

## The occurrence of natural hybrids between *Betula pendula* Roth and *B. pubescens* Ehrh.

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### ABSTRACT

The occurrence of atypical seedlings originating from seed collected from *Betula pendula* growing in various locations in Britain is reported. Chromosomal and physiological evidence is presented to support the claim that the seedlings are hybrids of the cross *B. pendula* × *B. pubescens*. In appearance the putative hybrids are not intermediate but resemble most closely *B. pubescens*. The possible hybrid nature of some of the original seed trees is also discussed.

### INTRODUCTION

As part of a programme designed to study the genetic improvement of the silver birch (*Betula pendula* Roth), seed was collected from 101 trees growing in east and north Scotland and from 24 trees growing mainly in England but including some in Norway and Finland. More than 1900 seedlings were raised and planted out in a progeny test. Despite the many well known difficulties that are often encountered when trying to discriminate between *B. pendula* and *B. pubescens* Ehrh. (the downy birch) it was ensured that all the seed parents were 'textbook' examples of *B. pendula* with respect to form, twig, leaf and bark characteristics. Under the conditions of greenhouse growth which the seedlings experienced for their first year it is initially very difficult to distinguish between the two species. But after a few weeks *B. pendula* seedlings produce progressively less hairy leaves and stems and typically, vigorous shoots become covered in white resin glands. Leaves develop relatively acute tips and obvious double toothing. Amongst the 1900 seedlings, 94 plants retained their pubescence and their leaves developed obtuse apices and had single rounded teeth - i.e. they resembled *B. pubescens* seedlings. It is these atypical progeny from typical *B. pendula* which are the focus of the investigations discussed below.

### NATURAL HYBRIDS

The occurrence of birch trees that are intermediate in character between *B. pendula* and *B. pubescens* has been reported in many British Floras and these intermediates are often assumed to be of hybrid origin (see e.g. Newton 1971, Perring *et al* 1964 and Webster 1978). According to Walters (1972) the intermediates are usually considered to be hybrids and are common and widespread in the British Isles but rare for example in Finland. Continental European and Scandinavian opinion on hybridization of these birches appears to depend very much upon whether the subject is approached purely from a morphological point of view or whether cytological and phenological data are also considered. Johnsson (1944, 1945) concluded on the basis of artificial crosses that hybridization is rare and further stated that the few natural F<sub>1</sub> individuals found are absolutely female sterile. Jentys-Szaferowa (1938) also agreed about the rarity of hybrids, basing her conclusions upon the lack of overlap in flowering times of the two species in Poland. Natho (1959) stated that "although a considerable proportion of material is hybrid in origin I have found more trees with the chromosome set of one or other species (*B. pendula* 2n=28, *B. pubescens* 2n=56) than with the intermediate 2n=42". Using a hybrid index based on scores for 16 traits he calculated that in the populations studied 30% of individuals were of F<sub>1</sub> or later backcross generations. He found, however, only one

birch which, using root-tip material, gave a count of  $2n=42$  (and that he says cannot be regarded as absolutely safe) and a few more trees had counts around 42. One group of trees from the area of Graal Muritz in middle Europe showed fluctuating chromosome numbers between 35 and 50. Vaarama (1969) in an extensive review of the literature concluded that only 2 trees recorded could be regarded as natural hybrids.

#### ARTIFICIAL HYBRIDS

It is generally agreed that there is a high degree of incompatibility in artificial crosses between the two species but that between certain individuals viable crosses are possible (Hagman 1971). As is usual in crosses between parents of different ploidy level most success is achieved when the pollen parent has the highest ploidy. The chromosome numbers of progeny from such crosses vary and counts of  $2n=28$ , 42 and 56 have been published. Johnsson (1945) obtained two seedlings with  $2n=42$ , both from the same cross, in a series of crosses involving two *B. pendula* seed parents and three *B. pubescens* pollen parents. This represented a yield of viable seed of 0.2%. In the reciprocal crosses one combination yielded ten seedlings with  $2n=56$ . Johnsson was inclined to attribute these counts to pollen contamination. Hagman (1971) and Eifler (1960) produced  $2n=28$  and  $2n=42$  seedlings from *B. pendula* × *B. pubescens* crosses and from the reciprocal crosses seedlings with  $2n=28$ , 42 and 56 were obtained. Hagman suggested that 50% of *B. pubescens* gametes are  $n=14$ , the other half having  $n=28$ .

#### THE GLEN GAIRN BIRCH POPULATION

This population has been extensively studied and reported (Brown & Tuley 1971; Brown & Al-Dawoody 1977, 1979; Aston 1975) but a brief review of the situation is relevant to the present study. Both arborescent species occur in Glen Gairn as well as morphologically intermediate trees. Adult trees with  $2n=42$  have been identified but, on the basis of external morphology, these cannot be distinguished from *B. pubescens* (Gardiner & Pearce 1979). The  $2n=42$  trees are fertile and have been crossed with each other and with *B. pubescens* (Williams unpublished). Progeny from wind pollinations have chromosome numbers of around  $2n=42$ . No evidence has been found to support the idea that these are of hybrid origin and, indeed, it seems likely that they are aneuploid *B. pubescens* (Williams unpublished). On the other hand, in contrast to the results of Jentys-Szaferow (1938), Aston (1975) showed that cross pollination in this area is possible because there is extensive overlap in flowering times of the two species.

