A STUDY OF CYTOLOGY AND SPECIATION IN THE GENUS POPULUS L.

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With four plates

INTRODUCTION

A STUDY of the extent of interspecific hybridization within a genus has both practical and theoretical importance. It is of value in showing to what degree hybridization may be utilized within a genus to establish improved types and, at the same time, it is indicative of the relationships of the species themselves. In order that the study of the extent of hybridization within the genus shall prove of maximum value, data on behavior of the F_1 and F_2 generations under controlled conditions should be available. Due to the time factor involved, such data are not available in most cases when dealing with forest trees.

The study of genetic behavior of interspecific hybrids is of greatest value when coupled with a comparative study of the cytology of those hybrids. Studies of this sort have yielded many pertinent facts, particularly as critical evidence for the establishment of probable interspecific relationships. In addition it has led to a better understanding of the actual methods by which isolation and speciation have taken place. The basic assumption underlying this method of approach to the problem of interrelationships of species is that the pairing of the parental chromosomes is a criterion of chromosome homology. This criterion of chromosome homology, based on a study of pairing relationships at meiosis in F_1 species hybrids, is particularly valuable when supplemented with a study of chiasmata frequencies, chromosome configurations, and pollen sterility.

The study of chromosome numbers, microsporogenesis, and the development of the male gametophyte of species within the genus supplements this program of research, making it more valuable from both points of view. The study of chromosome behavior and pollen sterility of the pure species serves as a basis with which to compare the cytological behavior of the hybrids. The chromosome numbers will indicate the degree of polyploidy within the genus and indicate the probable success of any attempt to induce polyploidy.

The present investigation is a study of the cytology of the genus *Populus* L. in general, and as such it is subject to the limitations imposed by the unavailability of certain species and hybrids for study. With these limitations in mind it is a study in particular of chromosome numbers and chromosome behavior in pure species and inter-specific hybrids, especially as these data are related to speciation within the genus.

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MATERIALS AND METHODS

The materials used in this study were obtained from two sources: (1) the *Populus* collection of the Arnold Arboretum, and (2) the *Populus* plantations of the Northeastern Forest Experiment Station at Frye, Maine. Cytological material of species and natural hybrids were obtained from the former location and material of artificial hybrids from the latter.

Branches with flower buds attached were collected during the latter part of February and early March, placed in the greenhouse, and allowed to develop. Catkins were collected at appropriate times and fixed in 3:1 alcohol acetic and stored in the fixative at 2° C. until needed. Male catkins of *Populus* make exceptionally favorable material for cytological study, since one catkin contains many successive stages in the development of the pollen grain.

Aceto-carmine smear preparations were used entirely in the study of meiotic chromosomes. Pollen mother cells in prophase stages were difficult to stain, and those having the chromosomes advantageously placed for study were rare. When favorable cells were found, the chromosomes were drawn with the aid of a camera ludica. The length of the chromosomes was then measured by the use of waxed threads.

For root tip studies, cuttings from the desired species were rooted in water or sand, the root tips collected and fixed in Navaschin's solution, embedded in paraffin, sectioned at 10μ and stained with crystal violet. A similar procedure of fixation, embedding, and sectioning was followed in the study of the development of the male gametophyte, except in this case the sections were stained with Haidenhain's iron-haematoxylin.

In the study of the development of the pollen tube, freshly shed pollen was sprinkled on slides which had received a thin coating of a sugar, agar, gelatin, and water mixture. The preparation of these slides has been described by Newcomber (1938). In this case a mixture of 2 gm. sugar, 0.5 gm. agar, and 0.5 gm. gelatin to 25 cc. of water was found to be satisfactory. After the pollen was planted, the slides were placed in a moist chamber until the pollen had germinated. It was then fixed with 3:1 alcohol acetic and stained with aceto-carmine.

CHROMOSOME NUMBER AND MORPHOLOGY

The first investigation of the chromosomes of *Populus* was made by Graf in 1921. He found the reduced chromosome number in *P. tremula* and *P. canadensis,* as determined from reduction divisions in the embryo sac mother cells, to be four. These counts have since proved to be erroneous. In 1924 Blackburn and Harrison, on the basis of chromosome number in seven species of *Populus* and seventeen species of *Salix,* established the fundamental reduced chromosome number in the Salicaceae as nineteen. In *Salix,* a polyploid series based on multiples of two, four and six was found. Since 1924 various workers have studied chromosome numbers in *Populus.* Table I summarizes the chromosome numbers which have been determined up to this time and lists the authority for each

TABLE 1.

PREVIOUSLY DETERMINED CHROMOSOME NUMBERS OF POPULUS SPECIES, WITH AUTHORITY AND DATE.

Species	Diploid Number	Authority
*P. alba	38	Von Wettstein (1933)
*P. alba	57	Peto (1938), van Dillewijn (1940)
P. alba var. nivea aureo-intertexta	57	Peto (1938)
P. balsamifera[1]	38	Meurman (1925)
P. canescens	38	Peto (1938)
P. canescens	57	Peto (1938)
P. deltoides var. missouriensis	38	Van Dillewijn (1940)
*P. Eugenei[2]	38	Blackburn (1926), Peto (1938)
P. gelrica	38	Van Dillewijn (1940)
*P. generosa	38	Blackburn (1926)
*P. grandidentata	38	Peto (1938)
P. lasiocarpa	38	Von Wettstein (1933)
*P. nigra	38	Blackburn and Harrison (1924), van Dillewijn (1940)
*P. nigra var. italica	38	Van Dillewijn (1940)
*P. robusta	38	Van Dillewijn (1940)
P. serotina[3]	38	Blackburn (1926)
*P. serotina	38	Van Dillewijn (1940)
P. Sieboldii	38	Nakajima (1937)
*P. Simonii	38	Meurman (1925)
P. tremula	38	Blackburn and Harrison (1924)
P. tremula	38	Von Wettstein (1933), Muntzing (1936)
P. tremula	57	Muntzing (1936), Tometorp (1937)
*P. tremuloides	38	Erlanson and Hermann (1927), Peto (1938)
*P. trichocarpa	38	Meurman (1927)

* Count confirmed in the present study.

[1] P. balsamifera L. = P. deltoides Marsh. var. missouriensis Henry.

[2] P. Eugenei probably = ×P. canadensis Moench, var. Eugenei (Simon-Louis) Schelle

[3] P. serotina Hartig = ×P. canadensis Moench, var. serotina (Hartig) Rehd.

count. Table II shows the distribution of chromosome numbers among the sections of the genus. Those determined by the writer are indicated in this table. In all, some forty-five species, varieties, and natural hybrids have been investigated.

An examination of these numbers reveals that no polyploid series similar to that found in *Salix* exists in *Populus*. All species exist in the diploid form with the diploid number of chromosomes equal to thirty-eight. However, in the section *Leuce,* three species are found which possess triploid forms also, with the unreduced chromosome number of fifty-seven.

It should be pointed out that, in most cases, the chromosome numbers for any particular species have been determined from the examination of but one or, at the most, a few specimens of the species. It is possible that the examination of a species throughout its entire range might reveal

TABLE 2.

A LIST OF CHROMOSOME NUMBERS OF POPULUS SPECIES AND VARIETIES, SHOWING THE DISTRIBUTION OF THESE NUMBERS AMONG THE SECTIONS OF THE GENUS.

Section	Species	Chromosome Number, 2N
Leuce	P. alba L.	38, 57
	P. alba var. nivea aureo-intertexta	57
	*P. adenopoda Maxim.	38
	P. canescens (Ait.) Sm.	38, 57
	P. grandidentata Michx.	38
	P. Sieboldii Miq.	38
	P. tremula L.	38, 57
	P. tremuloides Michx.	38
	*P. tomentosa Carr.	38
Leucoides	P. lasiocarpa Oliv.	38
Tacamahaca	*P. acuminata Rydb.	38
	**P. angustifolia James	38
	**P. candicans Ait.	38
	*P. cathayana Rehd.	38
	×P. generosa Henry	38
	*×P. Jackii Sarg.	38
	*P. koreana Rehd.	38
	*P. laurifolia Ledeb.	38
	**P. Maximowiczii Henry	38
	P. Simonii Carr.	38
	**P. Tacamahaca Mill.	38
	*P. Tacamahaca var. Michauxii (Dode) Farwell	38
	P. trichocarpa Hook.	38
	**P. trichocarpa var. hastata Henry	38
	*×P. Woobstii (Reg.) Dode	38
Aegeiros	*×P. Andrewsii Sarg.	38
	**P. angulata Ait.	38
	×P. barbantica Houtz.	38
	*×P. berolinensis Dipp.	38
	*×P. canadensis Moench	38
	*×P. canadensis var. erecta (Selys-Longchamps) Rehd.	38
	×P. canadensis var. Eugenei (Simon-Louis) Schelle	38
	*×P. canadensis var. marilandica (Poir.) Rehd.	38
	*×P. canadensis var. regenerata (Schneid.) Rehd.	38
	×P. canadensis var. serotina (Hartig) Rehd.	38
	*P. deltoides Marsh.	38
	P. deltoides var. missouriensis Henry	38
	×P. gelrica Houtz.	38
	P. nigra L.	38
	P. nigra var. italica Muenchh.	38
	*P. nigra var. betulifolia (Pursh) Torr.	38
	*P. nigra var. plantierensis (Simon-Louis) Schneid.	38
	×P. robusta Schneid.	38
	**×P. Rasumowskyana Schneid.	38
	*×P. Sargentii Dode	38

** Determination made by the writer from root tip preparation.
* Determination made by the writer from meiotic preparations.

polyploidy within that species. In Sweden, where *P. tremula* has been studied most extensively, nine clones of the triploid form have been discovered (Nilsson-Ehle, 1936; Muntzing, 1936; Blomquist, 1937; Tometorp, 1937; Johnsson, 1940). One tetraploid form of *P. balsamifera* (= *P. deltoides?*) was reported by Blackburn and Harrison in 1924. Meurman (1925) thinks it likely that some species other than *P. balsamifera* was examined. Although it is true that workers since 1924 have found this species to be a diploid, it is possible that it may also exist in the tetraploid form. Johnson (1939) lists *P. Simonii* as having the unreduced number of seventy-six chromosomes. However, Meurman (1925), who first investigated this species, stated that it is probably a hybrid and due to this fact frequently shows thirty-eight univalent chromosomes at meiotic metaphase. Material investigated in the present study showed a reduced number of nineteen.

