

Variation in Sex Expression in *Populus tremuloides* Michx.

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Introduction

The genus *Populus* comprises about 35 species that are naturally distributed throughout the northern hemisphere and also occur in east central Africa.

All species in the genus are classified as dioecious, with the exception of *Populus lasiocarpa* OLIV., a monoecious, self-fertilizing species of poorly known distribution in central and western China (ANONYMOUS, 1958). For this reason, the discovery of deviation from strict dioecism has attracted considerable interest. The self-compatibility of several individuals exhibiting hermaphroditism has been established (SEITZ, 1954; VALENTINE, 1960), thus making possible a variety of genetic and breeding studies. The apparent lability of sex expression also offers possibilities for investigation of environmental influences on differentiation of various sex forms.

The majority of reports on deviations from dioecism have dealt chiefly with the occurrence of hermaphroditic flowers and floral monstrosities. Floral monstrosities, or intersexes, are proliferations of floral tissue in bizarre arrangements that are of doubtful functionality in reproduction.

genetic, physiological, or environmental influences affecting sex expression.

Materials and Methods

During the autumn of 1960, 146 sample trees were located throughout western Connecticut. Identification of these trees as *Populus tremuloides* was based on twig, bud and leaf characteristics. Selection of more than one member of the same clone was precluded either by distance between trees, or by the fact that all sampled flowers of two trees in question were unisexual and of the opposite sex. The selected trees were mostly along road cuts or in old fields. Trees ranged in age from 8 to 40 years, averaging 17 years.

During February and March of 1961, four or five branches were cut from different levels in the crown of each tree on the side having the greatest area of branches bearing inflorescence buds. The approximate exposure of the sampled side was recorded. The branches were placed in a greenhouse with their bases immersed in water. In most cases the inflorescence buds opened within one to two weeks (Fig. 2 A, B) although branch collections from

Table 1. — Summary of reports on deviations from dioecism in *Populus* species.

Species	Reference
Section <i>Leuce</i> DUBY	
<i>Populus alba</i> L.	BAIL, 1869; MEEHAN, 1880
<i>Populus canescens</i> SM.	SEITZ, 1953
<i>Populus grandidentata</i> MICHX.	HASTINGS, 1918; CAVANAUGH, 1930
<i>Populus tremula</i> L.	BAIL, 1869; RUNQUIST, 1951; SCHLENKER, 1953; SAUER, 1954; GORJUNOVA, 1961.
<i>Populus tremula</i> var. <i>erecta</i> SYLV.	HELMQVIST, 1948.
<i>Populus tremuloides</i> MICHX.	ERLANSSON and HERMANN, 1927; SCHLENKER, 1953; SANTAMOUR, 1955; PAULEY and MENNEL, 1957; KLAHN, 1958; EINSPAHR, 1960; LESTER, 1961.
Section <i>Aigeiros</i> DUBY	
<i>Populus deltoides</i> MARSH.	MAY, 1959.
Section <i>Tacamahaca</i> SPACH.	
<i>Populus candicans</i> AIT.	SAUER, 1954.

The reports of deviations from dioecism in *Populus* have been summarized in Table 1. The majority of these reports are limited to descriptions of deviations in only one clone, or at most a few clones, that were discovered by chance.

This study was undertaken to provide a more comprehensive view of deviations from dioecism in *Populus tremuloides* MICHX. by an intensive sampling of deviant trees. The objectives of the study were to determine the types of variation in deviation from dioecism, to estimate the frequency of such deviations between trees over a considerable area, and to investigate the distribution of deviations, both within trees and within inflorescences. It was hoped that the establishment of distributional patterns within trees and/or within inflorescences could form a basis for speculation and further experimental work on possible

eight trees were discarded after all or most of the buds failed to open.

The final sample thus consisted of 138 trees (Fig. 1). In 75% of these trees, it was possible to examine 100 inflorescences from each tree. The number of inflorescences examined for the remaining trees was dependent on available material, but was in no case less than 50. Bud scales were removed from each inflorescence and, where necessary, bracts were removed so that flowers throughout the entire inflorescence could be clearly viewed under a dissecting microscope. The percentage of inflorescences showing deviation from the predominant sex of the tree was recorded and possible patterns of distribution were noted for the deviation within inflorescences.

Trees which exhibited deviation from dioecism in at least 10% of the examined inflorescences were selected for further analysis. In 15 inflorescences from each tree, the staminate, pistillate, and hermaphroditic flowers were counted.

To investigate the constancy of deviations between years, 17 trees which exhibited only unisexuality in the

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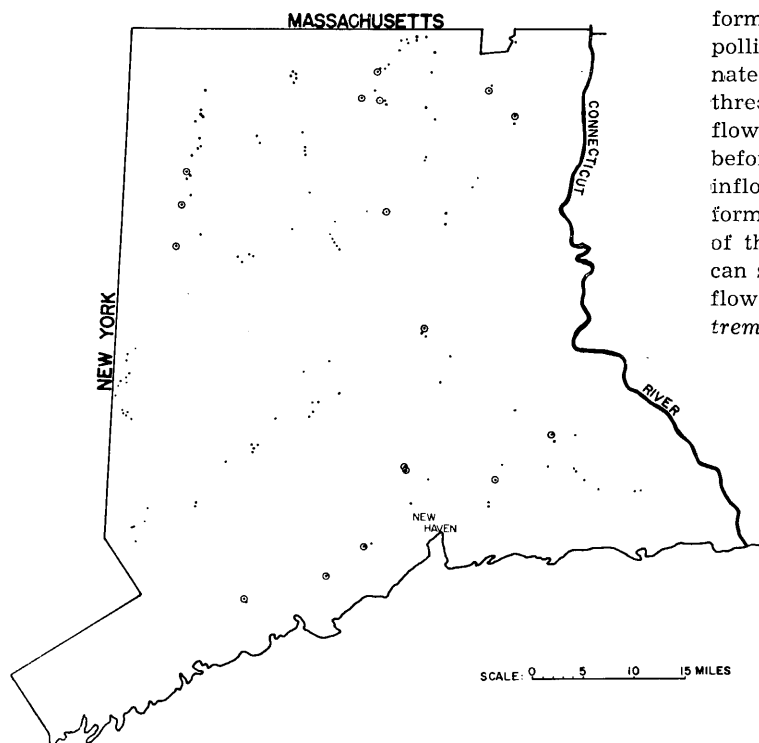


Figure 1. — Location of sample trees. The encircled dots represent trees that exhibited bisexual tendencies in at least 5% of the inflorescences examined in 1961.