With regard to Hagman's thesis that *B. pubescens* produces 50%  $n=14$  gametes, meiotic studies in Glen Gairn did not support this idea and the only extensive irregularities of meiosis found were in the  $2n=42$  cytotypes.

#### MATERIALS AND METHODS

Seed was collected in August and September 1977, sown in October in a heated greenhouse under an eighteen hour photoperiod and the seedlings were transplanted into a polytunnel in late spring 1978. By autumn they averaged around 1.5 m in height, having set buds by this time under a natural photoperiod. In spring 1979 the trees were planted out in a progeny test under forest conditions. Seed germination tests were carried out using Copenhagen tanks, where replicated batches of seed were germinated under constant conditions of light, heat and moisture. Before testing, the seed (strictly nutlets) were separated into those with fully developed embryos and those without. This was done using transmitted light under a low-power stereomicroscope.

All crosses were carried out either in the polytunnel or in a glasshouse. Female catkins were enclosed in paper bags before they became receptive and before anthesis of male catkins. Male catkins were collected just before anthesis and allowed to shed their pollen in a warm dry room. The pollen was applied to the female flowers by means of a fine camel hair brush at the time of highest receptivity when the stigmas were deep pink/bright red. Since not all the flowers in a catkin become

receptive simultaneously pollination was repeated several times. The paper bags were removed once all stigmatic surfaces had dried and withered.

Cuttings consisting of a leaf, axillary bud and internode were rooted in peat/sand mixture, under mist spray and 18 hour photoperiod. No rooting hormones were used (Kennedy *et al.* 1980).

Chromosome counts were made on cells of the bases of leaves sampled when emerging from the bud. The leaves were pre-treated with 8-hydroxyquinoline for three hours and then fixed in 1:3 acetic acid:alcohol. This was followed by hydrolysis and tissue softening in 5N HCl at room temperature for one hour. After rinsing, the leaves were stained in basic fuchsin for three hours. Squashes were prepared in 45% acetic acid.

## RESULTS

## MORPHOLOGY OF SEEDLINGS

Atypical seedlings were recognised following a period of several weeks growth after transfer of plants from greenhouse to polytunnel. It was recognised that certain individuals differed from their neighbours with respect to general leaf shape and the persistence of pubescence on leaves and stems. Because there is a high degree of variability within any birch population, the boundary between typical and atypical seedlings is hard to define and indeed may not exist if closer examination shows that there is a continuum of variation. Nevertheless, as a matter of convenience, two types of seedling were defined in terms of total scores for the three traits shown in Table 1. Seedlings scoring two or more were considered to have fallen outside the normal range of variation expected in *B. pendula* seedlings. In 1978, 77 seedlings were so identified but after planting out in the field and re-

TABLE 1. SCORING SCHEME FOR THREE LEAF TRAITS USED TO CHARACTERIZE TYPICAL AND ATYPICAL SEEDLINGS (1979)

Score	Leaf tip angle (degrees)	Hairiness	Toothing
0	≤60	glabrous	doubly serrate
1	61-65	axillary tufts (a)	intermediate
2	66-70	(a)+midrib hair (b)	biserrate
3	71-75	(a)+(b)+vein hairs (c)	intermediate
4	76-80	(a)+(b)+(c)+lamina hairs	serrate

TABLE 2. LEAF MORPHOLOGY OF BIRCH SEEDLINGS (1979)

Source	Seed tree no.	No. of seedlings		Mean leaf tip angle (degrees)±SE		Mean Score			
		a.*	b.*	a.	b.	a.	b.	a.	b.
Glen	1	18	5	52±1.42	63±2.8	0.0	1.4	0.0	0.8
Prosen	5	15	10	55±0.97	60±2.65	0.0	0.55	0.0	0.67
	6	3	7	55±1.33	57±1.05	0.0	1.7	0.0	0.43
	7	9	15	57±1.11	67±1.67	0.0	0.86	0.33	1.47
	8	17	7	52±1.32	60±1.90	0.12	0.86	0.12	0.56
Potarch	1	41	6	52±0.88	74±5.35	0.0	2.0	0.0	2.3
	2								
Forton	1	15	10	54±1.15	61±2.0	0.0	0.5	0.0	0.6
Kingsley	1	16	8	49±1.32	62±2.55	0.0	0.8	0.0	1.5
Common									
Three seed trees with no atypical progeny			75		54±2.35		0.0		0.0
<i>B. pubescens</i>			5		80±4.11		3.4		2.8

\* a-typical *pendula* seedlings

b-atypical seedlings

examination the scores of 22 were reduced to less than two. On the other hand 17 additional seedlings were found which had scores greater than two. Thus out of 1,900 seedlings a total of 94 atypical seedlings were found. In general, scores declined between the 1978 and 1979 growing seasons. This was probably due to a combination of developmental and environmental changes, showing that the seedlings tended to become *pendula*-like. Toothiness remained stable but leaf tips became more acute and pubescence declined. Table 2 shows, for those families where there were sufficient seedlings available for comparison, the traits of typical and atypical seedlings from the same families, the traits of seedlings from three families where no atypical plants were found, and the traits of five *B. pubescens* seedlings which were, by chance, grown along with, and under the same conditions as, the *B. pendula* progenies.

