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Genetic Analysis on the Hybrid Origin of *Populus Tomentosa* CARR.

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Abstract

Results supported the hypothesis that *P. tomentosa* CARR. was the natural hybrid of *P. alba* and *P. davidiana*. Evidence supporting hybrid origin came from studies in which comparisons of floral bracts of parents and progenies were made as follows: 1) from control-cross progenies of *P. alba* x *P. davidiana*, 2) from segregation studies of backcrossed progenies of both *P. alba* x *P. tomentosa* and *P. davidiana* x *P. tomentosa*, 3) from open-pollinated populations of female and male *P. tomentosa* plants possessing different bract types. Additional support for hybrid origin came from quantitative analysis of flower organ measurements.

Key words: P. tomentosa, P. alba, P. davidiana, control-pollination, backcrossing, open-pollination, origin, inheritance.

FDC: 165.1; 165.3; 165.71; 176.1 *Populus tomentosa.*

Introduction

P. tomentosa, belonging to the section of Leuce Duby, is a fast growing timber species native to China. It has been cultivated more than any other poplar species because of its fast growth, excellent wood quality and resistance to plant diseases and insect pests, especially the roundheaded borer (YANG, 1991). In China it is cultivated as timber species in Hebei, Shandong, Henan, Shanxi, Shaanxi, southeast Gansu, the north of Jangsu and Anhui (ZHANG, 1958). It is quite important to the economy of northern China. The species is researched in the provinces mentioned above and in Ningxia and Beijing. Most research concentrates on selection of plus trees and clonal testing.

The existence of *P. tomentosa* was first acknowledged as a species in Revue Horticole in 1867 (CARRIERE, 1867). Since then, many plant taxonomists have discussed its taxonomic position. CARRIERE (1867), LEE (1935), SCHENCK (1939), LIU (1955), and CHEN (1959) all consider it to be an independent species and call it *Populus tomentosa* CARR. HENRY (1903) also acknowledges it as an independent species but calls it *Populus peking* L. HENRY. MAXIMOWICZ (1879) and WESMAEL (1887) regard it as a variety of the white poplar. After morphologically comparing the variation in bracts of *P. alba, P. tremula, P. davidiana, P. x canescens* and *P. tomentosa,* BARTKOWIAK (1961) first suggests *P. tomentosa* originates from a natural hybrid. When comparing 25 characters of *P. tomentosa* with those of *P. alba, P. alba* var. *bachofenii, P. alba* var. *bolleana, P. tremula, P. davidiana, P. x canescens,* BIALOBOK (1964) suggests that *P. tomentosa* is a natural hybrid of *P. alba* and *P. davidiana.* This paper is an important and valuable taxonomic report about the taxonomic position of *P. tomentosa.* WAN and FANG et al. (1984) still believe that *P. tomentosa* is an independent species, but the hybrid origin of *P. tomentosa* is acknowledged by most scholars. BIALOBOK (1964) also points out *P. tomentosa* is similar to *P. adenopoda.* There are now 3 viewpoints about this origin. First, *P. tomentosa* originates from *P. alba* and *P. davidiana.* This view is supported by NIU and QU (1980), YU and ZHANG (1992). Second, *P. tomentosa* originates from *P. alba* x *P. adenopoda.* This view is supported by BAI and MA (1990). Third, *P. tomentosa* may result from the combination of several natural hybrids, mainly *P. adenopoda* x *P. davidiana* and its parents, also include *P. alba* and *P. hopeiensis.* ZHAO (1987) and XUE (1981) believe the last viewpoint. No genetic studies on *P. tomentosa* have been reported.

The studies mentioned above represent a valuable information source on the possible origin of *P. tomentosa.* Their conclusions, however, are inferred from morphological comparisons of *P. tomentosa* with assumed parents, or are judged on the basis of the overlapping distributions of assumed parents. AYALA and KIGER, JR. (1984) point out that "Nothing in biology is understandable except in the light of genetics." In the following paper, we investigated the origin of *P. tomentosa* by genetic studies.

Materials and Methods

The genetic analysis of the origin of *P. tomentosa* was made from 4 studies: (1) comparing floral bracts of control-pollinated F1 progenies from *P. alba* x *P. davidiana* with those of *P. tomentosa* and both parent species; (2) analyzing segregation of floral bracts of backcrossed offspring and comparing them with tentative parent species, *P. alba* and *P. davidiana,* and

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with *P. tomentosa;* (3) comparing and analyzing the plant components of both the female and the male section from open-pollinated populations, respectively; (4) quantitative analysis of above-mentioned plant materials.

The control-pollinated crosses of *P. alba* x *P. davidiana* were made by CHANGGENG MA and PEILIN LIU et al., in the 1950's. The backcrosses of *P. alba* x *P. tomentosa* were made by Professor PEIZHONG YE in the 1950's. Those hybrids were introduced into Shaanxi in 1960's. Backcrosses from *P. davidiana* x *P. tomentosa* were made by the genetic team at Northwest College of Forestry and PEILIN LIU in the 1950's. The materials studied for the open-pollinated populations came from the collection of Northwest College of Forestry in Shaanxi and were used from 1982 to 1993. All the living *P. tomentosa* specimens examined were growing at the central nursery in Hu county, Shaanxi. These specimens were identified taxonomically by YU and ZHANG and were published and reported as new taxonomic units (YU et al., 1992; ZHANG et al., 1987; ZHANG,

Table 1. – The identification numbers; types of female *P. tomentosa, P. alba,* and *P. davidiana;* and 10 measurements or ratios of measurements of catkin and bract traits.