1961 sampling were rechecked by the same procedure in 1962. In addition, trees which showed deviation in 10% of the inflorescences in 1961 were also sampled in 1962.

Results

Types of Variation

Morphological variation in the flowering habit of *Populus tremuloides* can be divided into two classes: 1) abnormalities of non-sexual structures, and 2) deviations from dioecism. The latter category includes intersexes, vestigial androecia and gynoecia, hermaphroditic flowers, and monoecious and trioecious floral arrangements.

Non-sexual Abnormalities. — Non-sexual abnormalities comprised any malformation of inflorescence structures other than gynoecia and androecia. These abnormalities were relatively rare, occurring in about 10% of the trees sampled. In most cases, non-sexual abnormalities were found infrequently within the tree, occurring in one to a few inflorescences.

Figure 2 C shows an inflorescence in which leaf primordia apparently were initiated prior to floral initiation. Flowers were sometimes formed in axils of such leaves. In one inflorescence of this type, pedicels and perianths were formed, but there was no evidence of pistil initiation (Fig. 2 D). Bottle grafts of twigs bearing abnormal inflorescences were made in an attempt to perpetuate the vegetative portion of the inflorescence. By this technique, the vegetative tissues remained sufficiently functional for buds to be formed in axils of the leaves.

In other deviant forms, the inflorescence possessed a more pronounced vegetative aspect. Figures 2 E and 2 F show inflorescences where many bracts had developed into transitional forms toward a leaf. These forms were dark green and contained the usual leaf structures. Functional pistillate flowers were present in axils of these transitional

forms (Fig. 2 F), and viable seed was produced after hand pollination. Of particular interest was the semi-determinate growth of these inflorescences. On grafted material, three such inflorescences continued to elongate, initiating flowers and transitional bracts for two to three months before they withered. After cessation of growth, the whole inflorescence died because axillary buds had not been formed along the elongated stalk. Although inflorescences of this type were observed only rarely, their occurrence can serve as a bridge over the barrier of non-synchronous flowering which separates the sympatric species *Populus tremuloides* and *Populus grandidentata* MICHX. Putative natural hybrids of these species are frequently observed, and seed collected by EINSPAHR and JORANSON (1960) from inflorescences of this type on *Populus tremuloides* yielded several seedlings with characteristics intermediate between the two species.

Deviations from Dioecism. — Floral monstrosities, or intersex forms, included antheroid growths on the perianth (Fig. 2 G), antheroid growths attached to the style or carpel wall, and a red-colored extension of the connective tissue of the stamens (Fig. 2 H). In trees exhibiting a low degree of deviation from dioecism, the few deviant flowers were often intersexual. Certain trees showing a high degree of deviation also contained intersexes comprising up to 5% of the deviant flowers. HESLOP-HARRISON (1924) attributed intersex forms in *Salix* to the presence of Eriophyid mites.

In the present study, no mites were observed, and none of the animals observed on flowers and inflorescences were specific to deviant material.

Branched inflorescences were occasionally found, though never with more than a 6% frequency in any tree. Sometimes the branches arose as separate inflorescences from floral primordia. In other instances, the branches appeared to represent a continued growth of a pistil primordium so that the differentiated structure was an inflorescence attached to a pedicel. Generally, the proximal portions of lateral inflorescences arising in the latter manner contained intersex forms, while the distal portions expressed the dominant sex of the tree.

Reduced or vestigial androecia and gynoecia were surprisingly infrequent. Only rarely were minute, shrivelled stamens, or short, slender pistils observed. No attempt was made to investigate the functionality of the vestigial structures. On a morphological basis, the development of stamens and pistils after initiation seems to be generally assured, for the vast majority of deviant flowers contained stamens and/or carpels of typical size and structure.

Regardless of the relationship between morphology and the functionality of the sporangia in deviant flowers, there were several obstacles to natural self-pollination in forced inflorescences from trees exhibiting bisexuality. The hermaphroditic flowers of predominantly pistillate trees were protogynous so that self-pollination within the same flower was precluded. The receptivity of stigmas proceeded in acropetal succession and as stamens are generally concentrated in the proximal flowers, pollen dehiscence usually occurred at a time when only the distal stigmas were receptive. Thus, a transport of pollen would be necessary for selfing to occur. Few stamens are present in most of the deviant inflorescences of predominantly pistillate trees so that selfing may be an infrequent occurrence despite the



Figure 2. — Anthesis and variation in sex expression: — (A) Dormant inflorescence buds on a twig, February, 2 \times . — (B) Anthesis of staminate inflorescences. — (C) Vegetative stalk subtending a pistillate inflorescence. — (D) Pedicels and perianths formed in the axils of leaves on a vegetative stalk, 3 \times . — (E, F) Semi-determinate inflorescence with transitional bracts. F. 3 \times . — (G) Antheroids differentiated from the perianth of a pistillate flower, 20 \times . — (H) Proliferation of the connective tissue of a stamen, 30 \times . — (I) Anthesis of a gynomonoecious inflorescence. The transition from bisexual expression in the proximal portion to pistillate expression in the distal portion is distinct. 3 \times . —

relatively high frequency of trees exhibiting bisexuality. Moreover, in anthers from predominantly pistillate trees, although the microspores appeared to be normal, dehiscence was limited and pollen often remained adherent to the ruptured pollen sac in a sticky mass.

In predominantly staminate trees, as well as in purely staminate flowers of largely pistillate inflorescences, anther dehiscence was free and synchronous with stigma receptivity. As self-compatibility has been established for individuals of the species (HONG, 1960; LESTER, 1961; VALENTINE, 1960), trees with both pistils and relatively large

numbers of stamens probably can produce selfed seed under natural conditions. The naturally occurring triploids of *Populus tremula* L. are hypothesized as arising from self-fertilization with an unreduced gamete in hermaphroditic individuals. SEITZ (1953) corroborated this hypothesis by producing triploids of *Populus canescens* through self-pollination of trees from a gynomonoecious clone.

Frequency of Deviations from Dioecism

Between Trees. — In Table 2, the observed frequencies of deviation from dioecism are presented on an individual

tree basis from a sample of 50 to 100 inflorescences per tree. A total of 34% of the trees sampled in 1961 exhibited some degree of deviation. This frequency is considerably higher than frequencies recorded in previous reports, and is probably attributable to the more intensive sampling used in the present study.