#### DISTRIBUTION

The atypical seedlings were irregularly distributed throughout the sample and, from 127 seed trees and 48 sites, only 23 trees on 13 sites produced such seedlings, as is shown in Table 3. At the Forton site the seed tree was a planted ornamental possibly of Continental origin and was surrounded by what appeared to be *B. pubescens* - also planted ornamentals. The Alice Holt tree originated from a cross made between two trees, one of native origin and the other East German. This particular birch was in the midst of a small stand of both native and exotic birches. The seed trees on all the other sites appeared to be of natural origin and indeed in many areas foresters and owners confirmed this to be true. The bulk of the birches on these sites were *B. pendula*, but there were a few *B. pubescens*. The exception to this was Glen Prosen and Kingsley Common, where there are fairly intimate mixtures of the two species. The most common variant of *B. pubescens* encountered was subsp. *pubescens*.

#### VEGETATIVE PROPAGATION

In 1979 seed was gathered from five trees at Glen Prosen that were known to produce high proportions of atypical seedlings and from three trees identified as *B. pubescens* on the same site. After growing the resulting seedlings for about ten weeks under extended photoperiod one *pendula*-type seedling and one atypical type was selected from each of the five *B. pendula* seed sources. One seedling was selected from each *B. pubescens* source. From each seedling, six to eight nodal cuttings were taken and inserted in the rooting beds. The results of rooting, root production and subsequent growth of the cuttings are shown in Fig. 1 and Table 4. In another test, cuttings from an atypical seedling from a Potarch seed tree rooted with a success rate of 85.7% compared with 69.3% rooting for *B. pendula* seedlings. These differences were significant at  $p=0.01$ .

#### FLOWERING

Birch is a genus in which precocious flowering can readily be induced by a variety of methods and

TABLE 3. ORIGIN AND DISTRIBUTION OF ATYPICAL SEEDLINGS

Site	Total no. trees sampled	Total no. seedlings	Atypical Seedlings		
			No. seed trees	No. seedlings	% Total seedlings
Potarch, S. Aberdeen, v.c. 92, GR,37/609.974	4	49	2	6	12.2
Banchory, Kincardineshire, v.c. 91, GR37/686.966	4	66	1	1	1.5
Ballogie, S. Aberdeen, v.c. 92, GR37/579.953	6	106	1	1	0.9
Glen Tanar, S. Aberdeen, v.c. 92, GR 37/483.966	8	110	1	2	1.8
Finzean, S. Aberdeen, v.c. 92, GR37/589.935	12	116	1	1	0.9
Pitlochry, E. Perth, v.c. 89, GR27/965.558	5	98	1	1	1.0
Alford, S. Aberdeen, v.c. 92, GR38/655.112	13	231	4	5	2.2
Forton, W. Lancs., v.c. 60, GR34/488.586	1	26	1	9	34.6
Baldyuin, S. Aberdeen, v.c. 92, GR38/595.165	2	34	1	3	8.8
Kingsley Common, Surrey, v.c. 17, GR41/793.383	1	24	1	8	33.3
Alice Holt, Surrey, v.c. 17, GR41/803.427	10	95	1	1	1.0
Glen Prosen, Angus, v.c. 90, GR37/394.586	8	129	8	56	43.4

TABLE 4. GROWTH PERFORMANCE OF ROOTED CUTTINGS OF DIFFERENT MORPHOLOGICAL TYPES AFTER 92 DAYS GROWTH

Cutting type	Mean no. roots/cutting	Mean no. lateral branches/cutting	Mean no. internodes/cutting	Mean final height/cutting (cm)	Mean aerial dry weight/cutting (g)
" <i>pendula</i> "	5.73 ]*	1.7 ]	16.4 ]	48.8 ]	1.77 ]
	P=0.001 ]	P=0.001 ]	P=0.05 ]	P=0.01 ]	P=0.02 ]
"atypical"	17.10 ]	4.7 ]	18.0 ]	41.7 ]	1.8 ]
	P=0.002 ]	P=0.001 ]	P=0.002 ]	P=0.001 ]	P=0.02 ]
" <i>pubescens</i> "	15.05 ]	4.7 ]	19.1 ]	52.05 ]	2.23 ]

\* lines connect values differing significantly at levels indicated.

TABLE 5. FLOWERING IN BIRCH OF DIFFERENT MORPHOLOGICAL TYPES

Morphological type	No. of trees	No. in flower	% flowering
Progeny test population (age 3 years)			
" <i>pendula</i> "	1633	10	0.6
"atypical"	61	11	18.0
Glen Gairn population (age 8 years)			
" <i>pendula</i> "	257	1	0.4
" <i>pubescens</i> "	118	16	13.6

