TAXONOMIC STUDIES OF *BETULA* WITH PARTICULAR REFERENCE TO *BETULA PENDULA* ROTH AND *BETULA PUBESCENS* EHRH.

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Contents.

Summary.

Tree birch from within the British Isles have been examined cytologically, morphologically and chromatographically. Studies have also been made of the reproductive biology of Betula.

Cytology confirms that difficulties are associated with chromosome number determination in Betula because of the small size of chromosomes in the genus. It is suggested that two cytodemes have been identified having chromosome numbers 2n=28 or 2n=56. The evidence for aneuploids is not persuasive from present studies in view of the ambiguity of some chromosome preparations. No plants with a chromosome number 2n=42 have been found in the samples studied.

Trees having normally accepted morphological characteristics of Betula pendula and B. pubescens together with trees apparently intermediate in terms of these characters have been found on the disturbed area of ground at Drumclog Moor near Glasgow. Various statistical methods have been used in the study of the morphology of birch. Both diploid and tetraploid trees have been found to be variable. In many cases the ranges of variation in a given character for the two groups show considerable overlap. Certain accepted criteria for distinguishing birch species have been found to be of little value in the present studies.

It has been established that cross-pollination may take place between B. pendula and B. pubescens in certain areas examined. This does not ensure, however, that fertilisation and the successful production of hybrids takes place in nature. Pollen studies have been made to investigate the possibility that "giant grains" are produced. In view of the size of the sample and the fact that there is a considerable overlap in the sizes of pollen grains of B. pendula and B. pubescens, firm conclusions cannot be drawn from this area of work. The viability of seeds has been

investigated by measuring seed germination. It has been found that the germination success of birch seeds is very variable.

A chromatography technique has been developed which is apparently capable of distinguishing leaf extracts of B. pendula from those of B. pubescens. In a majority of cases the classification of birch on the basis of chromatography has agreed with the classification made from cytology and morphology. In a few cases a tetraploid plant has had the chromatographic pattern which characterises the diploid.

It has frequently been stated in the literature that hybridisation occurs in birch, although supporting evidence is difficult to obtain. The present studies are consistent in several ways with the concept of hybridisation but it must be stressed that a relatively small number of plants have been examined. More work is required to determine the extent of variation in birch throughout its range.

CHAPTER 1.

Chapter 1.

Introduction.

The morphological variability of birch trees makes their taxonomy difficult. As a result they have been classified in a variety of different ways depending on the taxonomist's interpretation of the observed variation. In herbarium collections specimens are variously named as species, sub-species, varieties and hybrids, different authorities not always agreeing on the taxonomic position of a particular tree.

1.1 A brief history of the taxonomy of birch.

Linnaeus (1753) described three European taxa of Betula, Betula alba L., B.nana L. the dwarf birch and B.alnus L. the alder. B.alba L. was divided by Roth (1788) into two separate species namely B.pendula (Roth) and B.alba L.A distinguishing morphological character was said to be the occurrence of pendulous branches in B.pendula, as distinct from B.alba in which branches were erect and very straight. Roth described the leaves of both taxa as being ovate, acuminate and deeply serrate.

A different classification was suggested by Ehrhart (1791) who divided B.alba L. into species named B.pubescens Ehrh. and B.verrucosa Ehrh., the former having pubescent leaves and the latter glabrous ones.

Natho (1959) has noted that later examination of herbarium material appears to confirm that B.pendula Roth and B.verrucosa Ehrh. refer to the same taxon. It has been pointed out by Natho (1959) and Tuley (1973) that, following the International Rule of Botanical Nomenclature, the two species within B.alba L. should be called B.pendula and B.pubescens. Walters (1964) and Clapham, Tutin and Warburg (1962, 1968) have used these specific names. B.verrucosa , however, has been preferred by many taxonomists in other European countries. For the sake of clarity, B.verrucosa

appears in this thesis where reference is made to other birch studies in which that name was used.

In Species Plantarum (1805) edited by Willdenow, a further birch species, B. carpatica Waldst. et Kit., was named and described as a birch tree with doubly-serrate rhomboid leaves, glabrous petioles , ciliate catkin scales and catkin lobes oblong, obliquely truncate. Trees have been classified as this species by Jentys-Szaferowa (1949-51), Natho (1959) and Bialobrzeska and Truchanowiczowna (1960). According to Gardiner (1972a), Jentys-Szaferowa has identified material from Scotland as B. carpatica.

Later work has supported further sub-division of B. alba L. into species. For example Jentys-Szaferowa (1949-51) considered B. alba L. to comprise several species namely B. pubescens Ehrh., B. verrucosa Ehrh., B. tortuosa Ledeb., B. carpatica Waldst. et Kit., B. oycoviensis Bess. and B. obscura Kot. She devised a method which enabled comparison of a number of leaf characters from different trees simultaneously. Trees were identified by the resemblance of their graphed leaf measurements to those of previously classified specimens and, as such, the method was not objective. Furthermore, the position of possible hybrid trees, of intermediate appearance, was not clarified. Jentys-Szaferowa particularly noted that B. pubescens had more variable leaves than B. verrucosa.

Natho (1959), in his detailed examination of birch taxonomy, supported the division of B. alba L. into distinct species. Further support for such division comes from the work of Bialobrzeska and Truchanowiczowna (1960). Birch fruit characters were examined using a method similar to the one employed by Jentys-Szaferowa and their results suggested that the section B. nana L. could easily be distinguished from section B. alba L. The latter was said to include several species, B. verrucosa, B. pubescens, B. carpatica and B. tortuosa being named as examples.