A summary of data from reports by SANTAMOUR (1956), and PAULEY and MENNEL (1957) is presented in Table 3. Ten inflorescences per tree constituted the sample in both reports. The authors suggest that their results probably represent a low estimate, as there was much variation in the number of inflorescences containing hermaphroditic flowers. Bisexual expression in all flowers of all observed inflorescences has not been reported in *Populus*. Apparently in every tree one sex is predominant.

Table 2. — Frequency of sex types in a natural population.

Sex Type	Trees (Number)	Deviation within Sex (%)	Frequency in Total Population (%)
Unisexual			
Pistillate	41	—	29.7
Staminate	50	—	36.2
			65.9% dioecious
Bisexual			
Hermaphroditic or Monoecious			
Gynomonoecious	21	32.3	15.2
Andromonoecious	20	27.4	14.5
Intersexes only			
Gynomonoecious	3	4.6	2.2
Andromonoecious	3	4.1	2.2
Totals	138		34.1% deviant

Table 3. — Summary of reports on frequency of hermaphroditism in natural populations of *Populus tremuloides*.

Sex Type	SANTAMOUR (1956)		
	Trees (Number)	Deviation within Sex (%)	Frequency in Total Population (%)
Unisexual			
Pistillate	28	—	38.9
Staminate	39	—	54.1
			93.0% dioecious
Bisexual			
Gynomonoecious	3	10.7	4.2
Andromonoecious	2	5.1	2.8
Totals	72		7.0% deviant
Sex Type	PAULEY and MENNEL (1957)		
	Trees (Number)	Deviation within Sex (%)	Frequency in Total Population (%)
Unisexual			
Pistillate	46	—	22.3
Staminate	142	—	69.0
			91.3% dioecious
Bisexual			
Gynomonoecious	12	20.6	5.8
Andromonoecious	6	4.0	2.9
Totals	206		8.7% deviant

A comparison of Tables 2 and 3 reveals one striking difference. In the present study, the percent of individuals showing deviation within either sex was similar. This is in contrast to the large excess of deviations in predomi-

nantly pistillate trees shown in Table 3. However, this difference is of questionable significance when the degree of deviation within a tree is considered. If only those trees in Table 2 that show deviation in at least 5% of the sampled inflorescences are considered, the number of predominantly pistillate trees exhibiting deviation is twice that of predominantly staminate trees (13:6). In addition, pistils in staminate inflorescences are much more conspicuous than anthers in pistillate inflorescences, so that it is easy to overlook some bisexual flowers in pistillate trees having only a few such flowers. The present results thus support the conclusion of PAULEY and MENNEL (1957) that the control of sex expression is less rigid in pistillate individuals than in staminate ones. In the present study, the similar numbers of individuals deviating from the staminate or pistillate unisexuality are probably the result of more intensive sampling.

The significance of the frequency of deviation on a tree-to-tree basis is dependent upon the degree to which sex expression is a genetically controlled characteristic. Unfortunately, there is little published information on the inheritance of sex expression in *Populus*. SCHLENKER (1959) reported the predominance of the sex of the *P. tremula* parent in individuals from the crosses *P. tremula* × *alba*, *P. tremula* × *alba* var. *bolleana*, and *P. alba* × *tremula*, although all of the individuals in the various progenies were not examined. SCHLENKER suggested that the high frequency of bisexual expression in the hybrid progenies was probably attributable to precocious flowering. SANTAMOUR (1956) reported a higher percentage of hermaphroditic inflorescences in predominantly pistillate trees from an intraspecific cross with a bisexual female parent.

Some indication of the genetic control of sex expression might be expected from the ratio of staminate to pistillate trees in natural populations. Estimates of the sex ratio have been contradictory. Data presented by SANTAMOUR (1956) do not show a significant deviation from a 1:1 ratio in a sample of 67 trees from a wild population but the predominance of staminate trees is significant ($p = 0.40$) in a sample of 654 trees including the wild population and progenies from controlled crosses. PAULEY and MENNEL (1957) found a ratio of three staminate to one pistillate tree in a sample of 206 trees. EINSPAHR (1960) reported the absence of significant deviation from a 1:1 sex ratio in a sample of 91 trees. In the present study the ratio of predominantly staminate to predominantly pistillate trees agrees closely ($p = 0.66$) with a 1:1 ratio. Each of the reports on sex ratios in *P. tremuloides* is subject to the criticism that sampling was not at random. Only the trees with flower buds can be classified. EINSPAHR (1960) attempted to obtain a random sample by randomly choosing trees and then girdling non-flowering selections to induce flowering. The sample remained biased, however, for flowering was not induced in an unspecified number of the selected trees.

In 1962, 17 trees that had been unisexual in the 1961 sample were re-examined by a similar sampling technique. Five of the seven staminate trees, and seven of the ten pistillate trees again exhibited unisexuality. Of the two deviant staminate trees, one contained a single hermaphroditic flower in two inflorescences and the other had deviant flowers in 20 inflorescences. There was a deviation of only one intersexual flower in two of the pistillate trees, and in the third tree, six inflorescences each had one hermaphroditic flower or intersex.

These results suggest that there may be few, if any, individuals of *Populus tremuloides* in which unisexuality is so strongly established that formation of structures of the opposite sex is not possible. By including the deviations found in trees previously classified as unisexual, the overall percentage of trees exhibiting deviation from dioecism in 1961 and 1962 was 38.

Within Trees. — As a further measure of variability in sex expression, the percentage of deviant inflorescences within each tree exhibiting any degree of deviation from dioecism was determined. Various authors have noted that not all of the inflorescences from a deviant tree exhibit deviation. SAUER (1954) found 33 deviant inflorescences in a sample of 50 from one gynomonoecious individual of *Populus tremula*.

In the present study, based on the total of 12,900 inflorescences examined in 1961, the overall frequency of deviant inflorescences was 5.3%. The data on deviation within trees are summarized in Table 4. The percentages ranged from 1 to 92% of the inflorescences examined from each tree. The concentration of variation in the 1 to 10% class is even more striking when it is pointed out that in 17 trees only one flower in one inflorescence from a sample containing 100 inflorescences showed deviation from the predominant sex of the tree. Four of these 17 deviants were intersexes. From a theoretical point of view, trees with low frequencies of deviations can be classed as exhibiting bisexual potentialities. In practical terms, however, such trees are unisexual.

Table 4. — Variation in percentage of inflorescences showing deviation.

