight trees studied intensively, none of the Black cottonwood males showed variations in sex expression, whereas one of the two Black cottonwood females was abnormal in one year and both hybrid females in more than one year. This finding is in line with earlier studies in the genus *Populus* reporting greater lability in the female than in the male sex (Schlenker, 1953; Sauer, 1954; Santamour, 1956; Pauley and Mennel, 1957; Lester, 1963); and greater lability in hybrids than in more typical members of conventional species (Seitz, 1953; Jovanovic and Tucovic, 1964b; Melchior, 1967). Our cursory inspection of nine additional females precludes any generalization about the stability of sex expression in natural populations of Black cottonwood. It merely suggests that abnormalities are not frequent, probably less frequent than in Trembling aspen in the Eastern United States (Lester, 1963).

In both the hybrid female trees, the degree of deviation varied significantly from one year to another. One of the two was bisexual during four years, unisexual in the fifth. This shift was gradual and was not associated with any obvious major change, internal or external. Shifts between the unisexual and bisexual state of sex expression have been observed before (Lester, 1963) and are consistent with the suggestion that in the genus *Populus* every individual has both male and female potentialities, one of the two being usually repressed (Sauer, 1954; Klaehn, 1958; Lester, 1963). This notion is further supported by the apparent ability to function of the bisexual flowers in our material and above all, by the successful selfing conducted on hermaphroditic material in earlier studies (Seitz, 1952; Schlenker, 1953; Jovanovic and Tucovic, 1964a; and Zufa, 1962). If our findings of significant variation in sex expression between years, and between branches of the same tree in the same year, are typical for Black cottonwood and Black cottonwood hybrids, future studies on sex expression and sex distribution in natural populations would best be based on sampling from several branches per tree, repeated over several years.

As in most previous studies the bisexual tendencies expressed in our material clearly followed a quantitative variation pattern. Unlike any previously described material, abnormal catkins from our hybrid female trees showed an association between maleness and catkin curvature. Most hermaphrodite flowers were found on both moderately and sharply curved catkins, male flowers almost exclusively on sharply curved ones. The abnormal flowers typically occurred on the inside of the curve. Anatomical study of catkin primordia during development revealed that abnormal flower development clearly preceded catkin curvature, regions occupied by abnormal flowers elongating less than those occupied by normal female flowers. While it is conceivable that the first event caused the second it seems more likely that both events had a common cause. The relevance of auxin as the causative agent is obvious. The role of auxins in stem elongation has long been established (Audus, 1959). More recently, insight has been gained as to their role in flower development (reviewed by Heslop-Harrison, 1959; Lang, 1965). Of particular interest to our study is the association of high auxin level with femaleness, and low auxin level with maleness, as found in cucumbers (Peterson and Anhder, 1960; Galun, Jung, and Lang, 1962).

Thus we may view sex differentiation in the genus *Populus* as a process programmed by the genome, but mediated by auxin levels, absolute or relative to other substances, and thus more or less open to environmental stimuli, depending on the genetic program. The process may well be elucidated by the comparative study, biochemical and physiological, of sex- stable material on the one hand, and sex-labile, on the other.

Acknowledgments

This study was supported through grants from the University of Washington Graduate School Research Fund (NSF Institutional Grant, and Initiative 171) as well as from the McIntire-Stennis Cooperative Forestry Research Program. The technical assistance of Deanna L. West, Kathleen Hops, Marilyn Moser, Wei-Min-Chang, E. Howe, R. F. Piesch, A. N. Seigo, and G. K. Livingston; and the statistical advice of M. Hühn, Schmalenbeck, are gratefully acknowledged.

Abstract

Sex expression was studied for five consecutive years, 1964—1968, in four male and four female trees of the normally dioecious Black cottonwood *(Populus trichocarpa*, T. & G. ex Hook.). Two of the female trees were probable hybrids with *P. deltoides* Bartr. Each spring, catkins were sampled from permanently designated sample branches and examined, partly by the naked eye, partly under the microscope, several hundreds from each tree. All four male trees showed normal sex expression during the period studied. Three female trees and notably the two hybrids, had abnormal catkins, the proportion varying significantly between branches of the same tree, and between years. Abnormal catkins showed various degrees of maleness, ranging from a single hermaphroditic flower to several male flowers. In contrast to the straight axis of most normal catkins, most abnormal catkins ha a curved axis, the degree of curvature being associated with the degree of maleness. The abnormal flowers were typically on the inside of the curve, clustered near the zone of maximum curvature, suggesting that variation in sex expression may be correlated with variation in auxin level.