TABLE 6. SOMATIC CHROMOSOME COMPLEMENTS OF ATYPICAL SEEDLINGS FROM ALL SITES EXCEPT GLEN PROSEN

Seed parent location	Progeny no.	2n±SD		No. cells counted	Coef. var.	Morphological score
Potarch	1	41.3	2.5	9	6.3	6
	2	34.3	10.3	9	30.0	5
	3	35.7	3.7	7	10.4	6
Potarch	1	41.7	1.5	3	3.7	10
	2	36.3	7.1	14	19.6	4
	3	40.5	1.6	12	3.9	—
Banchory	1	38.5	4.4	27	11.4	4
Ballogie	1	41.2	1.4	10	3.4	6
Glentinar	1	39.2	3.2	11	8.2	3
Finzean	1	39.7	5.8	8	14.4	7
Pitlochry	1	34.3	4.8	7	13.8	3
Alford	1	36.6	2.9	12	8.0	—
Forton	1	35.5	6.7	24	18.9	4
	2	28.7	4.5	12	15.8	0
	3	36.2	6.5	8	18.0	3
	4	38.6	3.0	14	7.7	—
	5	31.4	3.9	11	12.3	2
Baldyvin	1	38.2	6.1	17	16.0	9
	2	40.3	2.3	7	5.7	—
Kingsley Common	1	38.0	5.2	15	13.5	5
	2	40.8	1.2	6	2.9	6
	3	32.1	5.3	19	16.4	3
	5	40.9	1.3	9	3.1	7
Alice Holt	1	39.5	4.2	21	10.6	—
Kingsley Common	4*	27.9	1.1	7	3.8	0
	6*	27.7	0.6	3	2.1	0

\* seedlings of typical *pendula*-type morphology.

one method simply involves the encouragement of rapid growth since readiness to flower and size of tree are directly correlated. Such early flowering occurred in some of the progenies and the production of catkins was more frequent in the atypical types than in the typical *B. pendula* seedlings (Table 5). These results have been compared with flowering in a nearby population of seedlings derived from seed collected in Glen Gairn. These were eight years old at the time of recording and were grown for one year in the greenhouse before planting out.

## SOMATIC CHROMOSOME NUMBERS

Table 6 shows somatic chromosome numbers for 26 atypical seedlings from all sources except Glen Prosen. Counts for two typical *pendula*-type seedlings are also included. The Glen Prosen results are shown separately in Table 7, along with the chromosome complements and morphological types of seed trees. In this table, seedlings 4(1) and 7(1), and all those with an associated morphological score, were amongst the original atypical seedlings. Seedlings of trees 4a, 11, 14, 17, OAP and A1 and progenies 3-7 and 5-6 of trees 8 and 9 respectively were chosen at random for counting from a separate sowing with no regard to morphology. Seedling 3 from parent tree 10 was counted because

TABLE 7a. SOMATIC CHROMOSOME COMPLEMENTS OF *PENDULA*-TYPE SEED TREES IN GLEN PROSEN AND THEIR PROGENIES RESULTING FROM WIND POLLINATION

Seed Trees					Progeny					
No.	$2n \pm SD$		No. cells counted	Coef. var.	No.	$2n \pm SD$		No. cells counted	Coef. var.	Morphological score
1	26.9	1.7	13	6.4	1	38.0	4.4	33	11.7	0
					2	43.7	5.5	9	12.6	3
					3	30.5	5.8	8	19.1	—
					4	35.8	4.0	6	11.1	—
					5	27.0	1.4	3	5.2	—
2	32.9	7.7	21	23.4	1	39.6	3.2	20	8.1	10
					2	40.0	1.3	12	3.4	—
					3	25.9	2.3	23	9.1	—
4a	30.0	5.2	20	17.5	1	27.3	0.8	4	2.8	—
					2	27.3	1.1	12	3.9	—
					3	27.7	0.6	3	2.1	—
					4	28.3	1.5	3	5.4	—
4	34.1	6.4	21	18.9	1	42.7	0.5	4	1.1	—
					2	41.3	2.2	17	5.5	5
					3	38.1	3.0	9	7.9	—
5	33.0	9.4	13	28.4	1	40.6	2.1	19	5.2	0
					2	44.2	5.6	13	12.7	1
					3	31.4	8.1	5	25.8	2
					4	39.0	5.4	8	13.8	0
6	27.0	7.7	32	28.6	1	42	—	1	—	1
					2	26.4	0.9	5	3.4	—
					3	33.2	6.1	27	18.4	—
					4	27	—	1	—	—
7	29.4	1.7	7	5.8	1	32.9	9.7	10	27.6	—
					2	28.8	2.5	6	8.6	4
8	26.9	1.1	13	4.2	1	31.9	4.4	16	13.9	5
					2	37.8	3.5	16	9.2	—
					3	27.4	0.7	8	2.7	—
					4	27.3	0.8	11	2.8	—
					5	27.3	0.8	6	3.0	—
					6	28	—	1	—	—
					7	27.0	0.6	7	2.1	—
OAP	—	—	—	—	1	27	—	2	—	—
					2	27.6	1.0	7	3.6	—
					3	26	—	2	—	—
					4	27.5	0.6	6	2.0	—



it had variegated leaves and stem, and seedling 1 from parent 11 was singled out because it was a *pendula*-type seedling from a *pubescens*-type mother. All other seedlings were counted because, on various occasions, they were identified as being atypical.

Due to the small size and relatively large number of birch chromosomes, which increases the chance of one obscuring another, and the tough cell walls that often prevent achieving a good spread of chromosomes on squashing, the error involved in counting is high (Brown & Al-Dawood 1977). In the higher ploidy birches it is estimated that experimental error causes a consistent under-estimation of chromosome number of at least two or three. There is also real variation to be found within leaf cells and it is not uncommon to find a cell with 28 chromosomes, adjacent to one with 35, 42 or 56. Results have been presented as mean chromosome numbers with a measure of variation.