Percentage of Inflorescences Showing Deviation	Trees (Number)
1—10	34
11—20	3
21—30	0
31—40	3
41—50	1
51—60	1
61—70	1
71—80	0
81—90	3
91—100	1

Within Inflorescences. — Variation in the degree of deviation from dioecism was evident within inflorescences as well as within trees. To assess this variation, 15 deviant inflorescences were chosen from each tree exhibiting deviation in at least 10% of the inflorescences examined. The numbers of staminate, pistillate, and deviant flowers were counted in each inflorescence and the percentage of deviation was calculated.

An estimate of the degree of deviation from dioecism, on the basis of individual flowers, was made for all of the material examined in 1961 (12,900 inflorescences from 138 trees). For this estimate, a conservative average of 125 flowers per inflorescence was assumed. Based on this assumption, 0.5% of all flowers examined were deviant.

The data from deviant inflorescences from several trees in 1961 and 1962 are presented in Figure 3 as mean deviations and standard error of the mean. From Figure 3 it is evident that lability in the differentiation of floral primordia within deviant inflorescences varied considerably between trees. With one exception (D 7), the average deviant inflorescence did not approach 50% deviation so that

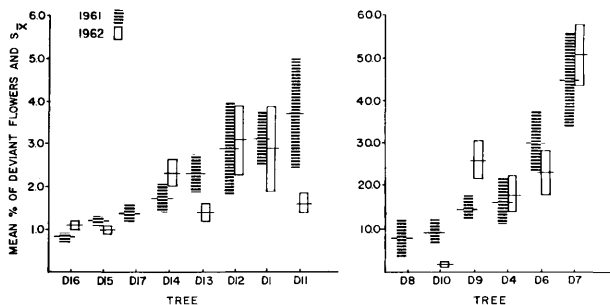


Figure 3. — Mean percentage of deviant flowers within deviant inflorescences and s_x for several trees in 1961 and 1962. Each mean is based on a sample of 15 inflorescences.

even within deviant inflorescences, the dominant sex of the tree was strongly expressed.

The mean deviations in 1961 and 1962 were compared by “t” tests. Data from samples composed of percentages of deviation less than 20 were transformed to $\sqrt{\text{percentage} + 1/2}$ for analysis and for data from samples including larger percentages, the angular transformation was used. Variances for the two samples from each tree were tested for homogeneity. For comparisons in which heterogenous variances were indicated (D 10, D 11, D 13), means were tested by COCHRAN’s approximate “t” test (SNEDECOR, 1956).

For five trees (D 9, D 10, D 11, D 13, D 16) the mean deviations from dioecism in the 1961 and 1962 sampling were significantly different at the 5% level. Whether these differences reflect insufficient sampling or actual differences in sex expression in 1961 and 1962 is uncertain. For most of the trees the relative degree of bisexual expression was similar in both years.

Table 5. — Comparison of the means from a sample of 15 inflorescences and the means from a larger and more extensive sample.

Tree	Sample of 15 Inflorescences	Larger Sample	
	Mean Deviation and s_x (%)	Mean Deviation (%)	and sample number (n)
D11	1.6 ± 0.2	3.1*	(74)
D14	2.3 ± 0.3	2.5	(118)
D6	22.8 ± 5.2	25.4	(63)
D4	18.2 ± 4.3	28.5	(37)
D7	50.2 ± 7.2	47.4	(30)

* significantly different at 5%

An indication of the reliability of estimates based on a sample of 15 inflorescences is provided in Table 5. In five trees, an estimate of the degree of deviation within deviant inflorescences was made using larger numbers of inflorescences taken from all sides and levels of the crown. In one tree (D 11), the means of the two samples were significantly different. The large sample from D 11 contained three inflorescences showing 23 to 34% deviation, a tenfold increase over the sample mean. For tree D 11 there appears to be a range of variation not encompassed by the sample of 15 inflorescences. The dissimilar means for samples of 15 and 37 inflorescences from D 4 likewise suggest that for some trees a sample of 15 inflorescences is indicative only of the relative degree of deviation for the tree.

The product of percentage of deviant inflorescences and mean percentage of deviant flowers within deviant inflorescences for the same tree provides an estimate of the frequency of deviant flowers in the tree as a whole. These estimates ranged from 0.1% in tree D 16 to 45% in tree D 7. There was no apparent association between the frequency

of deviant inflorescences and the frequency of deviant flowers within inflorescences.

Distribution of Deviations from Dioecism

Within Trees. — A chi-square test of data from the 1961 survey showed a non-significant relationship ($p = 0.5$) between orientation of the tree crowns from which branches were cut and the occurrence of deviations from dioecism. To further investigate possible quantitative relationships between deviations and orientation of the crown, two predominantly pistillate, and two predominantly staminate trees exhibiting a relatively high frequency of deviant inflorescences were chosen for more intensive study. Only three of the trees provided suitable material because the flowers on the fourth tree were severely damaged by insects.

The portion of the crown bearing inflorescence buds was divided into four cardinal directions and into three elevations: top, middle, and bottom. The frequency of deviant inflorescences and the number of deviant flowers were determined for these zones. Details of the sample trees are given in Table 6.

Table 6. — Details of trees sampled for data on distribution of deviations within tree crowns.

Tree	Sex Type	Crown Class	D.B.H. (cm.)	Height (m.)
D14	Gynomonoecious	Co-dominant	11	8
D11	Gynomonoecious	Dominant	23	22
D6	Andromonoecious	Dominant	16	12

Because the distribution of inflorescence buds within the crown was not symmetrical, estimates of deviation were not available for each of the 12 zones in each tree. Therefore, the test of differences between orientation and between elevations was limited to a test of heterogeneity in row by column tables using the proportions of deviant inflorescences or deviant flowers.

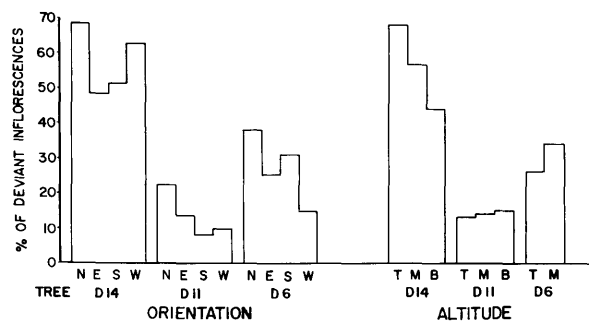


Figure 4. — Distribution of deviant inflorescences within tree crowns.