Polyploid forms of *P. tremula* have been produced by different investigators. A tetraploid form of this species resulted from the crossing of two triploid forms (Nilsson-Ehle, 1938). Similarly, tetraploids and individuals possessing all the chromosome numbers from nineteen to thirty-eight have been obtained from crosses of diploid and triploid forms of *P. tremula* (Johnsson, 1940).

The chromosomes of *Populus* are small and of varying size. Blackburn and Harrison (1924) made the first observations on size of meiotic chromosomes in *P. tremula*. Here they found that the chromosomes were ". . . of unequal dimensions; nine were small ones of more or less uniform size; nine others, larger than these, formed a graded series beginning with a member of just a little greater volume than the individual of the first group, and ending with one more than four times its volume. Lastly there was a single chromosome, obviously compound in structure, nearly always appearing in a flat plate as four-lobed, equalling in volume, if not exceeding, that of any two of the other eighteen." These observations were found to apply to *P. nigra* also. The studies were made on meiotic chromosomes at metaphase and anaphase I. Meurman (1925) thought there were two groups of nine chromosomes, nine smaller and nine larger, each group varying within itself. In *P. balsamifera* and *P. trichocarpa* one of the chromosomes was noted as being twice the size of any of the rest. Erlanson and Hermann (1927) saw a similar size classification in *P. tremuloides*. Muntzing (1936) and Johnsson (1940) agree that in *P. tremula* one of the bivalents is much larger than other chromosomes of the set. Nakajima (1937) speaks of a particularly long chromosome in *P. Sieboldii*, while van Dillewijn finds a "giant chromosome" in the meiotic configurations of *P. nigra*, *P. nigra* var. *italica*, *P. brabantica*, *P. gelrica*, *P. robusta*, *P. deltoides* var. *missouriensis*, *P. serotina*, and *P. alba*.

No measurements have been made during this investigation of the size of mitotic chromosomes of *Populus*. However, an examination of root tip chromosomes of *P. tremula*, as illustrated by Muntzing (1936) and Johns-

son (1940), would indicate that they range in size from approximately 0.75 to 2.1μ. Since the chromosomes are quite small and numerous, studies of chromosome morphology at mitotic divisions are extremely difficult. An attempt was therefore made to study the prophase stages of meiosis. *Fig. 1* shows a pachytene stage in the hybrid *P. nigra* × *P. trichocarpa*. Due to the difficulties involved in making accurate measurements, the lengths are to be regarded as approximations. However, it can be seen from the idiogram (*Fig. 2*) that there is no "giant chromosome" apparent at this stage. Neither can there be any division of the chromosomes into two groups of nine, one composed of small and one of large chromosomes. There is, rather, a gradual increase in size from about 8.5 to about 30.6μ in length at this stage. There are one group of three chromosomes and two groups of two chromosomes which are of exactly the same length, a fact which may be of some significance. The discrepancy in reports on the comparative lengths of prophase and metaphase meiotic chromosomes may be due to a differential rate of contraction of the chromosome during the prophase stages. The preparations did not stain in such a way as to show the spindle insertion point.

The genus *Populus* is dioecious. For this reason it might be expected to be the subject of investigation seeking to determine whether or not sex-chromosomes are present in plants. Blackburn and Harrison (1924) first described a heteromorphic chromosome pair in the meiotic chromosomes of *P. tremula* as consisting of a medium sized and a small member. In subsequent investigations the same was found to be true in the case of *P. nigra*. Meurman (1925) seems to have been the first to designate the larger of this chromosome pair as an X chromosome and the smaller as a Y. Thus the male in *Populus* is XY, the female XX, making the condition existing in this genus analogous to that prevailing in most animals. He was able to demonstrate a heteromorphic chromosome pair in the male trees of the species *P. trichocarpa, P. balsamifera,* and *P. Simonii*. Erlanson and Hermann (1927), investigating the cytology of perfect flowers in a specimen of *P. tremuloides,* found a heteromorphic pair present at the meiotic divisions similar to that which they saw in the monoecious form of the same species. Nakajima (1937) found that a heteromorphic chromosome pair exists in *P. Sieboldii*.

Muntzing (1936) points out that, since the triploid forms which exist in certain species of *Populus* must have arisen from the union of two gametes, one reduced and one unreduced, we might expect intersexual forms. None, however, has been found. Peto (1938) found no heteromorphic chromosome pair which was present consistently. However, heteromorphic pairs were found in hybrids, and in such instances he thinks that their presence might be explained on the basis of structural differences involved in species differentiation.

Although a special study of sex-chromosomes has not been made a part of this investigation, it may be said that no definitely heteromorphic chromosome pair has been found consistently in any of the species or

hybrids studied. *Fig. 10* shows what appears to be a heteromorphic bivalent similar to those used as illustrations of sex-chromosomes in *Populus* by different authors. It would seem that a demonstration of the presence or absence of a heteromorphic pair at meiosis in a pistillate tree might offer some proof of the existence of sex-chromosomes in this genus. Only one investigation of female trees has been made with this point in mind. Blackburn (1926) states, in reference to *P. tremula*, "I am able to state in regard to the female only that all pairs appear to be equal." A study of meiotic prophase chromosomes, where conditions are most favorable for the detection of morphological differences among the chromosomes, has thus far revealed no strikingly different homologues.

Lawrence (1931) suggests that in general those families with high chromosome numbers, such as the Salicaceae, will be found to be secondary polyploids. Secondary polyploids, according to the definition of Darlington and Moffett (1930), are ". . . homozygous allopolyploids in which some chromosomes of the basic set are present more frequently than others." Some examples of such genera are *Pyrus* (Darlington and Moffett, 1930), *Acer* (Meurman, 1933), and *Dahlia* (Lawrence, 1931).

Cytologically, secondary polyploids may be recognized by the appearance of a secondary association of chromosomes at either pro-metaphase I or II of the meiotic divisions. The presence of more than two genetically similar chromosomes will result in the formation of occasional multivalent chromosome configurations at the meiotic metaphase I. Further, if these chromosomes are genetically similar it might be expected that they would be morphologically similar, also.

Lawrence (1931) found evidence of secondary association of chromosomes on examination of illustrations of the meiotic chromosomes of species of *Salix* published by Blackburn and Harrison (1924). Van Dillewijn (1940), working on *Populus*, found secondary association of chromosomes in the case of *P. nigra* and *P. nigra* var. *italica*. On this basis he divides the chromosomes into three groups of three each, four groups of two each, and an additional group composed of the "giant chromosome" associated with a smaller chromosome. From this latter association he concludes that the giant chromosome is formed from the union of two smaller chromosomes and that the group as a whole is a group of three. There are thus eight groups in all, giving a basic ancestral number of eight to this genus. Of the original eight chromosomes, four have been duplicated and four triplicated, while within one group of three chromosomes, two have become fused.

Additional evidence that *Populus* is a secondary polyploid is given by the rare occurrence of trivalent formations in the meiotic metaphase of certain diploid hybrids (Table VIII) and the similar occurrence of occasional quadrivalents in the triploid form of *P. alba* (Table III). An examination of the idiogram (*Fig. 2*) does not reveal the similarity in chromosome size which one would expect from van Dillewijn's account of secondary association. Certain members of the chromosome complement are of exactly the

same length. However, on a purely morphological basis it is impossible to obtain the grouping of chromosomes to give eight as the basic number.

There seems to be evidence that the genus *Populus* is of polyploid origin, but obviously there must be a more detailed study before the actual basic number can be determined with any certainty.

CYTOLOGY OF SPECIES

According to Chamberlain (1897), the stamens of *Salix* pass the winter in the spore mother cell stage. He inferred that this was likewise the case in *Populus*. The present work confirms this, for catkins collected in December and early February of 1940 showed the pollen mother cells in a resting condition.

The rate of the meiotic divisions in all species is quite rapid. All stages from first meiotic division to the stage where free immature pollen grains are seen may take place within a space of from twenty-four to thirty-six hours.

The instigation of the meiotic divisions is usually correlated in this genus with the development of anthocyanin pigment in the anthers. Further, the sequence of these divisions and consequently of pollen formation within the catkin seems to be constant within the species and even within the sections of the genus. Erlanson and Hermann (1927) noted that in normal male catkins of *P. tremuloides* the divisions began in the stamens at the base of the catkin and continued regularly toward the tip.

In general there seem to be two types of order of flowering. In type I the meiotic divisions are instigated at either a central or basi-central position and proceed toward the tip and base of the catkin. In type II the divisions begin at the tip of the catkin and proceed toward the base. In the seventeen species and varieties examined, the type was constant within a section of the genus. Type I was found in the sections *Leuce* and *Tacamahaca,* while type II was typical of the section *Aegeiros*. The natural hybrid × *P. berolinensis* was aberrant, for while belonging to the section *Aegeiros* it was of type I.

Observations made at the time of anther dehiscence bear out these results, for it was noted that those species belonging to type I shed the pollen at the basal end of the catkin first, while in type II the reverse was true. Meiotic divisions within a pollen sac are usually at the same stage, although two successive stages may be present. In general, the same may be said of all the anthers in one flower. However, in hybrids some irregularities have been observed. Meurman (1925) noted that in *P. Simonii,* which is probably a hybrid, stages from prophase I to telophase I inclusive were often found in the same anther sac. Erlanson and Hermann (1927) found a similar condition in the anther sacs of perfect flowers in *P. tremuloides.*

The first and second meiotic divisions, with few exceptions, are regular in the true species of *Populus* (*Figs. 8–15*). The picture is diagrammatic in its simplicity and regularity.

TABLE 3.

CHROMOSOME PAIRING AND POLLEN STERILITY IN POPULUS SPECIES.

Species	Pairing Relationship				Sterility
	I	II	III	IV	%
P. acuminata	0.80	18.60	0	0	45
P. adenopoda	0.40	18.80	0	0	19
P. alba (diploid)	0	19.00	0	0	3
P. alba (triploid)	10.56	13.52	5.56	0.68	23
P. deltoides	0	19.00	0	0	2
P. grandidentata	0	19.00	0	0	2
P. koreana	0.50	18.75	0	0	12
P. laurifolia	0.40	18.80	0	0	40
P. nigra	0	19.00	0	0	3
P. nigra var. italica	0	19.00	0	0	4
P. Sargentii	0	19.00	0	0	7
P. tremuloides	0	19.00	0	0	2

TABLE 4.

CHROMOSOME PAIRING AND POLLEN STERILITY IN A SPECIES OF POPULUS FROM WHICH FLOWERING BUDS WERE FORCED IN THE GREENHOUSE FOR DIFFERENT PERIODS OF TIME.