## SEED YIELD OF PARENT TREES

The results in Table 8a are based upon the germination of replicated lots of 50 full seed. Variations in the proportions of full seed between trees is caused by a number of factors. Infestation of seed by insects is common and parent trees 9 and 11 suffered bad attacks. Parthenocarpy is a feature of birch but in artificial crosses variation in trees has been found in their ability to develop unpollinated catkins. This may reflect similar differences in the ability to develop catkins with different proportions of fruit containing viable embryos. Table 8b shows a comparison of the overall Glen Prosen germination results with those from various other sites. Ben Loyal, Morrone and Slugain are sites with only *B. pubescens*, Glen Gairn results are for a mixture of both species and Balnaguard results derive solely from *B. pendula*.

TABLE 7b. SOMATIC CHROMOSOME COMPLEMENT OF *PUBESCENS*-TYPE SEED TREES IN GLEN PROSEN AND THEIR PROGENIES RESULTING FROM WIND POLLINATION

Seed Trees				Progeny					
No.	$2n \pm SD$		No. cells counted	Coef. var.	No.	$2n \pm SD$		No. cells counted	Coef. var.
9	54.4	3.5	13	6.4	1	26.0	2.5	4	9.8
					2	26.5	1.9	17	7.3
					3	34.8	3.4	8	9.7
					4	36.4	8.6	10	23.5
					5	54.1	2.4	12	4.5
					6	52.0	2.6	3	5.1
					7	52.5	3.1	8	5.8
					8	51.4	2.1	3	4.0
					9	53.4	2.4	10	4.3
10	37.3	12.7	13	34.1	1	35.4	6.6	21	18.7
					2	30.0	6.0	5	20.0
					3	24.9	3.7	9	15.0
					4	55.7	1.5	3	2.8
11	56.0	5.4	11	9.6	1	28.2	1.5	6	5.2
					2	47.8	6.6	6	13.7
					3	51.5	2.5	4	4.9
					4	55.0	1.4	2	2.5
					5	54.0	1.8	4	3.4
					6	53.7	3.1	3	5.7
14	55.0	2.4	10	4.4	1	52	—	1	—
					2	53.7	1.0	6	1.9
					3	55.0	—	2	2.6
					4	53.7	1.6	3	2.8
18	52.0	8.0	4	15.4	1	53.0	1.5	8	3.3
					2	52	—	1	—
					3	50	—	1	—
					4	53	—	1	—
					5	51.3	3.5	3	1.8
A1	53.3	3.7	10	6.1	1	39.2	3.7	4	9.5
					2	50.8	4.2	6	8.3

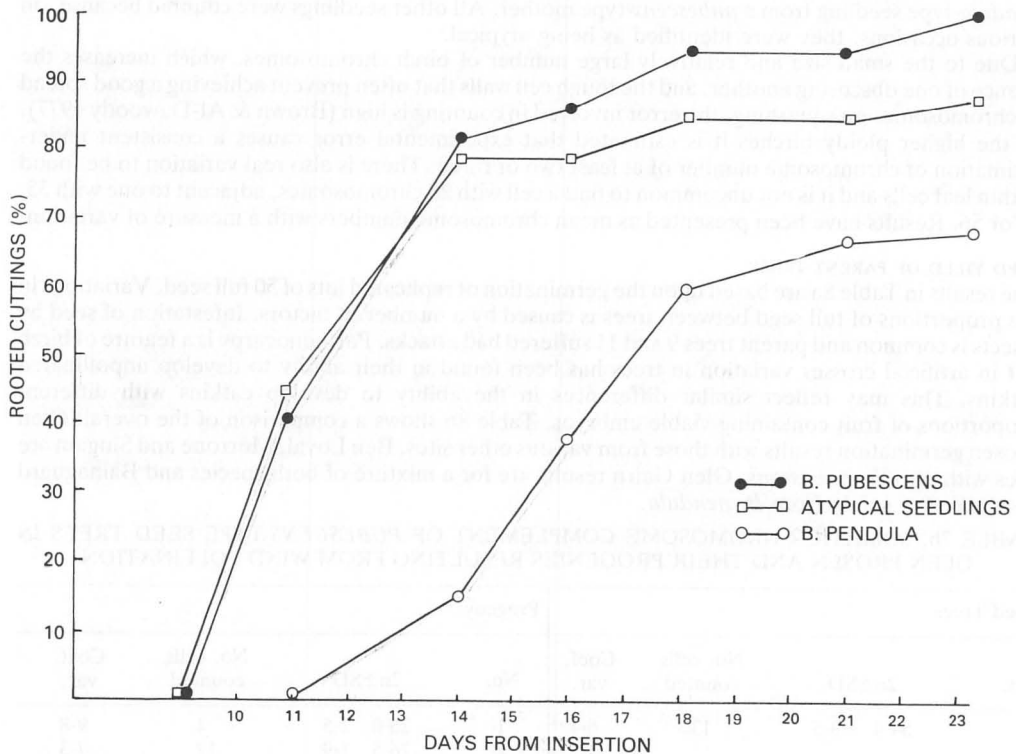


FIGURE 1. Rooting performance of cuttings from three morphological types of *Betula*.