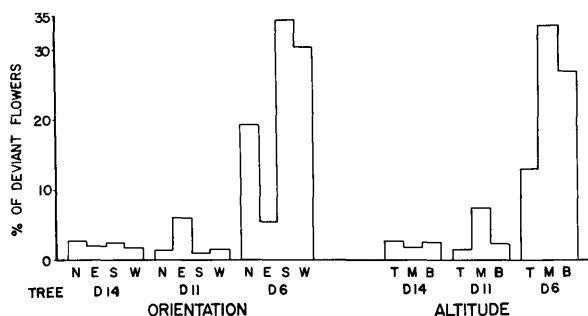


Figure 5. — Distribution of deviant flowers within tree crowns.

Figure 4 illustrates the distribution of deviant inflorescences for orientation and elevation in the crowns of the three trees. These values were calculated from samples of 90 to 380 inflorescences, depending on the availability of material. Although analyses of orientation showed significant heterogeneity in each tree, the only consistent tendency was for the highest degree of deviation to be present in samples from the north side of the crown. In one tree (D14), samples from different levels in the crown were significantly heterogeneous.

In Figure 5, the distribution of deviant flowers is represented. Heterogeneity within the crown was significant for each tree, both in orientation and elevation, but no systematic relationships were apparent.

From these results no pattern of variation within tree crowns can be established other than the tendency for the greatest number of deviant inflorescences to be located on the north side of the crown. This tendency is of questionable significance, for in the two gynomonoecious trees, the highest degree of deviation represents the greatest staminate expression, while in the andromonoecious tree, the opposite is true. The data do, however, point out some of the problems involved in attempting to investigate variability in sex expression. In tree D11, for example, Figure 5 shows an unusually high frequency of deviant flowers in samples from the east side and the middle of the crown. Both of these frequencies reflect the presence of four inflorescences in the middle zone on the eastern side. These inflorescences contained from 23 to 34% deviant flowers; values which considerably exceeded the total mean deviation of 3%. This interaction cannot be tested with the present data.

Within Inflorescences. — Several authors have observed that the distribution of deviant flowers in most inflorescences is not at random (ERLANSSON and HERMANN, 1927; RUNQUIST, 1951; SCHLENKER, 1953; SEITZ, 1953; SAUER, 1954). All observations on *Populus tremuloides* have indicated that in both predominantly staminate and predominantly pistillate trees, stamens are more common in proximal portions of inflorescences, while pistillate flowers are more common toward the apex (Fig. 2 I). In developmental terms, this suggests a transition from male expression to female expression as successive flowers are initiated in the inflorescence. This developmental tendency, however, is not universal in *Populus*. SCHLENKER (1953) reported a predominance of hermaphroditic flowers in proximal portions of gynomonoecious inflorescences of one *Populus tremula* and several hybrids of *P. tremula* and *P. alba*. This same *Populus tremula* was further investigated by SAUER (1954), who confirmed the distribution of perfect flowers at the apex of gynomonoecious inflorescences.

The tendency for a staminate to pistillate transition with time was substantiated in the present study. Initial classification was based on whether stamens appeared to be more numerous near the base or toward the apex of each deviant inflorescence examined in 1961. A summary of these estimates is given in Table 7.

Table 7. — Developmental tendency within inflorescences.

Direction of Staminate to Pistillate Tendency	Trees (Number)
Acropetal	32
Generally acropetal, some basipetal	3
Generally basipetal, some acropetal	1
Basipetal	3

To further investigate the apparent relationship between sex expression and development, three predominantly staminate trees and three predominantly pistillate trees were chosen for more intensive investigation in 1962. Among these trees were representatives of the first three classes in Table 7. None of the three trees in which a basipetal distribution was suggested contained sufficient numbers of deviant inflorescences to give reliable estimates of developmental patterns.

Deviant inflorescences from each tree were divided into segments of ten flowers, starting at the base of each inflorescence. The numbers of staminate, pistillate, and hermaphroditic flowers were determined in each segment from the base to the apex. The numbers for the corresponding segments were then totaled for all inflorescences and the average percent of deviation was calculated for each segment. The segmentation of inflorescences, to provide a numerical measure of development, was complicated by the variation in the total number of flowers in different inflorescences. Inflorescences in which the majority of deviations were restricted to the apical region had to be summarized in terms of approximately equal numbers of segments in order to reflect the actual pattern of distribution. To obviate this difficulty, data were taken only from inflorescences of average length. While this limitation excluded a few smaller and a few larger inflorescences, most of the examined inflorescences were included in the data.

Another problem was the choice of a common variable for all trees. In predominantly pistillate trees, staminate flowers were rare, or totally absent. Although pistillate flowers were not uncommon in predominantly staminate trees, their numbers were too low to adequately represent the developmental pattern. Because a hermaphroditic flower represents the expression of both male and female factors, the approach adopted consisted of determining the following proportion in each segment of ten flowers:

$$\frac{\text{Sum of } \text{♀} + \text{♀ flowers}}{\text{Sum of } \text{♂} + 2(\text{♀}) + \text{♀ flowers}}$$

This proportion is presented as percentage of "femaleness" in Figure 6. Each point represents the average percentage of "femaleness" in a particular segment of ten flowers, and all points are arranged in acropetal sequence for each tree. The sequence of values in each tree was placed along a roughly sigmoid curve suggested by the overall appearance of the data from the six trees.

The number of inflorescences from which the data were taken are listed in Figure 6. The last two plotted points for each tree do not represent the total number of sampled inflorescences. This is due to the previously mentioned problem of differences in total number of segments in different inflorescences. In other words, only the longer inflorescences are included in these points. In some cases, only a few flowers were available for calculation of the proportion for the last segment. This is the probable explanation for the exceptional location of the last point for tree D 7.

Within the limitations of the sampling procedure, Figure 6 represents the developmental pattern of floral differentiation in several different trees. The transition from staminate to pistillate differentiation is evident in each tree, regardless of the overall degree of femaleness in that tree. These data seem to substantiate the suggestion of THOMAS (1956), namely, that developmental patterns of sex expression may be similar in various sex forms of a species.