			Catkins		Bracts							
								Lacisions				
No.	Sex	Name	L	W	L	W	L:W	num.	D	D:L	D:W	Color[1]
			(cm)		(mm)				(mm)			
00	♀	P. tomentosa cl.'Laevicorticata'	5.25	0.66	4.21	3.69	1.14	8.85	0.93	0.22	0.25	2.00
01	♀	P. davidiana X P. tomentosa	4.74	0.62	4.18	4.24	0.99	9.38	1.92	0.50	0.45	0.50
02	♀	P. alba	4.10	0.45	2.75	1.65	1.67	0.00	0.00	0.00	0.00	3.00
03	♀	P. alba X P. tomentosa (Nanjing)	3.18	0.39	3.56	1.38	2.59	0.00	0.00	0.00	0.00	3.00
04	♀	P. davidiana	5.60	0.50	3.14	2.97	1.06	8.37	1.20	0.38	0.40	0.50
05	♀	P. alba X P. davidiana	4.70	0.43	2.85	2.47	1.15	6.35	0.71	0.25	0.29	2.00
06	♀	P. tomentosa (Tongyuanfang)	3.82	0.46	3.40	2.87	1.18	7.02	0.79	0.23	0.28	2.00
07	♀	P. tomentosa cl.'Rotundifolia'	5.20	0.70	3.89	3.25	1.20	6.93	1.32	0.34	0.41	2.00
08	♀	P. tomentosa cl.'Microphalla'"	4.80	0.70	3.40	2.02	1.68	4.45	1.04	0.31	0.51	3.00
09	♀	P. tomentosa cl.'Parvibracteata'	5.15	0.65	4.01	2.77	1.45	7.87	1.13	0.28	0.41	2.00
10	♀	P. tomentosa cl.'Yanglingensis'	6.27	0.73	4.00	2.88	1.39	8.80	1.27	0.32	0.44	2.00
11	♀	P. tomentosa cl.'Evitibracteata'	4.38	1.02	4.38	2.99	1.46	0.00	0.00	0.00	0.00	3.00
12	♀	P. tomentosa cl.'Hopeinica'	4.18	0.98	5.00	5.22	0.96	9.62	2.39	0.48	0.46	0.50

[1]) Color was scored qualitatively as 0.5 for dark brown, 2 for brown, and 3 for light brown.

Table 2. – The identification numbers; types of male *P. tomentosa, P. alba,* and *P. davidiana;* and 10 measurements or ratios of measurements of catkin and bract traits.

			Catkins		Bracts							
								Lacisions				
No.	Sex	Name	L	W	L	W	L:W	num.	D	D:L	D:W	Color[1]
			(cm)		(mm)				(mm)			
00	♂	P. tomentosa cl.'Longibracteata'	10.70	1.37	8.88	4.62	1.92	8.00	1.31	0.15	0.28	2.00
01	♂	P. tomentosa cl.'Glabricorticea'	13.27	1.09	6.39	4.64	1.38	6.98	1.82	0.28	0.39	2.00
02	♂	P. tomentosa cl.'Platybracteata'	13.37	1.25	7.41	6.20	1.20	11.75	1.91	0.26	0.31	2.00
03	♂	P. tomentosa cl.'Fastigiata'	9.51	1.05	6.82	5.34	1.28	9.13	1.90	0.28	0.36	2.00
04	♂	P. tomentosa cl.'Intermedia'	11.68	1.36	7.79	4.63	1.68	8.50	1.20	0.15	0.26	2.00
05	♂	P. tomentosa cl.'Stellibracteata'	11.87	1.12	5.35	5.04	1.06	8.10	2.18	0.41	0.43	0.50
06	♂	P. tomentosa cl.'Pallidibracteata'	13.31	1.13	7.44	5.16	1.44	7.07	1.47	0.20	0.28	2.00
07	♂	P. tomentosa cl.'Truncata'	11.33	1.12	7.45	3.82	1.95	7.02	1.88	0.25	0.49	2.00
08	♂	P. tomentosa cl.'Spathacea'	12.30	1.10	6.92	4.71	1.47	9.88	2.61	0.38	0.55	2.00
09	♂	P. tomentosa cl.'Ferruginea'	13.31	1.13	5.10	3.72	1.38	6.97	1.43	0.28	0.39	2.00
10	♂	P. tomentosa (Xiyuan)	11.27	1.05	7.00	3.77	1.86	3.62	1.07	0.15	0.28	2.00
11	♂	P. tomentosa cl.'Borealo-sinensis'	11.53	1.35	6.20	3.34	1.86	4.03	1.25	0.20	0.37	2.00
12	♂	P. alba X P. tomentosa (Nanjing)	5.57	0.81	5.26	3.02	1.74	3.27	0.79	0.15	0.26	3.00
13	♂	P. davidiana	5.66	0.65	4.45	3.88	1.15	7.63	1.54	0.35	0.40	0.50
14	♂	P. tomentosa cl.'Dentibracteata'	6.36	0.87	5.78	3.00	1.93	0.0	0.0	0.0	0.0	3.00
15	♂	P. alba	4.21	0.58	3.42	2.65	1.29	0.0	0.0	0.0	0.0	3.00

[1]) Color was scored qualitatively as 0.5 for dark brown, 2 for brown, and 3 for light brown.

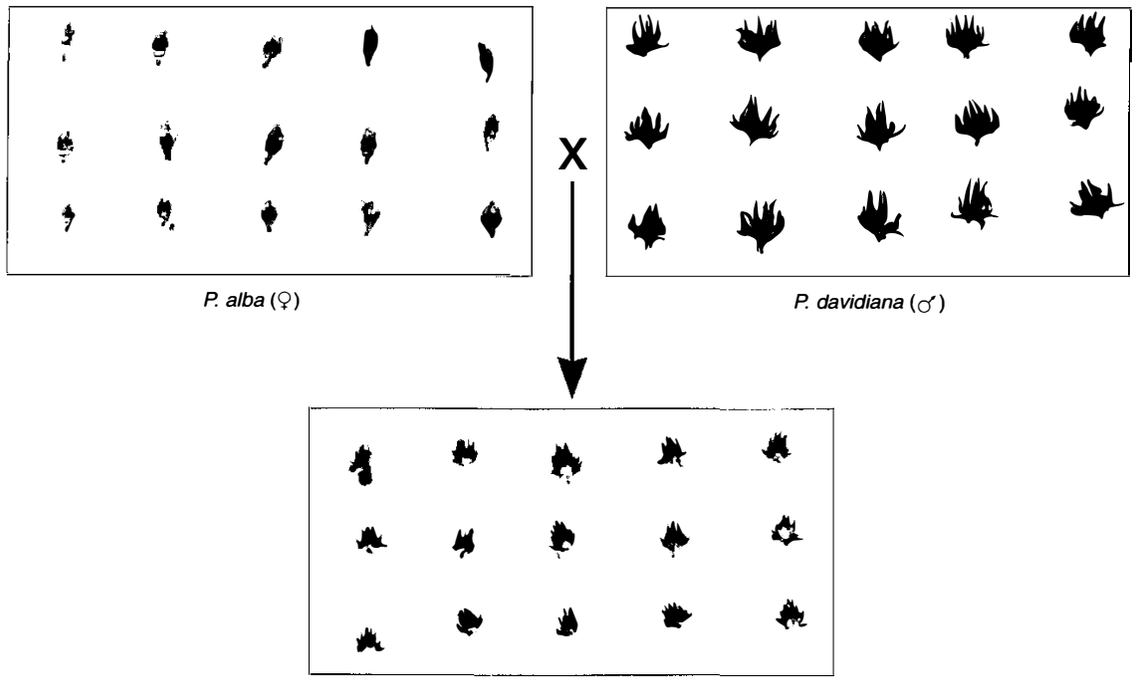

Figure 1. – Floral bracts of parent species and progeny from control-pollinations between *P. alba* X *P. davidiana.*