#### CROSSES

A number of crosses have been carried out using typical *pendula*-type seedlings and atypical ones. The germination of seed from such crosses was done only by estimation after sowing under greenhouse conditions. An atypical seedling from Glen Prosen parent number 6 produced 1% germinable seed when used as a pollen parent in a cross with a Kingsley Common seedling. As a seed parent, however, in crosses with three *pendula*-types no germinable seed were formed. A second atypical seedling used as pollen parent, from Glen Prosen parent number 7, produced no germinable seed when crossed with two typical *pendula*-types and an atypical seedling from Pitlochry. The Pitlochry seedling was only used as a seed parent in five crosses and again no seed resulted. The *pendula*-type seedlings when crossed amongst themselves as both pollen and seed parents were fertile to various degrees. Many catkins on the trees were left unisolated and exposed to wind borne pollen but no viable seed resulted from any atypical seedling.

#### DISCUSSION

The main questions raised by the foregoing observations and results concern the status of the atypical seedlings in general and the seed trees in Glen Prosen. Leaving aside the Glen Prosen seed trees at present, the object of the following discussion is to show that there is good reason to believe that some or all of the atypical seedlings have arisen through pollination of *B. pendula* by *B. pubescens*. Flowering times do overlap in nature in at least one area of Scotland and despite evidence for a degree of incompatibility there is published evidence for the production of hybrids under artificial conditions. Thus there seems to be no biological obstacle to the cross. Apart from the trees in Glen



TABLE 8a. SEED PRODUCTION AND GERMINATION FOR GLEN PROSEN SEED PARENTS

Parent no.	2n	Filled seed %	Germination filled seed %	Germination all seed %
OAP	—	23	47	10.8
1	26.9	22	48	10.6
6	27.0	16	70	11.2
8	26.9	27	71	19.2
5	33.0	12	41	4.9
4a	34.1	5	66	3.3
10	37.3	38	77	29.3
22	37.6	12	60	7.2
A1	52.9	20	70	14.0
9	56.4	6	30	1.8
11	56.0	21	81	17.0
14	55.0	29	62	18.0
18	52.0	31	56	17.4

TABLE 8b. SEED PRODUCTION AND GERMINATION OF SEED FROM NORTH, NORTH-EAST, CENTRAL AND CAIRNGORM REGIONS OF SCOTLAND

Location of seed trees	Collection year	Filled seed %	Germination filled seed %	Germination all seed %
Glen Prosen, Angus, v.c. 90, GR37/394.586	1979	19	57	10.8
Glen Gairn, S. Aberdeen, v.c. 92, GR38/342.985	1976	35	76	26.7
Ben Loyal, W. Sutherland, v.c. 108, GR29/588.516	1979	38	74	28.0
Morrone, S. Aberdeen, v.c. 92, GR38/135.903	1979	50	83	41.7
Balnaguard, E. Perth, v.c. 89, GR27/942.514	1980	44	56	24.6
Slugain, S. Aberdeen, v.c. 92, GR38/139.950	1979	12	62	7.5

Prosen, the seed trees have not been extensively investigated but they have all been unequivocally identified as *B. pendula* on the basis of their morphology and the bulk of their seedlings shows no deviation from the expected course of development.

The atypical seedlings were singled out on the basis of a few morphological traits. Such studies were not pursued since the seedlings were grown under artificial conditions and a description of form and morphology developed under such conditions would only have been of value if there were descriptions of comparable *B. pubescens* available. Unfortunately the only such seedlings were five half-siblings included in the progeny tests by chance. Since characters such as leaf size and pubescence change, for example, with age and exposure it will be more appropriate to provide full descriptions of the putative hybrids once adult foliage on long and short shoots has been produced under field conditions.

Between 1978 and 1979 the change in morphological score suggested a more *pendula*-like appearance of the seedlings. This is somewhat misleading because, while some traits like hairiness of leaf and stem become less marked with age, less easily quantifiable characters such as overall crown shape and branching patterns have developed so that, at the time of writing, the trees look much more like *B. pubescens* than *B. pendula*.

The experiment on rooting of cuttings not only showed differences in rooting abilities but also showed that the subsequent growth of the cuttings anticipated crown development of the seedlings. Differences in rooting ability and rate of rooting between the atypical seedlings and their half-siblings are marked but the cuttings from atypical seedlings were similar in rooting performance to cuttings from *B. pubescens*. There are no significant differences in the production of internodes or lateral branches between the atypical cuttings and those from *B. pubescens*, which suggests that they

will develop into bushier and shorter trees than their seed-parents. Further evidence of physiological similarities between the atypical seedlings and *B. pubescens* was in their readiness to flower when compared with *B. pendula* of the same age. Precocity of flowering in tree hybrids has been recorded previously and has, for example, been recorded in *Eucalyptus* (Venkatesh & Sharma 1976).

These morphological, developmental and physiological similarities between the atypical seedlings and *B. pubescens* strongly suggests that this species is indeed the pollen parent. But these very strong similarities are not compatible with the claims that hybrids between the native arborescent birches are of intermediate character. On the basis of chromosome number, however, 'intermediacy' is not necessarily to be expected. *B. pendula* has 28 somatic chromosome and *B. pubescens* has 56 so that a hybrid would be expected to have 42 chromosomes with two thirds of them coming from the *B. pubescens* parent. If the proportion of active genetic material in the hybrid is in proportion to the chromosomal material then there is reason to think that the hybrid would be closer in all respects to *B. pubescens*. It must be said, however, that Stern (1963) reported that two of fifteen hybrid progeny from the cross *B. pendula* × *B. pubescens* were found to have 28 chromosomes but looked in every respect like *P. pubescens*. Concerning hybrids involving *B. nana* ( $2n=28$ ), it has been found (Williams unpublished) that seedlings from the cross *B. pendula* × *B. nana* are intermediate in leaf form and growth habit while progeny from the cross *B. nana* × *B. pubescens* are *pubescens*-like.