Date of collection	Date at which meiosis took place	Chromosome pairing at meiosis			Pollen sterility
		I	II	III	%
January 24	February 2	7.92	15.04	0	70
March 9	March 12	7.32	15.28	0.04	72
March 23	March 23	7.80	15.10	0	65

Table III gives the pairing relationships observed in a number of species. The counts are based on the study of twenty-five metaphase or late diakinesis configurations for each species, while the sterility counts were made on the basis of a count of one thousand pollen grains for each species.

The material for study was obtained from the *Populus* collection in the Arnold Arboretum. Flowering branches were brought into the greenhouse over the period of February to April and forced into flower. The results might be criticized on the basis that forced material might not behave as normally developed material. However, Nohara (1924) found in pollen studies of *Salix* sp. that results obtained from forced pollen did not differ either in percentage of perfect grains or in viability from that collected in the field. That forcing does not affect chromosome pairing and pollen sterility in *Populus* may be seen from Table IV, where collections of *Populus* sp. (probably a hybrid) made over the period January to March are compared in regard to chromosome behavior and pollen sterility.

Chromosome pairing was complete in most cases where pure species

were studied. This pairing was so intimate that the different members of the pair could be distinguished only with difficulty. Occasional univalent chromosomes were encountered, as for example in *P. laurifolia* and *P. adenopoda*. Apparently this lack of pairing at metaphase I was the result of a precocious separation due to failure or early terminalization of chiasmata rather than to lack of homology between the chromosomes concerned. In this connection Johnsson (1940) reports that in sixteen clones of diploid *P. tremula* examined, four clones showed metaphase I plates having varying numbers of univalents. This is attributed to the influence of external conditions, such as temperature, on meiosis.

Diakinesis proved to be an advantageous stage for study, for at this stage the chromosomes are widely scattered over the entire area of the cell. The nucleolus is usually still present at this stage (*Fig. 8*) and may in some cases remain visible until the early metaphase. However, in those cases where the nucleolus persists until late diakinesis, it shows an irregular outline and a light staining reaction indicative of dissolution. Van Dillewijn (1940) finds that in *P. brabantica*, a natural hybrid, the nucleolus is still visible at the metaphase in some cells, either on the plate or near the plate, in the cytoplasm.

One chromosome is invariably associated with the nucleolus (*Figs. 1, 8, 16*) and is easily distinguished from the remaining chromosomes of the complement, since it is somewhat condensed and darker staining than the rest. The association of one particular chromosome of the complement with the nucleolus has been described by several writers, including Heitz (1931), Sax (1932), and Smith (1933).

In many of the species studied it was noted that the nucleolus possessed a protuberance or knob. Rarely more than one of these was present per nucleolus. A similar condition was described in *P. nigra* by van Dillewijn (1940). According to this writer no protuberance is visible on the nucleolus in the early prophase, but as the prophase progresses a bud develops until it sometimes reaches the dimensions of a nucleolus itself. It seems to the present writer that the knob is first visible in very early prophase and that it reaches its maximum development at approximately the pachytene stage. No difference in size of the knob was noticed between pachytene and early diakinesis. By late diakinesis the knob began to disappear along with the nucleolus proper. In all cases where the nucleolus possesses a knob, the associated chromosome is located at the junction of the knob and nucleolus (*Fig. 1*).

The percentage of nucleoli which showed knobs differs in different species. In *P. deltoides* about seventy-seven per cent, in *P. alba* (diploid) about forty per cent, and in *P. nigra* about eighty percent showed knobs. In *P. alba* the knobs are quite small and rarely approach those of *P. nigra* and *P. deltoides* in size.

The situation where one finds a knobbed protuberance of the nucleolus has been reported but rarely in the flowering plants. Selim (1930), Nandi (1936), and Parthasarathy (1938) report a somewhat similar condition

in *Oryza,* as do Paul (1937) in *Tamarindus* and Iyengar (1939) in *Cicer.* McClintock (1934) reports a reciprocal interchange in *Zea Mays,* produced by x-ray treatment involving the nucleolus organizer, which produces a condition similar in appearance to that described here.

Following telophase I the nuclei are reorganized and the second meiotic division follows in a regular manner.

As is the case in simultaneous pollen cell formation, the cell wall is formed by a furrowing process. This is the method most commonly found in the flowering plants. A condition similar to that which occurs in *Populus* has been described in *Nicotiana* by Farr (1916). The furrows form along the equator of each spindle, extending from the periphery to the center of the pollen mother cell, eventually cutting it into four microspores. Previous to the appearance of these furrows, there appears to be a more or less hyaline area present in the center of the cell, presumably caused by a migration of the protoplasmatic granules away from this particular region. This hyaline area extends in four arms from the center to the periphery, marking the future position of the furrows. This seems somewhat comparable to the condition found in *Melilotus* (Castetter, 1925), where similar hyaline areas appeared, caused by a vacuolation of the cytoplasm. Whether or not the hyaline area in *Populus* is caused by a vacuolation must be determined by a more critical examination aided by differential staining methods.

The furrowing process seems to be easily upset. In many cases in hybrid material and in the triploid form of *P. alba* it appears that only one furrow formed, thereby cutting the pollen mother cell into dyads instead of tetrads, although the dyads are themselves binucleate. Occasionally the furrowing process was observed to fail entirely, or it was of such a nature that three microspores instead of the usual four were formed. The significance of such aberrant furrowing is indeed great, if such spores are functional. Numerous examples of such irregularities in microspore formation in *Populus* have been described (Peto, 1938; van Dillewijn, 1940).

A fairly wide variation is seen in the pollen sterility of the pure species studied. In general, these species which showed some univalent chromosomes were the most sterile, but the univalent frequency is hardly sufficient to account for the sterility encountered. Apparently genetic and environmental factors are also involved.

The triploid form of *P. alba,* with an unreduced chromosome number of fifty-seven, shows, as one would expect, a varying number of univalents, bivalents, and trivalents. It also shows quadrivalent formation to some extent (Table III), the significance of which has already been commented upon. It is surprising that the pollen sterility of this triploid is less than that of some of the diploid species studied.

Pollen fertility reported for various triploid forms has been summarized in Table V. Peto (1938) has commented upon this high fertility. It is his opinion that this is merely apparent, and he assumes that the genetically unbalanced pollen grains deteriorate rapidly following their

TABLE 5.

POLLEN STERILITIES IN TRIPLOID FORMS OF POPULUS SPECIES.

Triploid Species	Per cent Fertility	Authority
P. tremula	58–75	Johnsson, 1940
P. tremula	44	Muntzing, 1936
P. alba	63	Peto, 1938
P. alba var. aureo-intertexta	94	Peto, 1938
P. alba	67	Smith, 1942

formation. In the pollen slides which he examined, he observed numerous tiny specks which he interpreted to be degenerate pollen grains. It has been shown, however, that in the cross of a triploid × a diploid and a triploid × a triploid, *P. tremula,* progeny with intermediate chromosome numbers varying from thirty-eight to seventy-six can be obtained (Johnsson, 1940; Bergstrom, 1940). This fact seems to show that unbalanced pollen grains in at least one species of *Populus* can survive. It has been shown that the genus *Populus* is probably a derived polyploid (van Dillewijn, 1940, and the present study). If this is the case, then certain chromosomes and thus certain combinations of genes are duplicated within the basic set. This condition would be exaggerated in the case of a triploid form, where the basic set of nineteen chromosomes is present three times. In a situation of this sort, it might be expected that pollen sterility due to duplications and deficiencies of whole chromosomes would not be apparent, for it is conceivable that spores lacking certain duplicated chromosomes would still have a functional set of genes.

On the basis of the pairing relationships of the chromosomes at meiosis, it is apparent that the triploid forms of diploid species thus far reported are autotriploids (Muntzing, 1936), which probably arose through the union of a diploid with a haploid gamete.

Studies of the first and second meiotic divisions and the development of the immature microspores have been made by different writers. However, little work has been done on the further development of the male gametophyte. Chamberlain (1897), working with *P. monilifera* (probably *P. deltoides*), reports that the division of the microspore nucleus into the tube and generative nuclei takes place in the pollen grain relatively early, before the tapetal cells of the anther sac degenerate. He describes a rather unusual condition in that two divisions of this pollen grain nucleus occur. On the conclusion of the first division, the smaller of the daughter nuclei is cut off from the other by a cell wall and degenerates. The remaining nucleus then divides again to form the generative and tube nuclei. He concludes by stating, "Since spores already upon the stigmas showed no further differentiation, the division of the generative cell which presumably takes place, although I was not so fortunate as to observe it, must occur after the pollen tube begins to form."

If the condition described by Chamberlain, in which an extra prothallial cell is produced in the pollen grain, is correct, then it is unique, as far as is known in the Angiosperms, and thus of very special interest. Consequently an attempt was made to follow the microspore development up to the production of the gametes. In this part of the work the species *P. deltoides, P. acuminata,* and *P. adenopoda* were used. It was possible to demonstrate the first microspore division by aceto-carmine smears (*Fig. 3*). However, shortly after this division occurs, the pollen wall becomes so opaque that further observation by this method becomes impossible. Further study was made by embedding in paraffin, sectioning, and staining with haematoxylin. A second division within the pollen grain was not observed. The mature pollen grains examined contained two nuclei and there was no visible remnant of a disintegrating nucleus. However, occasional cases, both in *P. acuminata* and *P. adenopoda,* showed a third nucleus within the pollen grain. Since these species show some irregularity at metaphase I (Table III), it is possible that these irregularities might account for this third nucleus.

The development of the pollen tube in the species *P. deltoides, P. laurifolia, P. acuminata,* and *P. adenopoda* was investigated by placing freshly shed pollen from these species on slides coated with a mixture of agar-agar, gelatin, and sugar, as described in the section on technique. The pollen germinates almost immediately. As a rule the tube nucleus emerges first and the generative nucleus follows. The appearance of the generative nucleus after its emergence from the pollen grain is such that the chromosomes may be distinguished within it. In certain cases it would seem that the term generative cell should be used rather than generative nucleus. The division of the cell takes place from ten to fifteen hours after germination of the pollen grain. Different stages in the development of the pollen tube are shown in *Figs. 4–7.*

HYBRIDIZATION IN POPULUS

Natural hybrids are of frequent occurrence within the genus *Populus.* Johnson (1939) lists sixteen such interspecific hybrids. Rehder (1940) lists sixteen hybrids which have been given species or varietal names but does not include all those listed by Johnson. In all, about twenty-five natural interspecific hybrids have been reported in the genus.