1988; ZHAO, 1978). All types of bracts were identified by CHEN WANG, a poplar specialist in the Forestry and Soil Institute at Shengyang. Floral organs were studied, especially the bracts, because they were more stable during development, and both parents and offspring had distinct and contrasting floral parts.

Bracts of male flowers collected from 3 old plants in Xiyuan were used as typical bracts of *P. tomentosa.* The plants had developed from root sprouts of a maternal plant that was of the same generation as the plant of the type specimen of *P. tomentosa* used by CARRIRER in 1867 (GU, 1983). The male specimens used by WANG and by NIU (1980) also had this type of flowers.

Female *P. tomentosa* plants are rare, so most poplar specialists, like BARTKOWIAK (1961) and BIALOBOK (1964), have only studied male plants. In the present study, we used female bracts collected from a young tree at Tongyuanfang as the typical female *P. tomentosa* bracts. The female bracts are identical to those of *P. tomentosa,* illustrated in the book "Poplars in Shaanxi" by NIU et al. (1980). They were identical in shape to typical male flowers mentioned above but were slightly smaller. The female *P. tomentosa* at Tongyuanfang is a very valuable specimen. Its bracts were identical to female bracts of the trees that are widely distributed today in Shaanxi province.

The female and male catkins studied were collected after the elongated male catkins had begun to drop from the trees. Sixty female and male catkins were usually collected for each of the 29 taxonomic units studied. In the study, one bract was picked at random from the middle of each catkin. The selected bracts were fastened on four slides, with 15 bracts mounted on each slide. The dimensions of bracts were measured to the closest mm. Measurements were made under the magnification of a dissecting microscope. The number and depth of lacinations, and length and width of bracts were counted or measured for each bract. Bract color was scored qualitatively as dark brown (0.5), brown (2.0), light brown (3.0). Matrices of original data of floral organs of the female and male taxonomic sections were presented in *table 1* and *2.*

For qualitative analysis, 15 bracts in each slide were compared among groups. With quantification theory III (DONG et al., 1979) as the genetic model, data processing was done with the analyst's program (Fuchitsu Company, 1985) on a FACOMM 340s computer. The 10 female and male catkin and bract traits of *table 1* and *2,* respectively, were the original variables used in the principal components analyses. The variables were standardized prior to analyses.

Results

Three F1 progenies from control-pollinations between *P. alba* and *P. davidiana* were kept (*Fig. 1*). All 3 plants were female. Their bracts were brown, roundish, and had long, white cilia on the margins. The incisions were not as deep as those found in bracts of *P. davidiana.* The bracts of the F1 offspring were remarkably similar morphologically to those of the typical female *P. tomentosa* from Tongyuanfang. In *table 1,* D:L and D:W of bracts of the F1 offspring (No. 5) were 0.25 and 0.29, while those of the typical female plant (No. 6) were 0.23 and 0.28, respectively. The F1 offspring had bract features that were almost identical to those of *P. tomentosa.*

Figure 2 and *3* are the backcrossing results from parents *P. alba* and *P. davidiana,* respectively, to *P. tomentosa.* The backcross from *P. alba* and *P. tomentosa* produced both female and male hybrid types. Two female plants from *P. alba* and *P. tomentosa* (Tianshui) and one of both female and male plants from *P. alba* and *P. tomentosa* (Nanjing) were kept and used for reforestation. All female bracts had light-brown color, long-oval shape, irregular sawteeth, no incisions, and long cilia on the margins. This combination of characters was identical to those found on *P. alba.* Male bracts of sample 12 in *table 2* had brown color, near rhomboid shape, irregularly laciniate, incisions of intermediate depth (D:L of 0.15 and D:W of 0.26), and with

long white cilia on the margins. This combination of traits was nearly identical to those of the typical *P. tomentosa* in sample 10 of *table 2* (D:L of 0.15 and D:W of 0.28). All 4 backcross hybrids, clones 21, 30, 39, and 45, from *P. davidiana* and *P. tomentosa* (QIU, 1992) in sample No. 1 of *table 1* had dark brown bracts, oblate shape, deeply lacinate (incisions had D:L and D:W ratios of 0.50 and 0.45, respectively), and with long, white dense cilia on the margins. This bract type was identical to that of *P. davidiana* in sample 4 of *table 1.*

Since *P. tomentosa* plants are dioecious, bracts from female and male sections of open-pollinated populations were compared in *figure 4* and *5,* respectively. To facilitate comparison, typical bracts of *P. alba, P. tomentosa,* and *P. davidiana* were put on upper row of each figure, while the hybrids were positioned by sex on the lower row of each figure. Bract data for both the female and male plants revealed the open-pollinated populations of *P. tomentosa* had 3 different bract types. The 3 bract types were as follows: (1.) light-brown color, long-oval shape, irregular sawteeth, and long, white cilia on the margins (identical to traits of *P. alba,* located left-middle of *figure 4* and *5*); (2.) dark-brown color, oblate shape, palmate and deeply lacinate, and long, white, dense cilia on the margins (identical