In principle it should be straightforward to determine, by counting chromosomes, whether or not a birch is a triploid hybrid. The occurrence of autotriploidy in *B. pendula* should be accountable for since it has been reported that such trees show 'gigas' or exaggerated features of normal *B. pendula* (Johnsson 1944). Any other types of triploid derived from a diploid tree can only be of hybrid origin. There is at least one reservation that should be expressed, namely that if there are autotriploids which do not show 'gigas' features then they would go undetected. Non-triploid hybrids are much more difficult to confirm. Those authors who have reported the occurrence of diploids in progeny of the cross *B. pendula* × *B. pubescens* cannot discount the possibility of pollen contamination and to our knowledge they have never published follow-up reports on the morphology and subsequent development of these diploid 'hybrids'.

The results of counts made on birch seedlings show that there is variation in chromosome numbers both between and within seedlings. Previous experience has shown that counts made on *B. pendula* are the least variable, possibly due to intrinsic stability and low error in counting the relatively small numbers of chromosomes. Mean counts for this species usually fall between 27 and 28 with a standard error of about 1.0. Amongst the seedlings in Table 4, Forton 2 as well as Kingsley Common 4 and 6 have the sort of counts associated with *B. pendula*. In Table 7a seedlings from *pendula*-type parents 4a (1-4), 6 (2 and 4), 7 (2), 8 (3-7) and OAP (1-4), all have typical *pendula*-type counts. It should be noted, however, that seedling 7 (2) has a morphological score of 4 and a high coefficient of variation and that seedling 6 (2) was originally examined because 'it looked intermediate'. All the others, however, appear to be normal *B. pendula*. *B. pubescens* is associated with a greater range of variation in chromosome numbers due, in part, to the technical difficulties associated with a large number of small chromosomes. In Table 7b it can be seen that for *pubescens*-type seedlings 9 (5-9), 10 (4), 11 (2-6), 14 (1-4), 18 (1-5) and A1 (2) chromosome number averages around 52 or 53. This is lower than previously published counts but is almost certainly due to variation between observers in, for example, decisions on elimination or retention of chromosome counts which are abnormally low or high, although counts of more than 56 are relatively infrequent.

Concerning the other seedlings from *pendula*-type parents, it can be seen from Table 6 that a range of mean counts from  $2n=32.1$  to 41.3 has been obtained and the coefficients of variation are relatively high. Similar counts were obtained for the atypical seedlings from the Glen Prosen *pendula*-type parents (Table 7a) but the highest mean count obtained here was  $2n=44.2$ , although this was from a seedling with a morphological score of only 1. If the variation in chromosome numbers within these atypical seedlings was due to counting errors it might be expected that there would be a regular relationship between mean chromosome number and the size of the error involved in counting. In Fig. 2 coefficient of variation has been plotted against mean chromosome number for all trees in Tables 6 and 7 showing that variation is lowest in trees with mean counts of around 28, 54 and, to a lesser extent, 42, while those trees with mean counts between 28 and 42 show relatively high variation in somatic chromosome counts. This suggests that in these latter trees there is real variability in chromosome complement from cell to cell. It is well known that in certain somatic tissues chromosome number varies from cell to cell but this is especially true of hybrids.

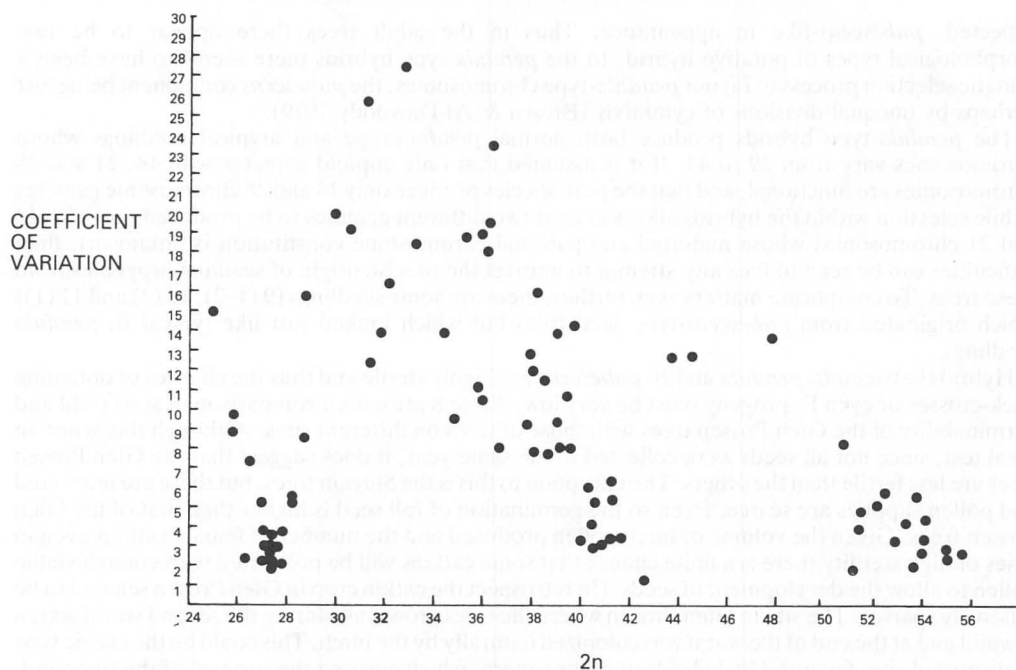


FIGURE 2. Distribution of coefficients of variation associated with mean somatic counts in all progenies.