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Isoenzyme Variation in Picea glauca (Moench) Voss Seedlings[1]

By Peter P. Feret[2]

Introduction

The analysis of genetic variation in forest trees has traditionally been approached by studying quantitative aspects of inheritance and natural variation for a wide variety of morphological, physiological, cytological and biochemical characteristics. The technique of disc gel enzyme electrophoresis (Davis, 1964) allows the analysis of the end product of a gene. Therefore, it is theoretically possible to achieve a very close approximation of allelic variation in a tree species because enzyme variation can be directly related to a heterozygous or homozygous state at a single locus (Hubby and Lewontin, 1969).

In consideration of this potential, the present investigation was initiated to: 1) develop suitable techniques for the isolation, electrophoretic separation, and analysis of isoenzymes in individual seedlings of *Picea glauca* (Moench) Voss, and 2) to investigate the usefulness of the electrophoretic technique to determine the genetic control of isoenzyme variation in *P. glauca*.

Literature Review

The technique of gel electrophoresis has been utilized to study seed-proteins in several forest-tree species. Lewis and Cech (1969) reported protein electrophoretic variation in *Prunus serotina* Ehrh. seeds collected from a single stand in West Virginia. Hare and Switzer (1969) analyzed seed-protein extracts of *Pinus echinata* Mill. and *P. taeda* L. and Durzan (1966, 1968) compared disc electrophoretic patterns of embryo and female gametophyte tissue proteins from *Picea glauca* (Moench) Voss, *Pinus banksiana* Lamb, and *P. strobus* L. In all cases, protein patterns differed between embryo and gametophyte tissue. Pickering and Fairbrothers (1967) studied soluble seed-proteins from four *Magnolia* taxa to determine their usefulness as taxonomic discriminators. A total of eleven proteins could be resolved on disc gels, but all taxa had identical protein patterns.

Pollen proteins have been investigated in several species of *Pinus*. The electrophoretic mobilities of several incompatible and compatible species of pine pollen were studied by Hagman (1967). Many protein patterns were common to all species and interspecific differences were not correlated with incompatibility patterns. Pine pollen proteins were also investigated by Bingham *et al.* (1964) in six species and two interspecific hybrids. Differences in major protein patterns were not apparent although weakly staining proteins did exhibit interspecific variation.

Material and Methods

Peroxidase and esterase enzyme variation in *P. glauca* was investigated in four open-pollinated (OP) half-sib families three families of selfed origin (S_1) and twelve full-sib (F_1) families. The families were represented by a total of 572 *P. glauca* three-year-old seedlings derived from four female parents (A—D) and six male parents (E—J) selected for height growth performance on the Chequamegon National Forest, Wisconsin. Seedlings were grown at the Hugo Sauer Nursery at Rhinelander, Wisconsin. The number of seedlings in the F_1 and open-pollinated progenies varied from 25 to 39; the S_1 progeny numbers varied from 13 to 17.

P. glauca parent and progeny needle tissue was collected July 17 and 18, 1969. Depending on the seedling and needle size, three to five laterals per seedling were sampled, placed in a polyethylene bag and stored, within one hour of collection, below 6° C. Samples were placed at —20° C within 36 hours after collection. A second collection of family

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[1] Research submitted as partial fulfillment of the Ph. D. degree in Plant Breeding and Plant Genetics at the University of Wisconsin, Madison, Wis. The author wishes to acknowledge Dr. James P. King of the U. S. Forest Service, North Central Region, for providing the *P. glauca* seedlings, and Dr. Gerald R. Stairs for his guidance and suggestions during the course of study.

[2] Assistant Professor of Forest Genetics, Division of Forestry and Wildlife, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.