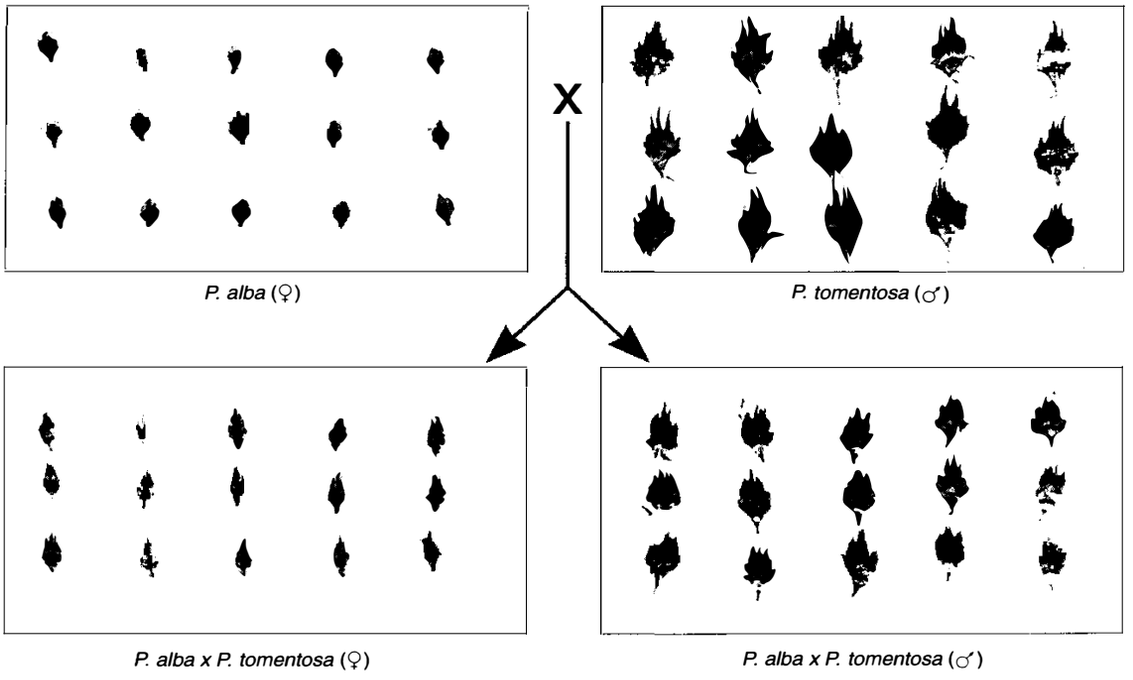

Figure 2. – Floral bracts of parent species and progeny from backcross testing *P. alba* X *P. tomentosa.*

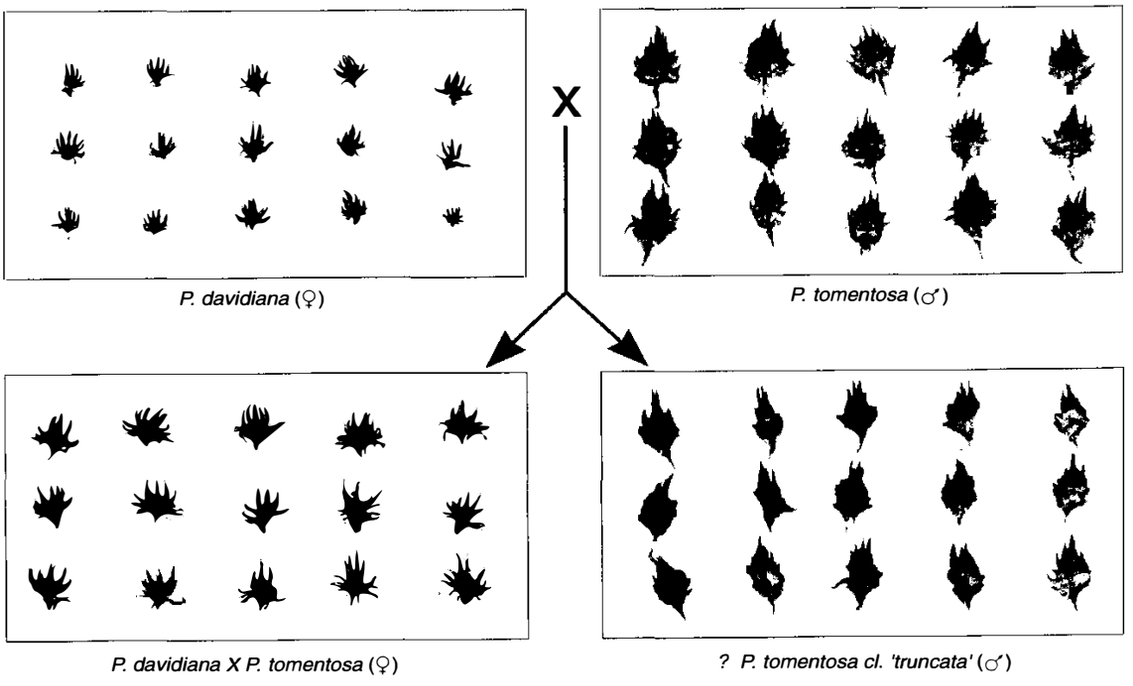

Figure 3. – Floral bracts of parent species and progeny from backcross testing *P. davidiana* X *P. tomentosa.*