Nielsen & Nath (1961) showed that counts tend to cluster around multiples of the basic chromosome number. Tai (1970) proposed that such a variation in hybrids is due to disturbances of the spindle caused by heterozygous spindle organisers existing in the same cytoplasm. Distribution of chromosome counts in birch follows the pattern shown by Nielsen & Nath and in artificial hybrids between *B. pendula* and *B. pubescens* the chromosome numbers vary between 28 and 42.

It is possible that some of the atypical seedlings result from back-crosses to *B. pendula*. The expectation is that back-cross progeny would be  $2n=35$ . If, as many authorities assume,  $x=7$  in *Betula* then this is a perfectly feasible combination. According to Stern (1963), however, artificial hybrids are not only highly sterile but show a high incidence of hybrid weakness. This being so, and assuming that natural hybrids are similarly sterile, then the chances of obtaining back-cross progeny would appear to be fairly slim. Although our atypical seedlings were not used extensively as pollen parents they too showed a relatively high sterility. Finally there is the hypothesis that the low-count atypical seedlings are aneuploid *B. pendula* but, if they are, then the fact that they look like *B. pubescens* merely deepens the mystery.

On balance the evidence strongly favours the conclusion that most, if not all, the atypical seedlings from *B. pendula* mother trees are of hybrid origin, the pollen parent being *B. pubescens*. Concerning the  $2n=28$  hybrids reported by such authors as Dieterich (1963), it is likely that they are of similar origin to the low-count atypical seedlings described here. In the absence of published arrays of counts or evidence for variation in chromosome number it is difficult to substantiate this idea, but on the other hand there is no direct evidence for doubly reduced gametes in *B. pubescens* whose existence has been postulated to explain the diploid hybrids.

Having concluded that the atypical seedlings are hybrids, the parent trees in Glen Prosen cannot be ignored. If the previous reasoning is applied to the adult trees, then it can be seen from Table 7a that only trees, 1, 7 and 8 could with certainty be judged to be pure *B. pendula*. Of the five other trees four have mean counts in excess of 28 and all have relatively high coefficients of variation and thus could quite conceivably be of hybrid origin. The fact that the adult trees show more variation in their somatic chromosome counts may be an indication that the re-assortment and redistribution of chromosomes continues with increasing age and thus increases variation between cells. In Table 7b tree number 10 and (possibly) tree number 18 are also likely hybrids but these are, as would be

expected, *pubescens*-like in appearance. Thus in the adult trees there appear to be two morphological types of putative hybrid. In the *pendula*-type hybrids there seems to have been a somatic selection process to favour *pendula*-type chromosomes, the *pubescens* component being lost perhaps by unequal divisions of cytomixis (Brown & Al-Dawoodly 1979).

The *pendula*-type hybrids produce both normal *pendula*-type and atypical seedlings whose chromosomes vary from 29 to 44. If it is assumed that only euploid gametes with 14, 21 and 28 chromosomes are functional, and that the pure species produce only 14 and 28 chromosome gametes (while selection within the hybrids allows at least two different gametes to be produced, i.e. with 14 and 21 chromosomes whose maternal and paternal chromosome constitution is unknown), then difficulties can be seen to face any attempt to unravel the precise origin of seedling progenies from these trees. To complicate matters even further, there are some seedlings (9 (1-2), 10 (3) and 11 (1)) which originated from *pubescens*-type seed trees but which looked just like typical *B. pendula* seedlings.

Hybrids between *B. pendula* and *B. pubescens* are highly sterile and thus the chances of obtaining back-crosses or even F<sub>2</sub> progeny must be very low. Table 8 presents a comparison of seed yield and germinability of the Glen Prosen trees with those of trees on different sites. Although this is not an ideal test, since not all seeds were collected in the same year, it does suggest that the Glen Prosen trees are less fertile than the others. The exception to this is the Slugain trees, but these are moribund and pollen supplies are scarce. Even so the germination of full seed is higher than that of the Glen Prosen trees. Given the volume of birch pollen produced and the number of female catkins even in cases of high sterility there is a finite chance that some catkins will be pollinated with enough viable pollen to allow the development of seeds. (In retrospect the catkin crop in Glen Prosen seemed to be relatively sparse). The site in Glen Prosen where the trees grow was, during the second world war, a sawmill and at the end of the war it was colonized naturally by the birch. This could be the classic type of disturbed site, favoured by hybrids of other genera, which ensured the survival of the trees and, despite the earlier argument against the atypical seedlings being back-crosses, has allowed them to produce further generations of hybrids of one sort or another. In conclusion, however, it must be said that until the chromosome numbers of these trees were actually counted, with the exception of one tree, there was no doubt in the minds of the investigators that they were normal, if handsome, specimens of *B. pendula* and *B. pubescens*!

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