Among the first interspecific hybrids to be described in this genus were many which occurred in botanic gardens when an introduced species was planted near a native species or when two introduced species were planted together. × *Populus berolinensis,* the hybrid *P. laurifolia* × *P. nigra* var. *italica,* which originated in the Botanic Garden of Berlin before 1870, is an example of this. × *Populus canadensis* and its varieties and × *P. robusta* are further examples. Peto (1938) reports similar hybrids in Canada between the European species *P. alba* and the native species *P. grandidentata* and *P. tremuloides.* It has been noted that female trees of species which have no male trees in the vicinity set viable seed. *P. Maximowiczii* is a good example of this fact, for in the Arnold Arboretum

it sets seed, some of which produce hybrid seedlings, although there are no male trees of this species in North America.

At different times natural hybrids have been described from the field. This has been especially true in eastern Asia and North America, where the number of species of *Populus* is most abundant. From China the "species" *P. Simonii* is probably a natural hybrid, and × *P. Woobstii* is thought to be the cross *P. laurifolia* × *P. tristis*. In order to determine the actual extent of natural hybridization among the North American species, a survey was made of the geographic distribution of the principal species and their natural hybrids, based upon a study of the herbarium sheets of *Populus* in the herbarium of the Arnold Arboretum.

× *Populus Andrewsii* represents the cross *P. Sargentii* × *P. acuminata*. It has been collected from two stations in Colorado, Welsenberg and Montrose, both within the southern part of the overlap range of the parent species, which are also represented from these stations. × *Populus Parryi* has been collected from three localities in California, Canada de las Uvas, San Bernando, and Owens Lake. It is the result of the cross *P. Fremontii* × *P. trichocarpa*. × *Populus Jackii* (*P. Tacamahaca* × *P. deltoides*) has been collected from some twenty localities scattered over southern Quebec and Ontario, Vermont, and New York. This area represents but a small part of the overlap range. In many cases both parents have been collected from the same localities as the hybrid. The hybrid *P. grandidentata* × *P. tremuloides* is somewhat similar in both these respects. It has been collected at twelve stations from Quebec and New England west to Ohio. The probable hybrid *P. acuminata* × *P. Wislizeni* has been collected from one locality, Silver City, New Mexico. The cross *P. candicans* × *P. Tacamahaca* is represented by one collection made in the Arnold Arboretum. Four of these natural hybrids are the result of intersectional crosses, while two are crosses between species belonging to the same section, *P. grandidentata* × *P. tremuloides* and *P. candicans* × *P. Tacamahaca*.

Any conclusion drawn from this study of natural hybridization are necessarily limited by the fact that only those herbarium sheets contained in the Arnold Arboretum herbarium were examined. In no case does the distribution of the hybrid cover the entire range of overlap between the parent species. In two cases the hybrid is represented by but two collections. From the numerous artificial hybrids reported one might expect to find natural hybrids widespread. Their relative scarcity may be due to several reasons. 1. These hybrids are capable of reproducing themselves only through vegetative means. 2. Hybrids are scarce in those regions in which relatively little collecting has been done and abound in those regions where abundant collections have been made. 3. The most probable reason, however, is that while growing in the same general geographic region, the parent species may occupy different ecological habitats and overlap rather infrequently. An attempt will be made in a later part of this work to account for this lack of hybridization on the basis of

TABLE 6.

NATURAL AND ARTIFICIAL INTERSPECIFIC HYBRIDS WITHIN THE GENUS POPULUS ARRANGED TO SHOW CROSSING WITHIN AND BETWEEN SECTIONS.

Leuce × Leuce	Leuce × Tacamahaca	Leuce × Aegeiros	Tacamahaca × Tacamahaca	Tacamahaca × Aegeiros	Aegeiros × Aegeiros
14	2	4	7	28	28

edaphic isolation. However, it can be shown that, in spite of such isolation, most of the possible hybrids occur between species of the same geographic region.

Artificial interspecific hybrids in this genus have been produced in abundance by different workers. Smith and Nichols (1941) list eighty-one artificial interspecific hybrids which have been produced and described by Henry (1914), Heimburger (1936, 1940), von Wettstein (1933), Schreiner (1934), and others. Table VI shows how these hybrids, along with the known natural hybrids, are distributed between and within the sections of the genus. The significance of this distribution will be discussed in relation to speciation in a later part of this work.

Material for a cytological study of some of the artificial hybrids produced by Schreiner and his collaborators for the Oxford Pulp and Paper Company was obtained from Dr. E. J. Schreiner of the Northeastern Forest Experiment Station. The number of hybrids investigated was unfortunately limited to those which happened to come in flower over the period 1939–1941. Most of these were not the wide crosses which were desired for study. Those hybrids from which collections were made are described in Table VII, along with the natural hybrids investigated. The latter were obtained from the collections in the Arnold Arboretum.

In all, twenty-five metaphase I, anaphase I and, in some cases, anaphase II plates were analysed to determine the extent of chromosome pairing and to study the various abnormalities which follow asynapsis. In each case the pollen sterility was determined by a count of two thousand pollen grains. In this connection it might be noted that the sterility was variable, differing somewhat with different collections from the same tree taken at the same or different times. Where possible, prophase stages of meiosis were studied to obtain some idea of chromosome pairing at the earlier stages. Table VIII summarizes the data on chromosome pairing and pollen sterility obtained from both the natural and artificial hybrids studied.

CYTOLOGY OF HYBRIDS

In order that chromosomes may pair at meiotic metaphase, three general conditions must be realized: first, that genetically similar chromosomes are present; second, that asynaptic genes do not influence the pair-

TABLE 7.

A LIST OF THE NATURAL AND ARTIFICIAL HYBRIDS OF POPULUS INVESTIGATED, WITH DESCRIPTIONS OF THE CROSSES WHICH THEY REPRESENT.

Name or number*	Cross	Description of cross
1. ×P. Andrewsii	P. Sargentii × P. acuminata	Between sections Tacamahaca & Aegeiros; both parents native to North America.
2. ×P. berolinensis	P. laurifolia × P. nigra var. italica	Cross within the section Aegeiros between geographically isolated species.
3. ×P. canadensis 4. ×P. canadensis var. Eugenei 5. ×P. canadensis var. regenerata	P. deltoides × P. nigra	Cross within the section Aegeiros between geographically isolated species, P. deltoides from North America, P. nigra from Europe & western Asia.
6. ×P. Jackii	P. Tacamahaca × P. deltoides	Intersectional cross Tacamahaca × Aegeiros; parents native to North America.
7. P. nigra var. plantierensis	P. nigra var. italica × P. nigra var. betulifolia	Within the section Aegeiros; both parents varieties of the same European species.
8. ×P. robusta	P. angulata × P. nigra var. plantierensis	Within the section Aegeiros between geographically isolated species.
9. P. Simonii	Supposed true species; native to north China.	
10. ×P. Woobstii	P. laurifolia × P. tristis ?	Cross within the section Tacamahaca; parents native to western Asia.
OP–64 OP–74 OP–113	P. charkoviensis × P. plantierensis	Both parents probably hybrids; cross might be written thus: (deltoides × nigra) × (nigra var. italica × nigra var. betulifolia).
OP–96 OP–97 OP–98 OP–118	P. nigra × P. laurifolia	Cross between sections Tacamahaca and Aegeiros; between geographically isolated species.
OP–99	P. charkoviensis × P. clone robusta	Both parents probably hybrids within section Aegeiros; (deltoides × nigra) × (angulata × nigra var. plantierensis).

TABLE 7 (*continued*).

Name or number*	Cross	Description of cross
OP–102	P. nigra × P. berolinensis rossica	Parents within the section Aegeiros; male parent hybrid; deltoides × nigra var. italica.
OP–103	P. nigra × P. nigra	Control cross.
OP–104	P. nigra × P. trichocarpa	Intersectional cross between geographically isolated species.
OP–105	P. nigra baatanicorum vitrum × P. volga	Crosses between 2 closely related varieties of P. nigra.
OP–106	P. nigra baatanicorum vitrum × P. plantierensis	
OP–109	P. Rasumowskyana × P. caudina	Female parent probably the hybrid P. laurifolia × P. nigra; parents probably belong to section Aegeiros.
OP–110	P. Rasumowskyana × unidentified cotton-wood	
OP–111 OP–114	P. charkoviensis × P. incrassata	P. charkoviensis probably hybrid P. deltoides × P. nigra; male parent closely related to P. nigra; both belong to section Aegeiros.
OP–112	P. deltoides × P. deltoides	Control cross.
OP–116	P. charkoviensis × P. berolinensis	Both parents probably hybrids; (nigra × deltoides) × (laurifolia × nigra var. italica); both belong to section Aegeiros.
OP–117	P. charkoviensis × P. deltoides	(nigra × deltoides) × deltoides; cross within the section Aegeiros.
OP–119	P. charkoviensis × P. caudina	Female parent probably hybrid; both within section Aegeiros.

* Entries numbered 1–10 are natural hybrids. The remaining entries are artificial hybrids; clone numbers and parentages supplied by Dr. Schreiner.

ing; and third, that, after pairing, chiasmata form in the pachytene chromosomes.

In the present study, while it is recognized that the latter two conditions may play a part in the pairing behavior of the chromosomes at meiotic metaphase, no attempt has been made to study the chiasmata formation in the parental species and hybrids due to the extremely small size of the chromosomes. In general, it was noted that the univalents tend to be the smaller chromosomes of the complement. At this time not enough is known of the genetics of these hybrids to determine to

TABLE 8.

MEIOTIC CHROMOSOME PAIRING, CHROMOSOME NUMBER, INVERSION BRIDGE FORMATION, AND POLLEN STERILITY IN NATURAL AND ARTIFICIAL HYBRIDS OF POPULUS.