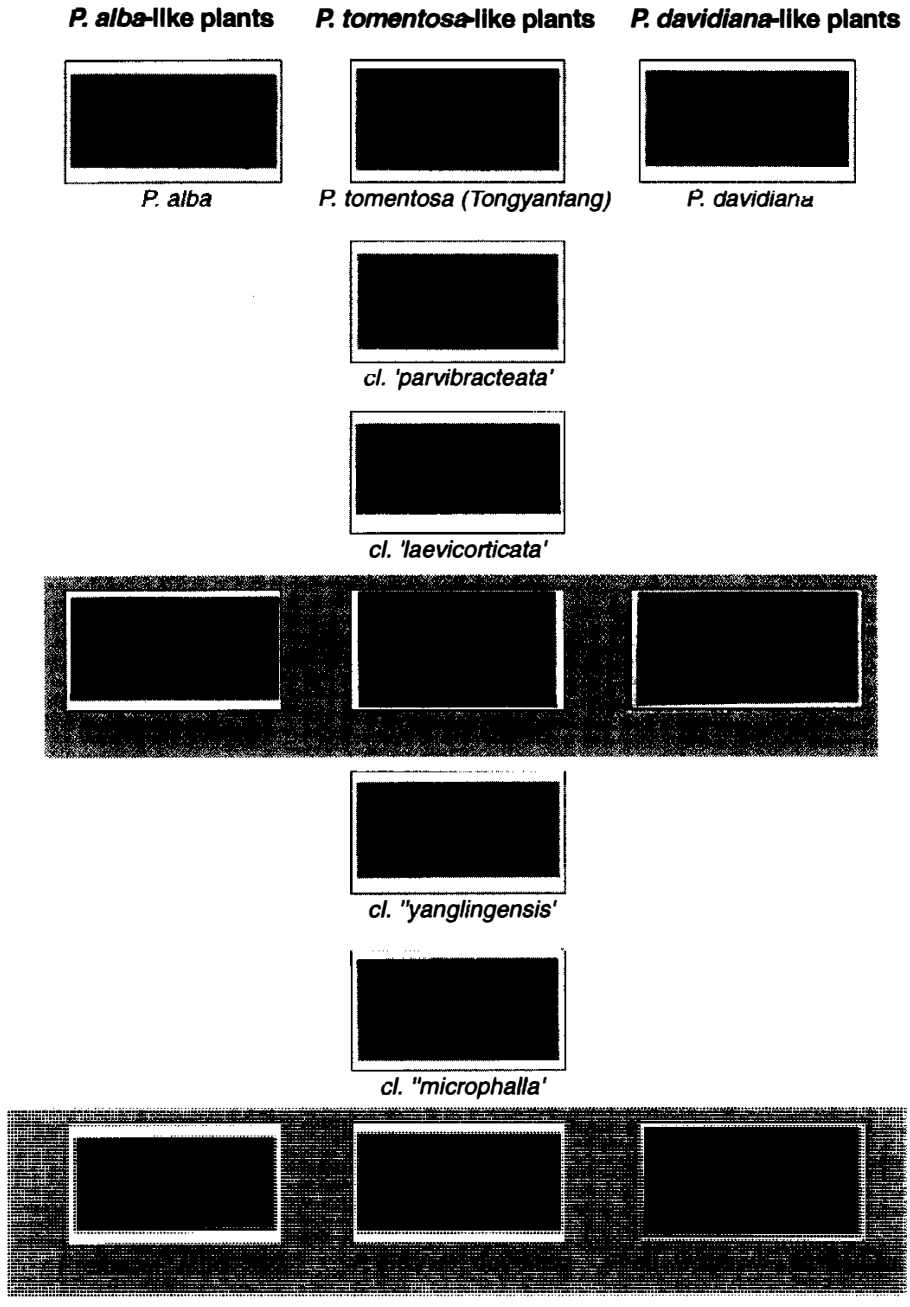

Figure 4. – Floral bract comparison of female trees in a *P. tomentosa* open-pollinated population with bracts of female *P. alba* and *P. davidiana.* Intermediates are *P. tomentosa*-like plants. The *P. tomentosa* allied to *P. davidiana* is located middle right, while the *P. tomentosa* allied to *P. alba* is located middle left.

to traits of *P. davidiana* bracts, located right-middle of *figure 4* and *5*); (3.) brown color (very few were light-brown bracts), roundish to oval shape, intermediate lacinations (one was deeply lacinated), and long, white cilia on the margins (intermediate type between the *P. alba* and *P. davidiana* types, shown in the middle of *figure 4* and *5,* respectively). Bracts of hybrids of the control-pollinations and the backcrosses also belonged to these same three categories.

Results of quantitative analysis using principal components analyses indicated that contributions of the first 2 main components were 91.8% and 88.1% for the female and male sections, respectively. The contributions of the first 3 main components were 95.3% and 92.9% for the female and male sections, respectively (*Table 3* and *4*).

Figure 6 showed the spatial distribution of the 13 female taxonomic units of *table 1,* while *figure 7* showed the spatial distribution of 16 male taxonomic units of *table 2.* Female and male taxonomic units on both figures separated nicely into 3 groups (*P. alba*-like on the right, *P. davidiana*-like on the left, and intermediates that were *P. tomentosa*-like in the middle) on the basis of the distance of every dot from the vertical coordinate axis (II). Each group of taxonomic units was delineated by a dotted line. The *figures 6* and *7* were rotated 90° to the right for ease of reading.

On *figure 6,* No. 2 was *P. alba,* while No. 15 on *figure 7* was *P. alba.* On *figure 6,* No. 4 was *P. davidiana,* while No. 13 on *figure 7* was *P. davidiana.* In the intermediate types, No. 6 on *figure 6* was the typical female *P. tomentosa* from Tongyuanfang and No. 10 on *figure 7* was the typical male *P. tomentosa* at Xiyuan.

On *figure 6,* No. 11 *P. tomentosa* cl. 'Evitibracteata' was in the group allied to *P. alba* and No. 12 *P. tomentosa* cl. 'Hopeinica' was in group allied to *P. davidiana.* On *figure 7,* No. 14 *P. tomentosa* cl. 'Dentibracteata' was located in group allied to *P. alba,* and No. 5 *P. tomentosa* cl. 'Stellibracteata' was in group allied to *P. davidiana.* This also indicated that some individuals in the open pollinated populations had characteristics of *P. alba,* while others resembled *P. davidiana.* These results and those from control-pollinations all supported the results of hybrid origin based upon morphological taxonomy.

Table 3. – Correlation, eigenvalues, percent and cumulative percentage of variation accounted for by the first 3 principal components (PC1, PC2, and PC3).

Sex	Axis number	Correlation coefficient	Eigenvalue	Variation	
				percentage	cumulative
Female	1	0.3551174	0.1261083	83.7	83.7
	2	0.1103625	0.0121799	8.1	91.8
	3	0.0730202	0.0053319	3.5	95.3
Male	1	0.2351540	0.0552974	75.5	75.5
	2	0.0962955	0.0092728	12.7	88.1
	3	0.0592138	0.0035063	4.8	92.9