In Figure 6, the points produced a pattern of development that was characteristic for each tree. It is suggested that these developmental patterns may be more reliable for comparing trees or treatments than determinations of mean percentage of deviation from dioecism. In trees D 1 and D 14, the developmental pattern was clearly defined. Trees with such characteristic patterns should be most useful in making yearly comparisons of deviation from

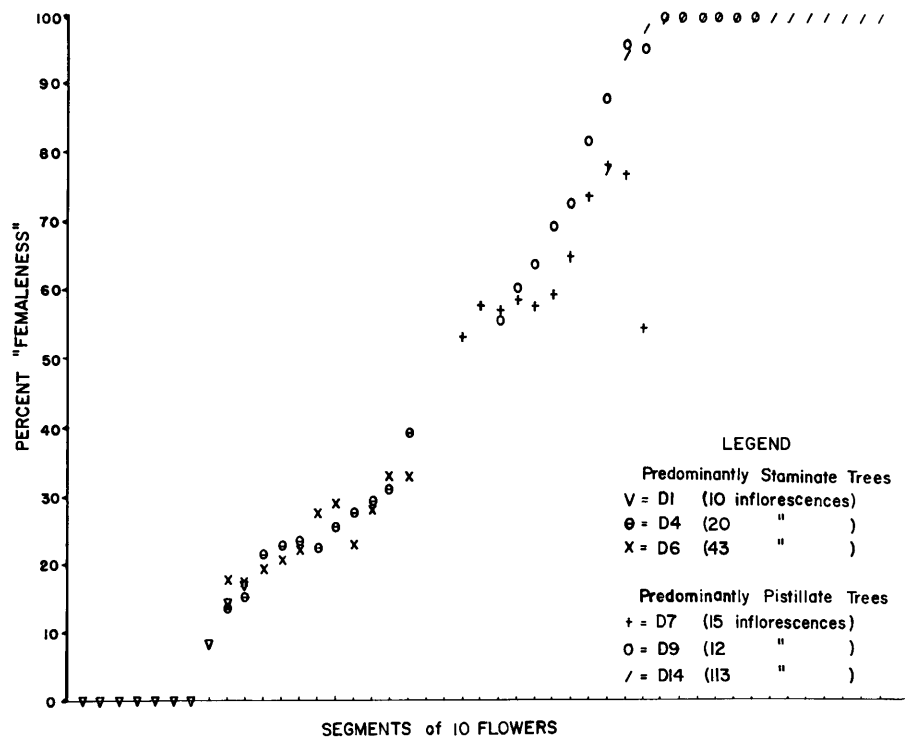


Figure 6. — Relationship between percentage of "femaleness" and the sequence of floral initiation within inflorescences of six trees.

dioecism, and as sources of material for experimentation on both environmental and genetic influences of sex expression.

Quantitative nature of sex expression

The quantitative nature of sex expression in *P. tremuloides* is illustrated in a number of ways. Among these, the considerable variability in the degree of bisexuality both within and between trees, and the developmental changes within deviant inflorescences have been discussed. In addition, quantitative influences are indicated by the numbers of staminate, pistillate, and hermaphroditic flowers in deviant inflorescences.

In all trees exhibiting deviations from dioecism in at least 10% of the sampled inflorescences, the staminate, pistillate, and hermaphroditic flowers were counted in each of 15 inflorescences. Counts were made in the same manner in both 1961 and 1962. The data for 1961 are presented in Figure 7. Data from 1961 only were graphically presented because, with one exception, there were no differences in the relative frequencies of staminate, pistillate, and hermaphroditic flowers between years.

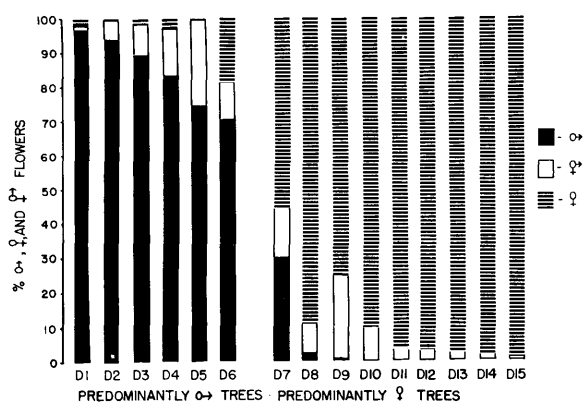


Figure 7. — Frequencies of staminate, pistillate, and hermaphroditic flowers in trimonoecious and gynomonoeious trees.

With three exceptions, Figure 7 demonstrates that in each tree the number of hermaphroditic flowers was intermediate between the numbers of staminate and pistillate flowers. Trees D7, D1, and D6 did not contain intermediate numbers of hermaphroditic flowers in 1961. While tree D7 had an intermediate number of hermaphroditic flowers in 1962, trees D1 and D6 contained more staminate and pistillate flowers than hermaphroditic flowers in both years. There was little difference between the percentages of pistillate and hermaphroditic flowers in tree D1. A possible explanation for the data from D1 is suggested by the developmental pattern shown in Figure 6. From this pattern it is clear that the transition from staminate to bisexual and pistillate floral initiation is abrupt. The periods of bisexual and pistillate expression seem to overlap. For this reason, as well as the small numbers of deviant flowers in each inflorescence, little significance can be attached to the slight excess of pistillate flowers in tree D1. Tree D6 then, remains the obvious exception to the intermediate nature of bisexual floral initiation. No explanation for this exception is apparent.

Figure 7 suggests that the factors controlling the initiation of stamens and pistils are quantitative in nature. A further indication of the quantitative nature of sex expression is the tendency for a decrease in the number of

stamens per hermaphroditic flower as the inflorescence develops. To illustrate this tendency each of 27 gynomonoeious inflorescences from one tree was divided into segments of ten flowers, proceeding in the acropetal direction. Within each segment, the number of stamens per hermaphroditic flower was determined. These data are presented in Figure 8. Although there is considerable scatter, suggesting the possibility of random environmental influences, the decrease in stamen number within each hermaphroditic flower, as a function of developmental time, is definite.

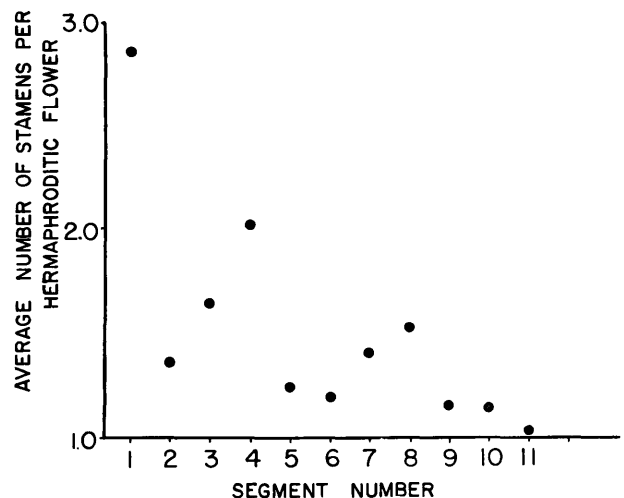


Figure 8. — Relationship between average number of stamens per hermaphroditic flower and the sequence of floral initiation.