Name or Number	Chro. No.	Metaphase I Analysis I	II	III	Anaphase I Analysis L	B	M	Pollen Sterility Per cent
1.	19	0.72	18.64	0	0.72	0.14	1	20
2.	19	5.30	16.20	0.10	2.00	0.12	1	57
3.	19	1.84	18.08	0	0.60	0.12	2	17
4.	19	4.92	16.48	0.04	2.90	0.33	3	63
5.	19	3.92	17.04	0	3.69	0.04	1	56.5
6.	19	2.40	17.80	0				62
7.	19	0	19.	0				6
8.	19	6.00	16.	0	2.50	0.13	2	75
9.	19	1.76	18.12	0				31
10.	19	14.24	11.88	0				80
OP–64	19	0.28	18.86	0	0.16	0.23	1	5
OP–74	19	0.64	18.68	0	0.20	0.08	1	24
OP–113	19	1.14	18.43	0	0.16	0.23	1	22.4
OP–96	19	0.40	18.80	0				20
OP–97	19	1.00	18.50	0	0.40	0.20	1	16
OP–98	19	0.20	18.90	0				10
OP–118	19	0.80	18.60	0	0.40	0	0	25
OP–99	19	1.00	18.50	0				26
OP–102	19	1.20	18.40	0				20.4
OP–103	19	0.20	18.90	0				5
OP–104	19	0.80	18.60	0	0.66	0.33	2	23
OP–105	19	0.20	18.90	0	0.26	0.06	1	10
OP–106	19	0	19.	0	0.06	0	0	6
OP–109	19	0.60	18.70	0	0.86	0.53	3	12
OP–110	19	2.20	17.90	0	1.25	0.30	2	40.5
OP–111	19	0.20	18.90	0	0.40	0	0	
OP–114	19	0.40	18.80	0				
OP–112	19	0.20	18.90	0	0.46	0	0	6
OP–116	19	3.00	17.50	0				
OP–117	19	1.40	18.30	0	1.00	0.26	3	
OP–119	19	0.76	18.62	0	0.44	0.12	2	35

I, II, and III under metaphase analysis refer to the average number of univalents, bivalents, and trivalents per cell. L, B, and M under anaphase analysis refer to the average number of lagging chromosomes, average number of inversion bridges and maximum number of inversion bridges per cell.

what extent asynaptic genes influence pairing relationships. Environmental factors are also known to cause asynapsis.

Two classes of genetic dissimilarity of the chromosomes are recognized. The first, which is purely genic and presumably arises through gene mutation, is usually not assigned a large role in asynapsis. The second type of dissimilarity is structural and is brought about by rearrangements of genic material within the chromosome. It is usually assumed that this type of dissimilarity plays the major role in asynapsis.

Darlington (1937) discusses the classification of hybrids at some length, dividing them into seven classes: numerical, structural, undefined structural, complex, polyploid, numerical-structural, and Mendelian hybrids. Under this classification the interspecific hybrids in *Populus* considered here would be placed in the undefined structural hybrid class, these " . . . resulting from the union of gametes dissimilar as a result of changes which cannot be defined . . . simply because the structural differences between their chromosomes are too slight or too numerous to be detected."

The undefined structural hybrids are further broken down into those which show potentially complete pairing at metaphase I, those which show partial pairing, and those which have a potentially complete failure of pairing. Evidently the interspecific hybrids of *Populus* investigated thus far might be placed in the first two groups, although the larger number belongs to the second, for these hybrids show a partial and always variable pairing.

Numerous examples may be cited for each of the hybrid classes listed above. Among the undefined structural hybrids which show potentially complete pairing are *Salix viminalis* × *S. caprea* (Haakanson, 1929), *Platanus orientalis* × *P. occidentalis* (Sax, 1933), and *Catalpa bignonioides* × *C. ovata* (Smith, 1941). The hybrids *Viola arvensis* × *V. rothmagensis* (Clausen, 1931) and *Ribes nigrum* × *R. Grossularia* (Meurman, 1928) are examples of undefined structural hybrids which show partial pairing.

By the cytological examination of the interspecific hybrids from pachytene stage of prophase onward it is possible to demonstrate that a varying number of bivalents and univalents are present (*Figs. 1, 18, 21*). Within a single hybrid the number of univalents present per cell at metaphase I may vary from none to thirty-eight (*Figs. 18, 19, 21*). Considering the hybrids studied as a whole, the number of normal cells, normal in the sense that they contained nineteen bivalent chromosomes at metaphase I, varied from four to ninety-six per cent, with an average of fifty-three per cent.

Univalent chromosomes were present in varying numbers in all the hybrids examined. Usually these univalents lie on either side of the metaphase plate (*Fig. 18*), come onto the plate after the bivalent chromosomes have divided, and then divide (*Fig. 21*). There is some evidence that some univalent chromosomes do not divide at anaphase I but go to the poles without lagging or dividing. On the basis of an examination of five hundred each of metaphase I and anaphase I figures (twenty-five each of twenty hybrids), it was found that more univalents were present at metaphase I than appeared as lagging univalents at anaphase I. The average was 1.65 univalents at metaphase I as compared to 0.96 univalents at anaphase I. Since no univalent chromosomes were observed dividing at anaphase II it seems likely that those univalent chromosomes which did not divide at anaphase I behave in a normal manner at the following division.

After dividing, the lagging univalents may or may not reach the poles

TABLE 9.

A COMPARISON OF SUPERNUMERARY SPORES AND UNIVALENT CHROMOSOMES IN SPECIES AND HYBRIDS OF POPULUS.

Species or hybrid	Univalents per cell	Supernumerary spores per cell
P. deltoides	0	0
P. alba (diploid)	0	0
P. nigra	0	0
×P. Andrewsii	0.72	0.55
×P. berolinensis	5.30	0.32
×P. robusta	6.00	0.47
×P. canadensis	1.84	0.30

in time to be incorporated into the daughter nuclei. If they do not reach the poles they are lost in the cytoplasm (*Fig. 23*). If they are included in the dyad nuclei they are distributed at random to the poles at anaphase II or are lost in the cytoplasm. On the basis of one hundred anaphase II figures analyzed in two hybrids it was found that three times as many univalent chromosomes were lost at anaphase I as there were at anaphase II. The ultimate fate of these univalent chromosomes seems to depend upon the rapidity with which they progress to the poles at both anaphase I and II. In both cases if they reach the poles before the daughter nuclei are formed they are included in these nuclei, otherwise they are left behind in the cytoplasm, where they form either micronuclei or supernumerary spores. Just what determines their fate is not clear. It does not seem to depend upon the number of univalents available, for the nucleus of the supernumerary spore is as small as the micronucleus. The factor determining this may be the position of the univalents at the time of the cell wall formation, those near the microspore nuclei becoming micronuclei and those farther out becoming supernumerary spores.

That the presence of supernumerary spores is correlated with the presence of univalent chromosomes is shown by Table IX.

An anaphase analysis of these interspecific hybrids revealed in nearly every case a varying number of inversion bridges (Table VIII; *Figs. 24–31*). The condition in which a portion of a chromosome is present in the inverted state is one of the most frequently encountered meiotic aberrations. This condition can be detected in plants when a crossover occurs within the heterozygous inversion region, for as a result chromatin bridges are formed at anaphase I. A loop pairing at pachytene is also characteristic. The occurrence of inversions in both plants and animals has been reported by many writers, among them Muntzing (1934), Richardson (1936), and Stebbins (1938). Structural hybridity has not previously been reported in the genus *Populus*. Haakanson (1929) has reported a case of reciprocal translocation in *Salix*.

Following the occurrence of a crossover within the heterozygous inversion, a dicentric chromatid and an acentric fragment should be pro-

duced at anaphase I. The fragment usually lies in the cytoplasm in the vicinity of the bridge and varies in size with the length and position of the inverted region. Only five cases were encountered in these hybrids where the fragment could be seen associated with the bridge (*Figs. 25, 30*). This is not an unusual condition, for Swanson (1940) finds that in *Tradescantia* fifty per cent of the bridges studied lacked visible fragments. Sax (1937) and Darlington (1937) report a somewhat similar condition. These investigators account for the lack of fragments on the basis of the presence of small subterminal inversions, which result in fragments which are below the limit of visibility or are obscured by other chromosomes of the complement.

It is possible that the number of bridges observed was but a fraction of those which actually occurred, since the bridges formed in the smaller chromosomes would break in very early anaphase or stretch so thinly that they could not be seen. Usually the inversion bridge breaks and the two parts of the chromosome reach the poles, but occasionally the bridge fails to break and remains in the cytoplasm following the first division (*Fig. 31*).

No bridges were seen in material from control crosses or from the species of *Populus* examined.

It is of interest that van Dillewijn (1940) notes the presence of chromatin strings stretching between the two anaphase plates at late anaphase I in *P. robusta*. Meurman (1933) figures the heteromorphic sex-chromosome pair in *P. Simonii* (a probable hybrid) as lagging at anaphase I and resembling a chromosome bridge. It is possible that what in the past have been taken to be sex-chromosome pairs at anaphase are in reality inversion bridges. This seems especially likely since the members of the heteromorphic pair seem to differ but slightly in size. In the hybrids studied here, the possibility that what appears to be inversion bridges are in reality dividing sex-chromosomes seems to be ruled out in those cases where two or more bridges were seen in a single cell.

In general it may be said that pollen sterility is due to one or more of three possible factors: purely genetic, structural, and environmental. It is not possible to separate these three causes in the case of interspecific hybrids of *Populus*. From an examination of pure species within this genus it is apparent that pollen sterility varies from 2–7 per cent in those species showing complete chromosome pairing and from 19–45 per cent in those species showing a varying number of univalent chromosomes at metaphase I. Presumably the sterility in the first group was due largely to genetic causes, while that of the second group was due to both genetic and environmental causes. Presumably in the case of the interspecific hybrids all three factors contribute to sterility. At present it is impossible to determine to what extent each of these exerts its influence. There does seem to be a correlation between univalent formation and pollen sterility, as had already been noted in the case of the species studied. In general those hybrids with the higher number of univalents show the higher sterilities. The correlation is high, since r equals 0.88.

TABLE 10.

A COMPARISON OF CERTAIN NATURAL AND ARTIFICIAL INTERSPECIFIC HYBRIDS OF POPULUS IN REGARD TO DATE OF ORIGIN, UNIVALENT CHROMOSOME FORMATION, AND POLLEN STERILITY.

Name or number	Cross	Probable date of origin	Univalents per cell	Percentage of sterility
Natural Hybrids:				
×P. berolinensis	P. laurifolia × P. nigra var. italica	1870	5.30	57
×P. canadensis var. Eugenei	P. deltoides × P. nigra	1850	4.92	63
Artificial Hybrids:				
OP–67	P. nigra × P. laurifolia	1925	1.00	16
OP–114	P. nigra × P. trichocarpa	1925	0.80	23

The failure of chromosome pairing in these hybrids, followed by a loss of chromosomes or unequal distribution of chromosomes to the microspores, results in deficiencies and duplications of entire chromosomes. In the case of deficiencies, at least, this would lead to the sterility of those microspores deficient for one or more chromosomes. The loss of fragments of chromosomes through the formation of inversion bridges would also result in deficiencies for parts of chromosomes.

Peto (1938) notes that the pollen sterility in the hybrids within the section *Leuce* compares favorably with the sterility of the parent species. In the case of the hybrids considered here, it seems that the natural hybrids have an average sterility which is considerably higher than that of the parent species. The artificial hybrids, on the other hand, possess sterilities corresponding to those of the species, ranging from 5–40 per cent with an average of 19.7 per cent. The natural hybrids, however, range from 6–80 per cent with an average of 46.7 per cent pollen sterility.