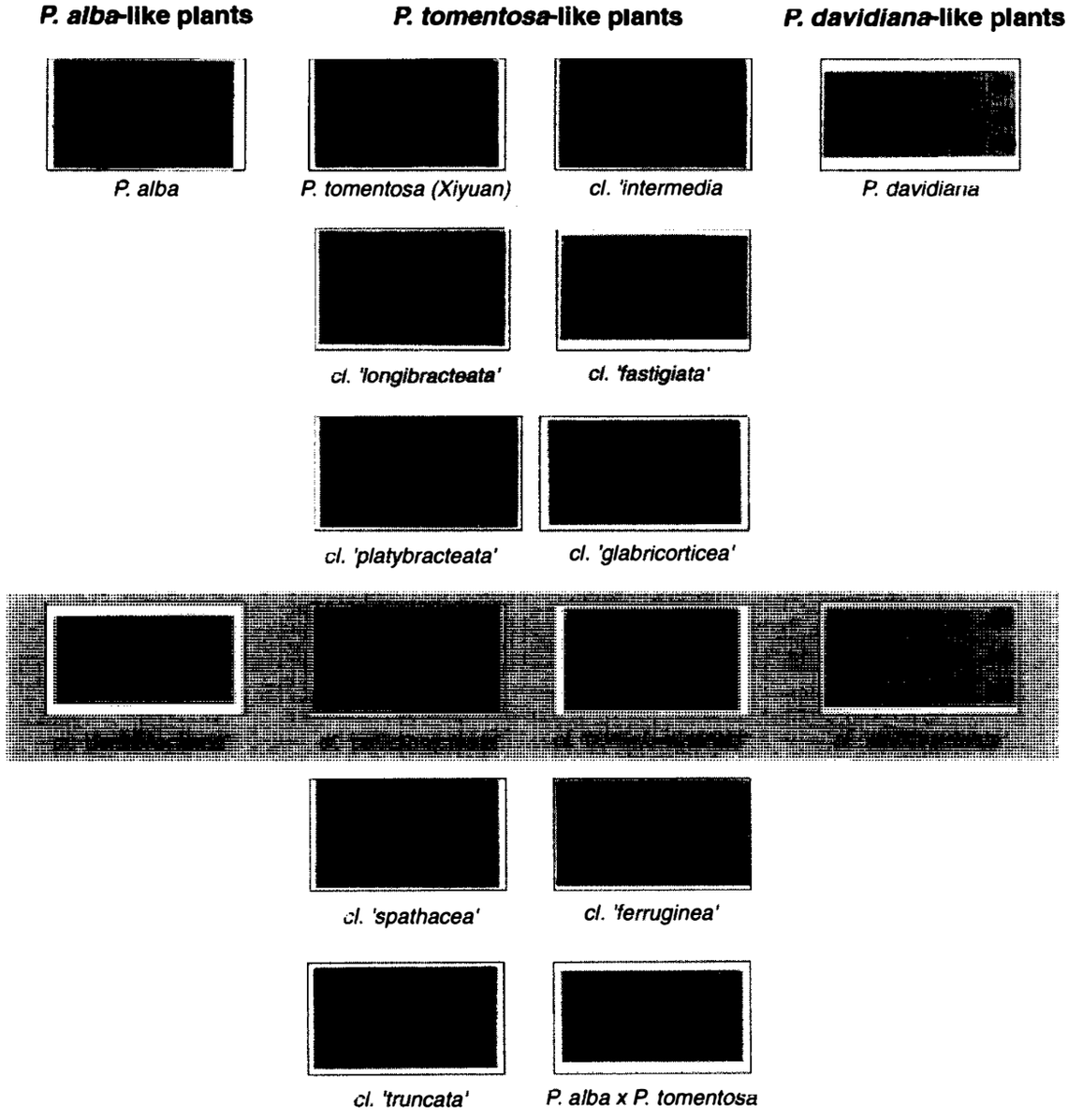

Figure 5. – Floral bract comparison of male trees in a *P. tomentosa* open-pollinated population with bracts of female *P. alba* and *P. davidiana.* Intermediates are *P. tomentosa*-like plants. The *P. tomentosa* allied to *P. davidiana* is located middle right, while the *P. tomentosa* allied to *P. alba* is located middle left.

Table 4. – Weight values and counts of the principal components used with *table 1, table 2,* and *table 3* values to calculate the male and female values displayed in *figure 6* and *figure 7.*

Sex	Metric variables	Count	Axis 1	Axis 2
Female	1	61.4	0.3499	-0.5695
	2	8.3	0.5012	1.8757
	3	48.8	0.4901	0.9277
	4	38.4	- 0.1285	1.6594
	5	17.9	1.4619	-0.6985
	6	77.6	-1.2149	-0.7597
	7	12.7	-1.3213	1.0486
	8	3.3	-1.2344	-0.0240
	9	3.9	-1.0187	-1.2313
	10	25.5	1.8965	-1.0402
Male	1	165.2	0.0382	-1.3992
	2	17.0	0.4599	-0.3959
	3	101.7	0.5479	-0.3712
	4	67.5	0.1077	0.7594
	5	24.6	1.3969	0.4330
	6	101.9	-1.4528	0.9607
	7	22.4	-1.3498	-0.1196
	8	3.5	-1.5122	0.0910
	9	5.0	-1.0816	-0.3668
	10	32.0	2.4238	1.3926

Discussion

One procedure to determine the parentage of a natural hybrid is to make control-pollinations between the assumed parents and then compare the F1 progenies with the natural hybrid. This was the way *P. x canescens* was determined to be a hybrid between *P. alba* and *P. tremula* (BARTKOWIAK, 1961). In the present study, bracts of offspring from control-pollinations of both assumed parents, *P. alba* and *P. davidiana,* were identical to the type specimen of *P. tomentosa* at Tongyuanfang. This indicated that *P. tomentosa* probably originated as a natural hybrid of *P. alba* and *P. davidiana.* It also suggests that the bracts used by WANG, on the illustration "Flora reipublicae popularis sinicae", were not the typical bracts of *P. tomentosa* female plants.

It is particularly important in testing for hybrid origin to study artificial backcrosses of each parent with the hybrid (ANDERSON, 1949). Backcrosses from *P. alba* and *P. tomentosa* produced the expected 2 phenotypes: 3 *P. alba*-like progenies and 1 *P. tomentosa*-like progeny. In addition, the reciprocal backcross of *P. tomentosa* x *P. alba* var. *bolleana* produced 2 hybrid progenies that had bracts that were the same as *P. tomentosa* (YUAN, 1985; MA, 1993), and 3 hybrid progenies that had bracts that were the same as those of *P. alba* (MA, 1993). This demonstrates that *P. alba* was one of the parents of *P. tomentosa.* The bracts of the male parent, *P. alba* var. *bolleana,* were identical to those of *P. alba.*

Four of the *P. davidiana*-like offspring from backcrosses of *P. davidiana* and *P. tomentosa* were kept for reforestation purposes because backcrossed plants with bract type similar to that of *P. tomentosa* were not obtained. To provide additional insight to compensate for this deficiency of data, we propose that the *P. tomentosa* cl. 'Truncata', selected by FU and WAN (1975) is an example of a backcross hybrid of this combination. This hybrid had less pubescence on the leaves, and specialists have sometimes mistaken it for aspen. Its second distinguishing feature was that it had the highest pollen viability (40%) (ZHU, 1987). Recent research proved that the backcross progeny was a multiclonal variety and included *P. tomentosa* cl.