Chemical Test for Assessing Variation in Sex Expression

The several approaches to variation in deviations from dioecism already presented have in common the problem of obtaining a reliable estimate of deviation from dioecism for comparing trees, treatments, or yearly variation. To date, the only measurable variables known are the actual deviant inflorescences or flowers. The necessity of large samples to insure reliability further complicates the already tedious process of counting flowers. However, other potential methods of evaluation appear to hold little promise at present. It seems doubtful that secondary sex characteristics, if present, could be sufficiently discriminatory to reflect the low frequencies of deviation noted for most of the trees in this study. A chromatographic technique which seemed of considerable potential value was investigated with the following results.

BLAKE, *et al.* (1960) reported that by chromatographic analysis of vegetative buds, the sex of mature trees of *Populus tremuloides* could be determined. Based on that report, and on personal correspondence (BLAKE, 1961 a), an attempt was made to quantitatively estimate male and female potency in trees deviating from dioecism using a similar chromatographic method.

During October, 1961, vegetative buds from six staminate trees¹⁾, six pistillate trees, and four trees of varying degrees of gynomonoeism were collected. The buds were finely chopped, and allowed to steep in 95% ethanol, using concentrations of 0.2 gm. (fresh wt.) / 4 ml., 0.5 gm. / 5 ml., and 1.0 gm. / 5 ml. Extraction was allowed to proceed at room temperature for at least one week and the extracts

¹⁾ The sex of each tree was evaluated by examination of 100 inflorescences in 1961.

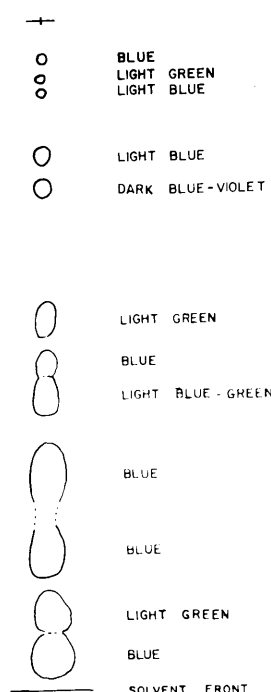
The general pattern of fluorescent spots for all trees is represented in *Figure 9*. Particularly striking was the absence on all chromatograms of the brilliant orange spot that was reported by BLAKE, *et al.* (1960) for extracts from buds from pistillate trees. In addition, the elongated light-green region, reported as characteristic of staminate individuals, was noted in extracts from some pistillate trees as well. As indicated in *Figure 9*, this region could be sepa-

Thus, at present, examination of the flowers remains the only approach to determining the sex of trees in *Populus*. Estimation of the degree of deviation from dioecism is likewise limited to observations of flowers and flower counting.

It was shown that deviation from dioecism in *Populus tremuloides* is a phenomenon of considerable variability at all levels investigated. The basis for that variability is uncertain. Sexual lability showed no apparent association with topography and no systematic distribution could be established for bisexual inflorescences within the tree. It appears that the factors which initiate an expression of bisexuality have no rigid control over the probability that flowers in a given inflorescence in a specified portion of the tree will exhibit bisexuality. However, the probability for deviation from dioecism in the tree as a whole is considerably greater in certain trees.

The developmental pattern of sex expression in bisexual inflorescences of *P. tremuloides* seems similar to patterns described for a variety of monoecious plants, especially the cucurbits. NITSCH, *et al.* (1952) distinguished six types of flowers in *Curcubita pepo* (Acorn squash) in a developmental sequence from underdeveloped staminate flowers to parthenocarpic pistillate flowers. The same authors demonstrated, by experiments in controlled environments, that photoperiod and night temperature profoundly influence the duration of developmental stages, but not the sequence of floral types in *C. pepo*.

The influence of systematic changes in photoperiod and temperature during the period of floral differentiation in *P. tremuloides* is questionable. In the vicinity of New Haven, Connecticut, the formation of pistils and stamens from undifferentiated meristems occurred over a period of three weeks, from June 25 to July 15, 1961 (LESTER, 1963). During that period, day length changed approximately five minutes (ANONYMOUS, 1960). A summary of mean daily temperatures for the last 50 years at the weather bureau in New Haven shows an average rise of 1.7° C for the period (ANONYMOUS, 1962). However, the maximum, minimum, and mean daily temperatures from June 25 to July 15, 1961



rated into light-green, blue, and light blue-green areas. Extracts from certain individuals were characterized by differences in intensity and size of particular fluorescent areas, but these differences were not associated with sex differences. Chromatograms on which separate extracts of bud scales and the inner bud tissue were run, suggested that most of the fluorescent substances were contained in the bud scales.

BLAKE (1961 b), in subsequent correspondence, noted that his investigations of material other than that from the trees on which the original publication was based, gave unreliable results. In his later tests, the characteristic orange spot appeared in 50% of the pistillate trees and in 30% of the staminate trees investigated. He rechecked the buds from the original trees and again found the technique reliable for distinguishing staminate and pistillate individuals in the original sample. However, continued experimentation with different systems for development and identification of possible sex differences by chromato-

showed no apparent relationship to the developmental pattern of sex expression in gynomonocious trees examined in the New Haven area. Although the relative influence of genotype and environment on the developmental pattern of sex expression must be determined from experiments in controlled environments, systematic climatic changes appear unlikely to exert a direct influence on the pattern.

Additional evidence of the quantitative nature of sex expression is provided by the generally intermediate numbers of hermaphroditic flowers and by the decrease in number of stamens per hermaphroditic flower as the inflorescence develops. The quantitative nature of the transition from staminate to pistillate floral differentiation in deviant inflorescences suggests a mechanism of sex determination mediated through changes in the absolute amount of a specific substance, or, more likely, through the relationships between substances within the developing inflorescence. After summarizing the literature on environmental influences on sex expression in flowering plants, HESLOP-HARRISON (1957) advanced the tentative hypothesis that the reported modifications in sex expression associated with various environments can be explained in terms of auxin levels. According to this hypothesis, floral morphogenesis is directed by genetic factors whose activity can be regulated by auxin levels in the differentiating meristem. The auxin levels are, in turn, subject to environmental influences affecting the development of the plant as a whole. Staminate floral differentiation is thought to be promoted at lower auxin levels and pistillate floral differentiation by higher levels.