It is known that the mutation rate increases with age (Cartledge and Blakeslee, 1934, and 1935). It might be expected that those physiological changes in the cell which condition this increased mutation rate might cause an increase in susceptibility to environmental influences, and, in addition, that structural changes occurring over a long period of time might accumulate to produce a greater pollen sterility. Certain natural and artificial interspecific hybrids of *Populus* are compared in Table X.

Some of the common meiotic abnormalities found in the interspecific hybrids of *Populus* have been mentioned. Among these were asynapsis, with subsequent lagging univalent chromosomes at anaphase I and II, and irregularities in cytokinesis within the pollen mother cell which result in dyads, triads, and number of microspores in excess of four. A pre-

TABLE 11.

A COMPARISON OF INTERSECTIONAL WITH INTRASECTIONAL CROSSES OF POPULUS, AND OF CROSSES BETWEEN NON-GEOGRAPHICALLY ISOLATED SPECIES WITH CROSSES BETWEEN GEOGRAPHICALLY ISOLATED SPECIES.

Class of hybrid	Number of hybrids	Average number univalents Metaphase I	Average number bridges Anaphase I	Average Per cent Sterility
Crosses within the section	13	3.22	0.19	37.9
Crosses between sections	7	0.90	0.17	25.0
Crosses between geographically isolated species	10	2.52	0.16	36.25
Crosses between non-geographically isolated species	3	5.78	0.14	54.0

cocious furrowing was a common abnormality of this sort. It was not uncommon to find that the furrowing process was well advanced before the completion of the second meiotic division. Muntzing (1936) and van Dillewijn (1940) describe cases of spindle fusion during the meiotic divisions in *Populus,* which result in microspores with the unreduced number of chromosomes. This is a possible cause for the occurrence of autotriploids within the genus. What appears to be a case of a third division in the pollen mother cell before microspore wall formation is shown in *Fig. 22*. Four spindles have formed and the thirty-eight chromosomes present have apparently been distributed to the poles at random.

DISCUSSION

Cytological and genetical studies of interspecific hybrids have been used by many investigators to establish probable interspecific relationships in plants and animals. The cytological study of species hybrids based upon chromosome configurations and sterility counts has been particularly useful, since it can be used as a basis for the determination of the manner in which speciation has taken place.

In the one varietal hybrid of *Populus* studied, *P. nigra* var. *plantierensis* (*P. nigra* var. *italica* × *P. nigra* var. *betulifolia*), the chromosome behavior and pollen sterility is comparable to that of a pure species.

Since *Populus* has been subdivided into four sections, and since the species within a section resemble one another more than they resemble those of other sections, it might be supposed that intrasectional hybrids would be more easily obtained and would show a lower percentage asynapsis and pollen sterility than intersectional hybrids. Table XI compares crosses between and within the sections *Tacamahaca* and *Aegeiros,*

which are perhaps less distinctly set off from one another than from the other sections of the genus. From the table it is clear that there is no significant difference in regard to asynapsis and pollen sterility between crosses within and those between these sections.

A glance at Table VI will show that the majority of the crosses are inter- and intrasectional crosses involving species belonging to the two sections *Tacamahaca* and *Aegeiros*. This is to be expected, since these two sections contain more species than do the others. Further, the species producing the better timber trees of the genus are placed in these sections. No hybrids are known either within the section *Leucoides* or between species of this section and those of the other sections of the genus. Interspecific hybrids are known, however, within and between the sections *Leuce* and the sections *Tacamahaca* and *Aegeiros*. Table VI seems to indicate that crosses within the section are more easily obtained than are intersectional crosses (except *Tacamahaca* and *Aegeiros*). Heimburger (1940) sees a definite limitation to species hybridization based on genetic affinities which cause crossing to follow a series similar to the series aspens — silver poplars — cottonwoods — balsam poplars. However, Johnson (1939) states that there appears to be little limitation to species hybridization within the genus, as far as artificial hybridization is concerned.

A comparison of the cytological behavior of hybrids between geographically isolated species is of interest, inasmuch as it permits a study of the effects of isolation upon speciation over long periods of time. Table XI compares hybrids which result from crossing of geographically isolated species with hybrids resulting from the crossing of non-geographically isolated species, in regard to asynapsis, inversion bridge formation, and pollen sterility. It is apparent that there is no significant difference between the two groups. Species of *Populus* native to North America cross readily with European and Asiatic species to produce hybrids which are as fertile as those resulting from crosses between native North American species.

The term ecospecies, defined by Turesson (1922) as uniform types between which crossing is possible with a relatively high degree of fertility but which commonly are prevented from doing so by isolating barriers, either edaphic or geographical, would seem to apply to species of *Populus*. An examination of hybrids between certain species reveals a considerable amount of sterility; however, the F_2 and backcross generations which have been obtained show a segregation indicative of an exchange of genes between the two species.

Species as discrete units can exist as such only by means of some isolating mechanism. Various classifications of these mechanisms have been made (Sax, 1936; Dobzhansky, 1941). In general they may be divided into five classes: edaphic (adaptation to particular local habitats), geographic, physiological (probably genetic, but in this case referring to flowering time), chromosomal (either numerical or structural), and purely genetic.

TABLE 12.

HABITATS AND BLOOMING TIMES OF CERTAIN SPECIES OF POPULUS WHICH OCCUPY THE SAME GEOGRAPHIC REGION.

Species	Physiological blooming time in Arnold Arboretum	Edaphic ecological habitat
P. grandidentata	April 20/40	Moist sandy soil, gravelly hillsides
P. tremuloides	April 20/40	Rich moist soil, borders of streams and swamps
P. deltoides	May 5/40	Low, river-bottomlands
P. Tacamahaca	May 4/40	Low, often inundated river-bottomlands, swamp borders

Considering the three sections of the genus which have been studied, it is clear that artificial hybrids may be made in any direction. The F_1 hybrids have proved to be relatively fertile. However, at this time insufficient data are at hand to determine with certainty the viability of the second generation. It would seem that genetic isolation itself could not be the major factor in isolation of the species.

Autotriploid forms of a number of species within the section *Leuce* have been described. These, though relatively fertile, are unable to preserve their identity. No other polyploid forms are at present known in this genus. Polyploidy (numerical chromosomal isolation) is ruled out as a factor in speciation within this genus. Evidence has been advanced to show that *Populus* is a derived or secondary polyploid, so polyploidy may have played a part in the ancestral differentiation of the genus. The discovery of inversion bridges in the F_1 interspecific hybrids and the asynapsis present in most hybrids indicate considerable structural differentiation of the chromosomes of the different species. But, as in the case of genetic isolation, it does not appear to prevent the crossing of the species or the production of a relatively fertile F_1 generation.

A striking example of physiological isolation is found in this genus. The species included within the section *Leuce* are earlier in blooming time by two to three weeks than are those of the other species of the genus. They are thus definitely set off in nature. This isolation may be overcome artificially (Smith and Nichols, 1941). However, the major factor in isolation of the species of *Populus* seems to be isolation of a geographic and edaphic nature. Both types are essentially the same, geographic isolation being perhaps more complete and on a larger scale than edaphic isolation.

The species *P. grandidentata, P. tremuloides, P. deltoides,* and *P. Tacamahaca* occupy a somewhat similar area in northeastern United States and the adjoining region of Canada. Table XII summarizes their habitats and blooming times.

Populus grandidentata and *P. tremuloides* are set off from the remaining two species by their time of blooming. Presumably they are themselves isolated by edaphic factors, since their habitats differ somewhat. However, the hybrid *P. grandidentata* × *P. tremuloides* does occur. Similarly the hybrid × *P. Jackii* (*P. Tacamahaca* × *P. deltoides*) occurs rather frequently, although presumably the parental species are separated by edaphic isolation.

A consideration of evidence available would indicate that the first step in speciation in *Populus* may have been a physiological isolation of a group or groups of species from the general population, since the major geographic groups of *Populus* species all contain species belonging to the section *Leuce*. This physiological isolation was in turn followed by geographic and edaphic isolation. The structural-chromosomal differentiation which has taken place since that time has been insufficient to prevent interspecific hybridization and the production of reasonably fertile hybrids.

The slow accumulation of genetic differences through mutation has probably been the major factor in the differentiation of the species of *Populus,* but apparently these species can exist as discrete units only as a result of physiological isolation, in the case of the section *Leuce,* and geographic or edaphic isolation in the case of the individual species.

ACKNOWLEDGMENTS

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SUMMARY

The chromosome numbers of thirty-eight species, varieties, and natural hybrids of *Populus* have been determined, twelve of which were in confirmation of previous work by other investigators. All species exist as diploids with an unreduced chromosome number of thirty-eight. In the case of three species, all within the section *Leuce,* triploid forms with an unreduced chromosome number of fifty-seven exist.

The chromosomes of the hybrid *P. nigra* × *P. trichocarpa* at the pachytene stage of the meiotic prophase vary in length from 30.6 to 8.5 μ in length. One group of three and two groups of two chromosomes are of exactly the same length. No heteromorphic chromosome pair which might be interpreted as a sex-chromosome pair has been seen consistently.

The occurrence of secondary association of chromosomes has been noted by other workers. Additional evidence for secondary polyploidy is found in the fact that morphological similarities exist among certain groups of chromosomes and that occasional trivalent associations of

chromosomes are found in hybrids and quadrivalent associations in the triploid form of *P. alba*.

The instigation and progress of the meiotic divisions within the catkin are consistent within the species and within the section of the genus. These divisions are regular as a rule, but in a few species a certain amount of asynapsis and abnormal pollen sterility is encountered. This is not interpreted as due to any genetic dissimilarity between the chromosomes.

One chromosome of the complement is invariably associated with the nucleolus at meiotic prophase. In the majority of cases the nucleolus possesses a knob which projects from the point of attachment of the associated chromosome.

The triploid form of *P. alba* shows a varying number of univalent, bivalent, trivalent, and quadrivalent chromosome configurations at metaphase I. The fact that the pollen sterility of the triploid forms is somewhat less than that to be expected on the basis of univalent formation is accredited to the fact that the basic set of chromosomes is triplicated and thus genetic unbalance, due to the loss of whole chromosomes, is less likely to occur.

Cytokinesis is of the type usually found in cases of simultaneous pollen cell formation. The process seems to be easily upset in the case of triploid forms and interspecific hybrids of *Populus*.