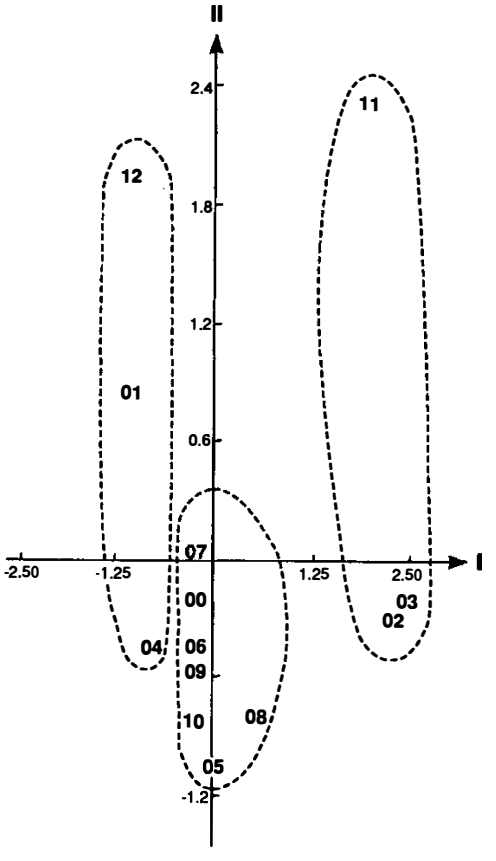

Figure 6. – Spatial distribution of 13 female taxonomic units based upon the first 3 principal components. The units separate into 3 distinct groups (*P. alba*-like, *P. davidiana*-like and *P. tomentosa*-like).

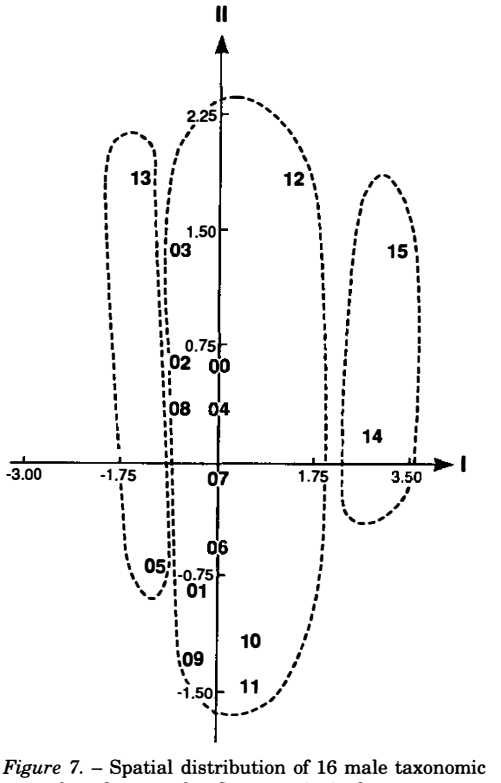

Figure 7. – Spatial distribution of 16 male taxonomic units based upon the first 3 principal components. The units separate into 3 distinct groups (*P. alba*-like, *P. davidiana*-like and *P. tomentosa*-like).

'Truncata'–14, –25, and –30 (FU et al. 1994). The backcross produced the expected 1:1 ratio of progeny with the parent phenotypes. In addition, we examined 11 plants of *P. tomentosa* cl. 'Truncata', located along the road by the gate of Northwest College of Forestry. Those trees, based on phenology, also appeared to be members of 3 different clones. The accumulated backcross evidence indicated that *P. davidiana* was likely a parent of *P. tomentosa.*

P. adenopoda has been suggested by some researchers to be a parent of *P. tomentosa,* but study of floral bracts indicates otherwise, at least for typical *P. tomentosa* at Xiyuan, Beijing. The *P. adenopoda* bracts are tassel-shaped, thus quite different than bracts of *P. davidiana and P. tomentosa* (ZHANG et al., 1988). *P. x pseudo-tomentosa* is a hybrid of *P. adenopoda* and *P. tomentosa* (WANG et al., 1979). The hybrid, *P.* x *pseudo-tomentosa,* should either be similar to *P. adenopoda* or *P. tomentosa.* However, results of quantitative taxonomic study with the SPEARMAN method on morphological characters of *P. tomentosa* and its relative species indicate that *P.* x *pseudo-tomentosa* is distant from *P. adenopoda* and even more distant from *P. tomentosa* at Xiyuan, Beijing. *P. adenopoda,* therefore, is not regarded to be a parent of typical *P. tomentosa.* It is a special hybrid (ZHANG et al., 1991).

We found female and male *P. alba*-like plants, female and male *P. davidiana*-like plants and female and male *P. tomentosa*-like plants in *P. tomentosa* open-pollinated populations. This provided additional evidence supporting the hybrid origin of *P. tomentosa* from *P. alba* and *P. davidiana.* The bract data also showed simple inheritance.

The quantitative analysis for progenies and plant materials of control-pollinated, backcrossed, and open-pollinated populations positioned each taxonomic unit as expected. All hybrids of control-pollination from *P. alba* and *P. davidiana* were located in *P. tomentosa*-like group. Parent-like plants produced by backcrosses of *P. alba* and *P. davidiana* to *P. tomentosa* were located in *P. alba*-like and *P. davidiana*-like groups, respectively. Both results provide strong evidence that *P. alba* and *P. davidiana* are parents of *P. tomentosa.* Individuals in the open-pollinated population positioned in *P. alba*-like, *P. davidiana*-like, *and P. tomentosa*-like groups. Again, this supports the hypothesis that *P. tomentosa* is the natural hybrid of *P. alba* and *P. davidiana* and supports the conclusions of BIALOBOK (1964).