The results of auxin applications on plants which have been induced to flower generally support HESLOP-HARRISON's hypothesis (LAIBACH and KRIBBEN, 1949; NITSCH, *et al.* 1952; HESLOP-HARRISON, 1959). Results from treatments with gibberellic acid have further indicated the influence of growth regulators in sex expression (PETERSON and ANHDER, 1960; HASHIZUME, 1961). Similar experiments on *P. tremuloides* might indicate the degree of sexual lability in apparently unisexual trees, as well as those expressing bisexuality, in addition to illuminating the relationship of sex expression to the genotype and to the development of the tree as a whole.

It is important to recall that this study was restricted to sex expression as represented by floral structures of the sporophyte. No final conclusions can be drawn from these data to apply to questions of the place of deviations from dioecism in the reproductive cycle of *Populus* until the functionality of these deviations under natural conditions is investigated further. In addition, it is well to re-emphasize that, although a considerable number of the trees investigated exhibited bisexual potentialities, the majority of those trees were overwhelmingly dioecious in the initiation and development of individual flowers.

Summary

Examination of inflorescences from 138 trees in Connecticut revealed the following frequencies of deviation from dioecism:

- 38 % of the trees showed varying degrees of bisexuality.
- 5.3% of the total number of inflorescences examined contained deviant flowers, and
- 0.5% of the total number of flowers examined were deviant.

Considerable variability was found in the frequency of deviant inflorescences in samples from different trees and in the frequency of deviant flowers within inflorescences from the same tree, as well as from different trees. In all trees, one sex was predominant.

Variation in the distribution of deviations in different positions within the tree crowns did not form any systematic pattern. Variation in numbers of deviant flowers within inflorescences exhibited a relationship in which increasing degrees of pistillate floral differentiation were associated with the acropetal sequence of development in inflorescences. The characteristic developmental patterns for each tree were arranged graphically to support an hypothesis of overall developmental change from staminate to pistillate floral differentiation. Of the various approaches to measurement of deviation from dioecism for comparative purposes, the pattern of development within the inflorescence appears particularly promising.

Observations which suggest a genetic influence on variability in sex expression include the generally similar degree of deviation in most trees in 1961 and 1962, and the characteristic transition from staminate to pistillate expression in all deviant inflorescences. An intermediate number of hermaphroditic flowers in trimonoecious inflorescences and the decrease in number of stamens per hermaphroditic flower as the deviant inflorescence develops further illustrate the quantitative nature of sex expression.

Zusammenfassung

Titel der Arbeit: *Variation im Geschlechtsausdruck bei Populus tremuloides Michx.*

Die Untersuchung der Infloreszenzen von 138 Bäumen in Connecticut ergab folgende Abweichungshäufigkeiten von der Diözie:

- 38 % der Bäume zeigten abweichende Bisexualitätsstufen,
- 5,3% der untersuchten Infloreszenzen enthielten abweichende Blüten und
- 0,5% aller untersuchten Blüten waren abweichend.

Beträchtliche Unterschiede fanden sich bei der Häufigkeit abweichender Infloreszenzen in den Proben von verschiedenen Bäumen und bei der Häufigkeit abweichender Blüten innerhalb von Infloreszenzen eines Baumes und auch verschiedener Bäume. Bei allen Bäumen war aber ein Geschlecht stets vorherrschend.

Die Mannigfaltigkeit in der Verteilung der Abweichungen innerhalb der Baumkronen zeigte kein systematisches Muster. Die Variation der Zahl abweichender Blüten innerhalb der Infloreszenzen zeigte dagegen insofern eine Beziehung, als die zunehmenden Ausbildungsstufen des Fruchtknotens in den Blüten mit der akropetalen Entwicklungsfolge in den Infloreszenzen assoziiert war. Die charakteristischen Entwicklungsmuster jedes Baumes wurden graphisch aufgetragen, um so eine Hypothese für eine allgemeine Entwicklungsänderung von der männlichen zur weiblichen Blütendifferenzierung zu finden. Von den verschiedenen Versuchen, die Abweichungen von der Diözie für Vergleichszwecke zu messen, scheint das Entwicklungsmuster innerhalb der Infloreszenz besonders geeignet zu sein.

Ein genetischer Einfluß auf die Variabilität im Geschlechtsausdruck läßt sich bei denjenigen Beobachtungen vermuten, bei denen generell ähnliche Abweichungsstufen bei den meisten Bäumen in den beiden Jahren 1961 und

1962 und ebenso die charakteristische Umwandlung vom männlichen zum weiblichen Ausdruck bei allen abweichenden Infloreszenzen gefunden wurden. Die intermediäre Anzahl hermaphroditer Blüten bei trimonözischen Infloreszenzen und die Abnahme der Staubgefäßzahlen je hermaphrodite Blüte bei solch abweichenden Infloreszenzen zeigt ferner die quantitative Eigenart des Geschlechtsausdrucks.

Résumé

Titre de l'article: *Variations dans la manifestation du sexe chez Populus tremuloides Michx.*

L'examen d'inflorescences de 138 arbres, dans le Connecticut, a permis d'établir les fréquences suivantes de déviation par rapport à la dioicité:

38 % des arbres sont bisexués à des degrés variés,

5,3% du nombre total des inflorescences examinées contiennent des fleurs aberrantes et,

0,5% du nombre total de fleurs examinées sont aberrantes.

La fréquence des inflorescences aberrantes dans les échantillons des différents arbres est très variable et on retrouve cette même variabilité à l'intérieur des inflorescences du même arbre, aussi bien que de différents arbres. Chez tous les arbres un des sexes est dominant.

La variation de la distribution des anomalies suivant la position dans la couronne de l'arbre ne suit pas une règle systématique. Pour la variation dans le nombre de fleurs aberrantes à l'intérieur d'une inflorescence, on constate une liaison.

Le développement caractéristique de chaque arbre est traduit graphiquement sur la base d'un changement complet dans la différenciation des fleurs mâles en fleurs femelles.

Dans le cadre des diverses études de mesures comparatives de la déviation à partir de la dioicité, le schéma de développement à l'intérieur de l'inflorescence paraît particulièrement intéressant.

Certaines observations suggèrent une influence génétique sur la variabilité de l'expression du sexe: Le taux de déviation pour la plupart des arbres est à peu près le même en 1961 et 1962; la transition des fleurs mâles en fleurs femelles est caractéristique dans toutes les inflorescences apparentes. La nature quantitative de l'expression du sexe est illustrée par le nombre intermédiaire de fleurs hermaphrodites dans les inflorescences trimonoïques et par la diminution dans le nombre d'étamines des fleurs hermaphrodites au fur et à mesure du développement de l'inflorescence aberrante.

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