A study of the development of the male gametophyte from the first microspore division up to the time of fertilization shows this to be typical of the process as found in the Angiosperms.

The geographic distribution of the North American species of *Populus* has been investigated, especially as related to the occurrence of natural hybrids. The natural and artificial hybrids which have been reported are arranged in tabular form to show the extent of crossing within and between sections of the genus.

The study of chromosome behavior at meiosis in ten natural and twenty-one artificial interspecific hybrids shows them to be variable in regard to synapsis. They are probably structurally undefined hybrids under Darlington's classification. It has been demonstrated that the chromosomes of the different species have been differentiated structurally and that inversion bridges are of relatively frequent occurrence. Pollen sterility within the artificial hybrids is of the same order as that within the parental species, but that of the natural hybrids is somewhat higher. An attempt is made to explain this fact on the basis of an accumulation of structural changes within the chromosomes over the relatively longer period of time these natural hybrids have been in existence as clones. No significant difference in pollen sterility, univalent formation, or inversion bridge frequency is found when intersectional crosses are compared with intrasectional crosses. Similarly a comparison of crosses between geographically isolated species and non-geographically isolated species reveals no significant difference.

A review of the isolating mechanisms which may operate in this genus seems to indicate that geographic and edaphic isolation are those which

separate the species, although one entire section is set off from the other sections of the genus by a physiological isolation. It seems from the evidence available that differentiation of the species of *Populus* has been brought about by a slow accumulation of genetic differences through mutation. These species can exist as discrete units, however, only through the operation of geographic, edaphic and, in a few cases, physiological isolation. Genetic and structural-chromosomal isolation plays a relatively minor part in species isolation.

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DESCRIPTION OF PLATES

All drawings are at a magnification of × 2090.

PLATE I

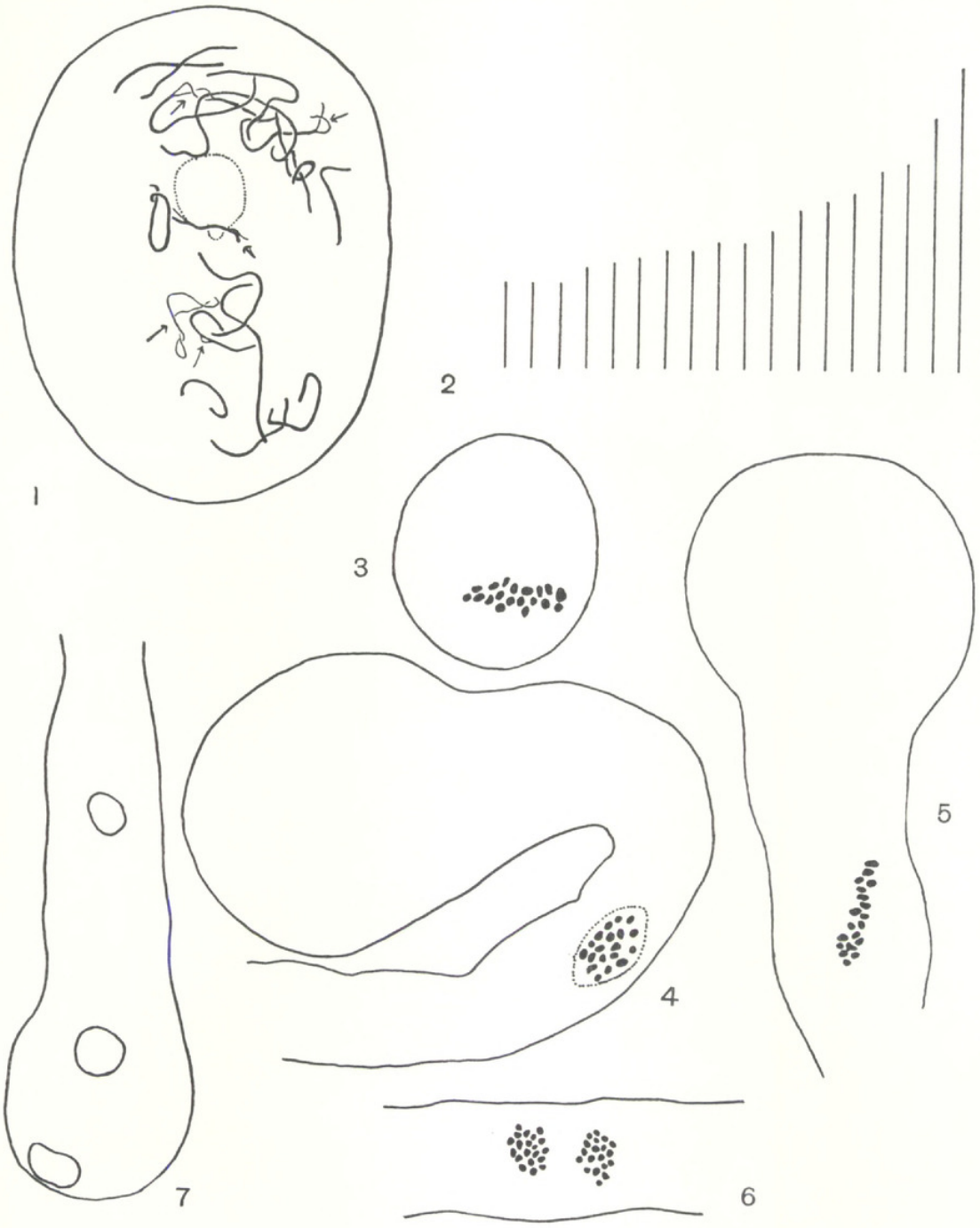
FIG. 1. Pachytene stage of meiosis of the hybrid *P. nigra* × *P. trichocarpa*. One chromosome is associated with the nucleolus at the junction of the knob and the nucleolus proper. Failure of synapsis is indicated by arrows. Camera lucida drawing. FIG. 2. Idiogram of pachytene chromosomes shown in figure 1. One group of three chromosomes and two groups of two chromosomes are of exactly the same length. FIG. 3. Metaphase of the first microspore division in *P. acuminata*. FIG. 4. Generative nucleus in the pollen tube of *P. laurifolia* twelve hours after germination. FIG. 5. Metaphase of the division of the generative nucleus in the pollen tube of *P. laurifolia*. FIG. 6. Anaphase of the division of the generative nucleus in the pollen tube of *P. laurifolia*. FIG. 7. Pollen tube showing tube nucleus (at lower end of tube) and two gametes of *P. deltoides*.

PLATE II

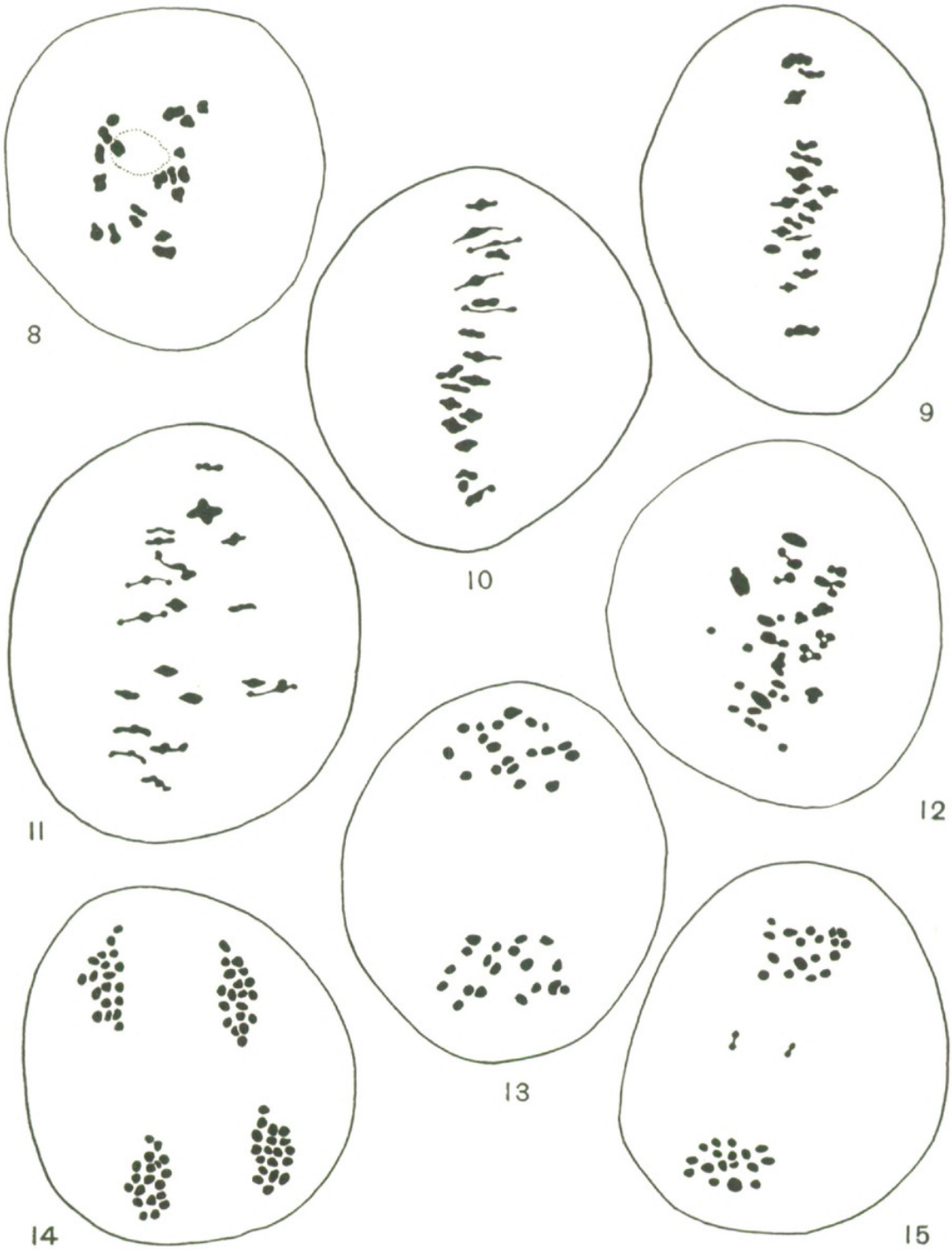
FIG. 8. Late diakinesis in *P. Sargentii*. Nineteen bivalent chromosomes present, one of which is associated with the nucleolus. FIG. 9. Metaphase I in *P. acuminata*, showing nineteen bivalent chromosomes. FIG. 10. Metaphase I in *P. adenopoda*, with "heteromorphic chromosome pair" at lower side of cell. FIG. 11. Metaphase I in *P. alba* (diploid), with nineteen bivalent chromosomes. FIG. 12. Metaphase I in *P. alba* (triploid). Nine univalent, seven bivalent, ten trivalent, and one quadrivalent chromosomes are present. FIG. 13. Anaphase I in *P. laurifolia*, showing nineteen chromosomes at each pole. FIG. 14. Anaphase II in *P. alba* (diploid). FIG. 15. Anaphase I in *P. acuminata*. Two lagging univalent chromosomes are shown in division.