Populations of *P. tomentosa* revealed considerable phenotypic variation, with *P. alba*-like, *P. davidiana*-like, and *P. tomentosa*-like plants making up those populations. Plants that are like the hypothesized parent species are rare, while the *P. tomentosa*-like plants are abundant. Within the *P. tomentosa*-like group, there was considerable phenotypic variation in floral morphology. We believe this variation may occur because of the following: (1.) *P. tomentosa* was a hybrid derived from several different provenances of *P. alba* and *P. davidiana* (BIALOBOK, 1964) and their parents may have been relatively heterozygous or possibly, had different alleles for the same traits, (2.) *P. tomentosa* in China was widely propagated by grafting and cuttings, thus existing variation was perpetuated by clonal propagation, (3.) the chance sexual reproduction of *P. tomentosa* trees in nature may have introduced additional heterozygosity that was later preserved by clonal propagation.

Selection of proper characters or traits for genetic study is very important. Flower characteristics are often used because they remain stable over a range of environments, thus they are valuable for taxonomic classification and genetic study of both individuals and populations. Fortunately, trees of *P. alba, P. davidiana,* and *P. tomentosa* had distinct and contrasting floral traits, which make them suitable for genetic study. Twenty-eight of the 29 taxonomic units of *P. alba*-like, *P. davidiana*-like, and *P. tomentosa*-like plants appeared to be classified correctly simply from the ratio of depth of lacisions to bract length. The sole exception was of *P. tomentosa* cl. 'Spathacea'.

Breeding procedures for *P. tomentosa* have been studied by many poplar specialists. Five of the fastest-growing types, *P. tomentosa* cl. 'Hopeinica' and 4 *P. tomentosa* cl. 'Truncata', were used as contrasts in the study of *P. tomentosa* breeding in China. All proved to be allied to *P. davidiana.* YABLOKOV and VERESIN (MOLOTKOV, 1982) point out that *P. tremula* is an excellent female parent for backcrossing. Some taxonomists regard *P. davidiana* as a variety of *P. tremula.* Such observations suggest that backcrossing *P. davidiana* to *P. tomentosa* may be a good method of producing fast-growing trees. If this does occur, a more discerning view should be taken of future use of the backcross between *P. alba* (including *P. alba* var. *bolleana*) and *P. tomentosa.* The latter cross does not appear to have as favorable growth attributes as does the backcross with *P. davidiana.*

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Sister Chromatid Exchanges in Coniferous Forest Trees

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Summary

A significant increase of the sister chromatid exchange (SCE) frequency is typical for mutagen-treated cells and widely considered as an indicator of genotoxic environmental impacts. SCEs were studied in 3 widespread gymnosperms: the spruce *Picea abies,* the pine *Pinus sylvestris,* and the larch *Larix decidua,* each with 2n=24 chromosomes.

Unifilarly BrdUrd-substituted chromosomes revealed, by means of the fluorescent plus Giemsa (FPG) technique, a basic SCE frequency of 36.9/cell in *Picea abies,* 36.2/cell in *Pinus sylvestris,* and 27. 6/cell in *Larix decidua.*

Seedlings from damaged *P. abies* trees revealed significantly more SCEs/cell than seedlings from healthy trees of the same area.

Key words: Picea abies, Pinus sylvestris, Larix decidua, sister chromatid exchanges, genotoxicity testing, forest damage, environment.

FDC: 165.3; 181.45; 425.1; 425.3; 174.7 *Picea abies;* 174.7 *Pinus sylvestris;* 174.7 *Larix decidua.*

Zusammenfassung

Die Häufigkeit von Schwesterchromatidenaustauschen (SCEs) kann durch Mutagenbehandlung drastisch erhöht werden. Eine erhöhte SCE-Frequenz gilt auch als Indikator für genotoxische Umwelteinflüsse.

Die SCE-Frequenzen für 3 weitverbreitete Gymnospermenarten (*Picea abies, Pinus sylvestris, Larix decidua*) mit je 2n=24 Chromosomen wurden untersucht.

Einsträngig mit dem Basenanalogon BrdUrd substituierte Chromosomen ergaben nach 'Fluoreszenz-plus-Giemsa'-Färbung eine SCE-Frequenz pro Zelle von 36,9 für die Fichte, 36,2 für die Kiefer und 27,6 für die Lärche.

Sämlinge von deutlich geschädigten Fichten wiesen durchgängig signifikant mehr SCEs/Zelle auf als Sämlinge benachbarter gesunder Bäume.

Introduction

Sister chromatid exchanges result from recombination events between the identical DNA double strands of sister chromatids detectable by methods recording a bias of incorporated base analogues. The mechanism(s) of origin and the biological significance are still a matter of controversial discussion (for review see SCHUBERT, 1990). Nearly all genotoxic influences clearly enhance the basic frequency of SCEs in all systems investigated (TAKEHISA, 1982). Though SCEs are usually no mutagenic events themselves, they represent a sensitive, reliable and simple means to study experimental/environmental genotoxic influences.

The conifers *Picea abies* (L.) KARSTEN, *Pinus sylvestris* L., and *Larix decidua* MILLER are forest trees with similar chromosome complements (2n=24). They are widespread throughout the Northern hemisphere and endangered by anthropogeneous environmental impacts in several areas. Here, the basic frequency of SCEs is reported for these three species and compared for seedlings of closely neighbouring healthy and damaged individuals of *P. abies* trees from the same area.

Material and Methods

Seeds were harvested in spring and germinated on wet filter paper for 7 days at 24 °C. The detection of SCEs was as described by SCHUBERT and RIEGER (1994). Briefly, root tips (1 cm in length) were incubated for 17 h in BrdUrd (100 µM) + FdUrd (0.1 µM) + Urd (5 µM) and for 19 h in dThd (100 µM) + Urd (5 µM), submersed in 1% colchicin for 15 h, fixed in ethanol:acetic acid (3:1 at 4 °C over night), softened in 1% pectinase +1% cellulase at 37 °C for 0.5 h, squashed in 45% acetic acid, and differentially stained by the FPG technique (SCHUBERT et al., 1979).

(This procedure is also suitable for root tips of older seedlings grown on perlit, provided possible changes of cell cycle duration are being considered).

Results and Discussion

The base-line value in unifilarly BrdUrd-substituted chromosomes (TB/TT) was 36.9 SCEs/metaphase for *Picea abies,* 36.2 SCEs/metaphase for *Pinus sylvestris,* and 27.6 SCEs/metaphase for *Larix decidua* (*Tab.1, Fig. 1*). This corresponds to ~1.5 SCEs/chromosome for *Picea abies* and *Pinus*