PLATE III

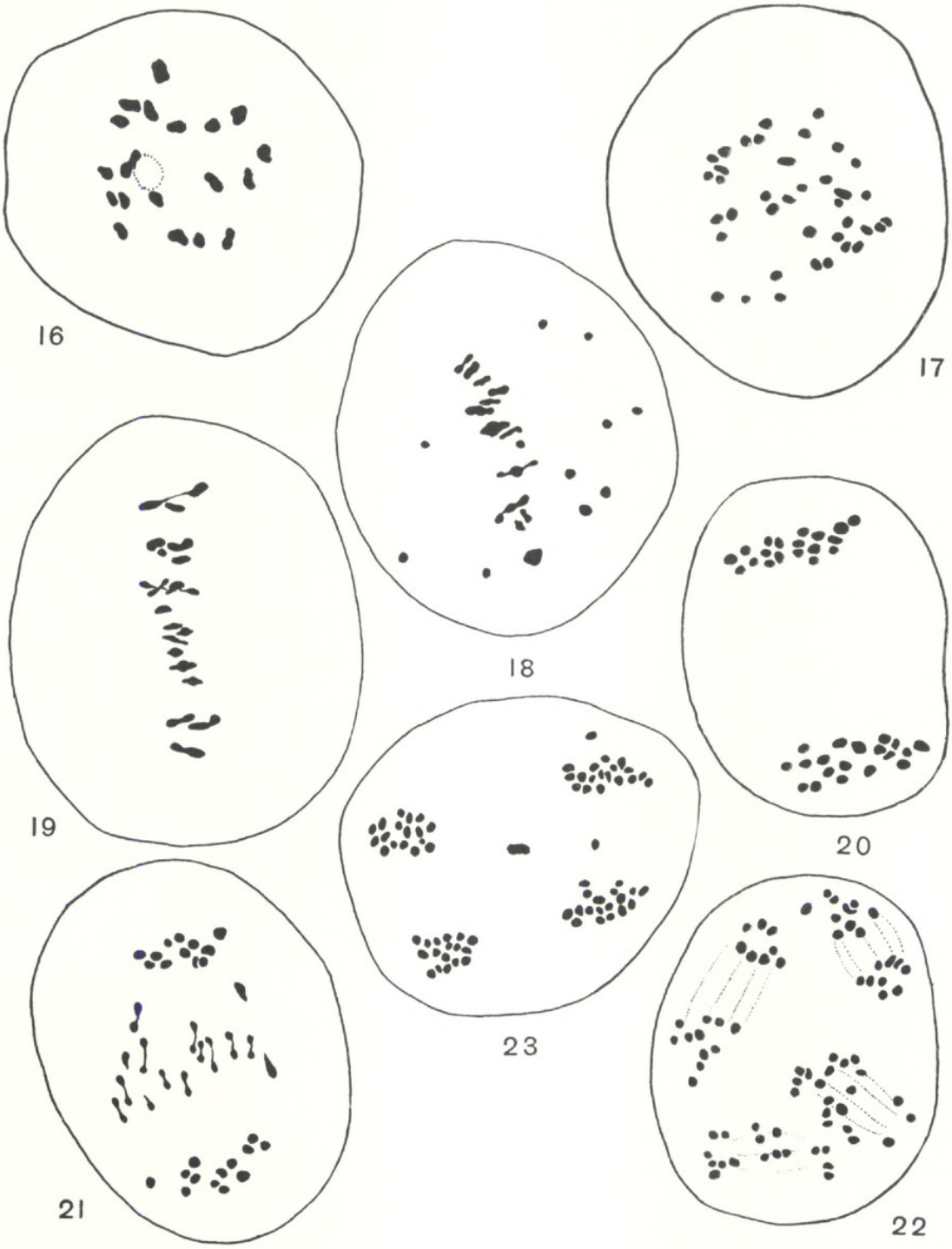
FIG. 16. Late diakinesis in the hybrid × *P. Rasumowskyana* × *P. caudina*, with nineteen bivalent chromosomes present. FIG. 17. Metaphase I in × *P. Woobstii* showing thirty-eight univalent chromosomes. FIG. 18. Metaphase I in *P. nigra* × *P. laurifolia*, showing fourteen bivalent chromosomes on the plate and ten univalent chromosomes scattered at the sides. FIG. 19. Metaphase I in *P. nigra* × *P. laurifolia*, showing nineteen bivalent chromosomes on the plate. FIG. 20. Regular anaphase in *P. nigra* × *P. laurifolia*, with nineteen chromosomes at each pole. FIG. 21. Irregular anaphase I in *P. nigra* × *P. laurifolia*; thirteen univalent chromosomes dividing on the plate; irregular distribution of chromosomes to the poles. FIG. 22. A third division within the pollen mother cell in × *P. charkoviensis* × *P. deltoides*, with a random distribution of the chromosomes to the poles. FIG. 23. Anaphase II in × *P. Rasumowskyana* × *P. caudina*. Apparently two lagging chromosomes have been lost at the first division and one is lagging at the second division.



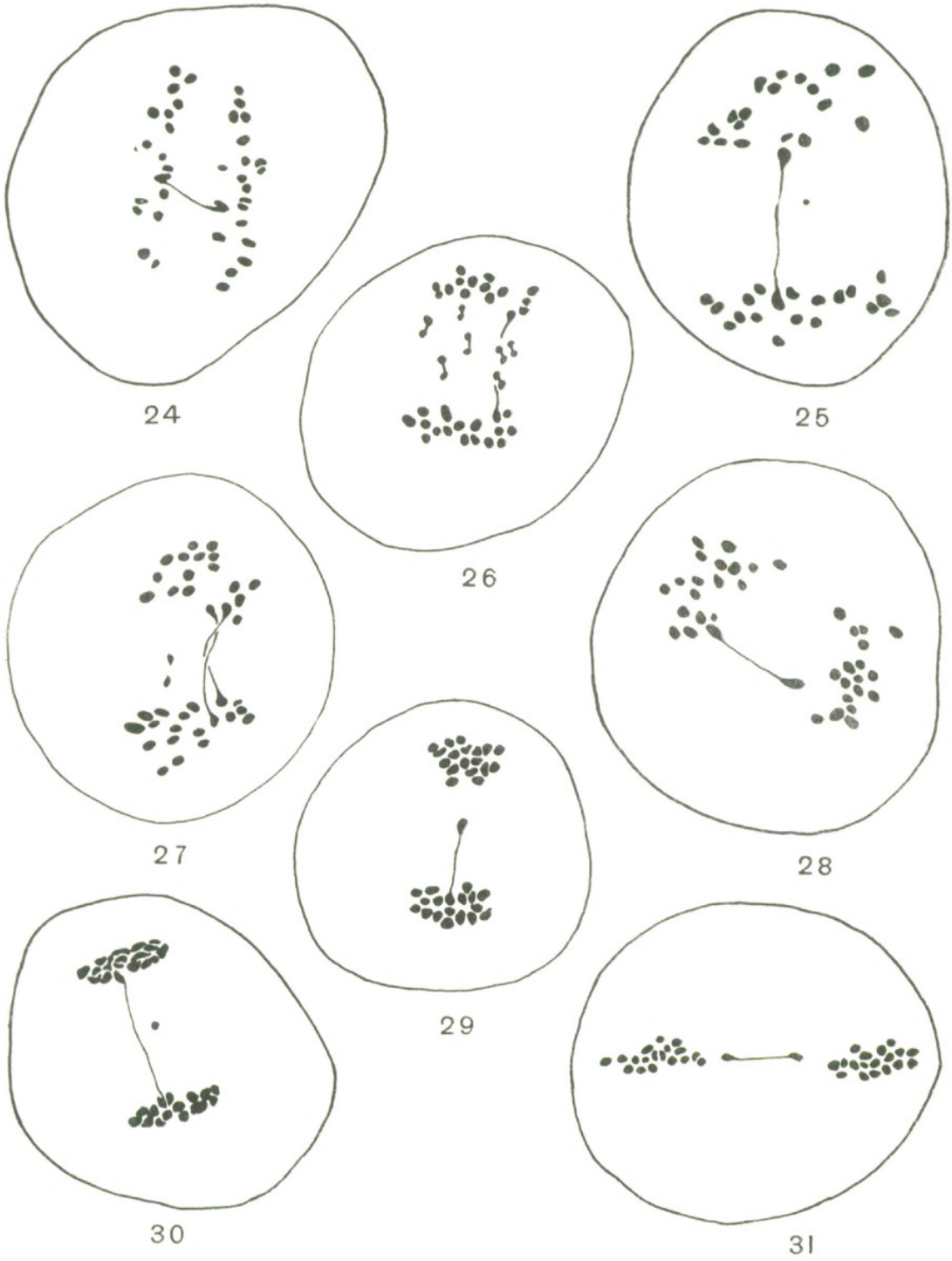
CYTOLOGY AND SPECIATION IN POPULUS



CYTOLOGY AND SPECIATION IN POPULUS



CYTOLOGY AND SPECIATION IN POPULUS



CYTOLOGY AND SPECIATION IN POPULUS

PLATE IV

FIG. 24. Early anaphase I in ×*P. charkoviensis* × ×*P. berolinensis,* showing an inversion bridge without a visible fragment. FIG. 25. Anaphase I in ×*P. charkoviensis* × ×*P. berolinensis,* with an inversion bridge and fragment. FIG. 26. Anaphase I in ×*P. charkoviensis* × ×*P. robusta.* Remnant of an inversion bridge. FIG. 27. Anaphase I in ×*P. Rasumowskyana* × *P. caudina,* showing two inversion bridges and one dividing fragment. FIG. 28. Anaphase I in ×*P. Rasumowskyana* × unidentified cottonwood. Bridge, no fragment visible. FIG. 29. Anaphase I in *P. nigra* × *P. laurifolia.* Inversion bridge, no fragment. FIG. 30. Late anaphase I in ×*P. charkoviensis* × ×*P. berolinensis.* Bridge and fragment. FIG. 31. Unbroken inversion bridge left out in cytoplasm at the first meiotic division in ×*P. Andrewsii.*

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