Certain 19th century British botanists did not divide B. alba L. into the distinct species recognised by taxonomists in other European countries. For example Hooker and Walker-Arnott (1860) considered that varieties of B. alba L. existed. Sowerby (1868) recognised sub-species of B. alba L. naming them ssp. verrucosa and ssp. glutinosa Fries. The latter ssp. was further divided into var. pubescens and var. denudata Gren. et Godr., a classification with which Hooker (1884) agreed.

In more recent British taxonomic works the existence of two species within B. alba L. has been accepted, namely B. pendula and B. pubescens. These species have been recognised by Clapham, Tutin and Warburg (1962, 1968) and Walters (1964). There have been, however, different approaches to further division of B. pubescens into sub-species.

Clapham, Tutin and Warburg (1962) described two sub-species namely ssp. pubescens and ssp. odorata Bechst., the latter including B. tortuosa. This classification has apparently been widely accepted. For example Burnett (1964) described the distribution of the sub-species in Scotland, ssp. pubescens being confined to Argyll, Perthshire and the south and ssp. odorata being more widespread. Gardiner and Jeffers (1962) and Gardiner (1972a) measured leaf characters and analysed results by means of a computer. Principal component analysis indicated that a reduction in the number of leaf characters used by Jentys-Szaferowa could be made without serious loss of information. The analysis supported a division of B. pubescens into ssp. pubescens and ssp. odorata (including B. tortuosa). In a recent paper by Forbes and Kenworthy (1973) B. pubescens ssp. odorata was treated as a taxon.

Walters (1964) gave details of a "minimum framework" for the taxonomy of birch. B. pendula was considered to be a species with a possible sub-species (described by Lindquist 1947). B. pubescens and B. oycoviensis

were also listed as species, the former having three sub-species namely ssp. carpatica (including B. odorata), ssp. pubescens and ssp. tortuosa. The taxonomic descriptions given are ssp. carpatica having glabrous young twigs, leaves often less than 3cm. and being a small tree; ssp. pubescens having puberulent young twigs and fruit wings about $1\frac{1}{2}$ times the width of the nutlet, leaves being 3-4cm.; ssp. tortuosa having young twigs and leaves puberulent and wings as wide as nutlets, leaves being less than 3cm.

Clapham, Tutin and Warburg (1968) renamed the sub-species of B. pubescens as ssp. pubescens and ssp. carpatica (including B. odorata) which agrees with Walter's classification. B. tortuosa, however, is apparently not recognised as a British taxon in this Flora.

Since B. odorata has been included in ssp. carpatica in one Flora (Clapham, Tutin and Warburg 1968) and in another has itself been given sub-specific rank (Clapham, Tutin and Warburg 1962) and has been said to include B. tortuosa, the taxonomy of these trees obviously requires careful study to determine their relationships to one another. It is of interest to note that Gardiner (1972b) found European samples identified as B. carpatica and B. tortuosa to be distinct from one another and from British samples of B. pubescens and B. pendula. B. carpatica and B. tortuosa could otherwise be taken to be one species based on their association in certain Floras with B. odorata.

In a study of Scottish birch by Brown and Tuley (1971), the taxonomic status of trees was found to be uncertain since very few had a majority of characters of either B. pubescens or B. pendula. Tuley (1973) has since suggested that B. alba L. be regarded as a species and that B. pendula and B. pubescens be sub-species. Vaarama and Valanne (1967), with reference to the uncertain taxonomic status of trees, are of the opinion that it is not possible to determine the exact number of species within the genus Betula.

1.2 (a) The interpretation of variation in birch as developmental, environmental and genecological.

Examples of developmental variation within individual birch trees have been given by Jentys-Szaferowa (1959). For example, leaves on fruiting shoots were observed to differ from those on vegetative ones, the presence of catkins appearing to have an effect on their morphogenesis. It was also found that the shape of leaves depended on the position from which they had been taken in the crown of the tree (Jentys-Szaferowa 1955). The author stated that further study was required to elucidate the situation.

In studies of B. pendula and B. pubescens , statistical evidence was obtained by England (1963) which suggested that temperature was a major factor in the determination of leaf length. Furthermore, England pointed out that intraspecific variation in this character largely disappeared when plants were grown under the same environmental conditions. This evidence suggested that genetic differences were slight and that the variation mainly resulted from the phenotypic plasticity of the plants. It should be borne in mind, however, that lack of variation observed in the experimental garden need not indicate genetic uniformity since conditions there might not allow the expression of genetic differences. Briggs and Walters (1969) have pointed out that great care must be taken before attributing variation to a specific factor.

Brown and Tuley (1971) have made reference to genecological variation. In addition to the possibility that characters such as leaf size may respond to changes in environmental conditions, they have suggested that it may also be the case that examples of intraspecific variation in response to the environment have appeared as a result of natural selection. One example quoted by these authors is the greater hairiness observed on leaves of plants growing in relatively severe environmental conditions. This could be of adaptive significance in reducing water loss resulting

from transpiration. Evidence of the existence of birch showing possible adaptations to relatively extreme conditions comes from the work of Elkington (1968), Forbes and Kenworthy (1973) and Vaarama and Valanne (1973).

Forbes and Kenworthy (1973) observed changes in the growth habit of B. pubescens sspp. associated with increases in altitude. The shrubby habit, found to be a feature of birch at higher altitudes, was said to persist in cultivation. This being the case, it may be postulated that this variation has a genetic basis.

Vaarama and Valanne (1973) noted variation in leaf characters such as leaf length and leaf apical angle which distinguished B. tortuosa samples from B. pendula ones. The differences persisted under experimental conditions and were regarded as being of adaptive significance as responses to the environment.

One explanation of the origin of birch showing adaptations connected to a particular environment could be hybridisation. In this way plants with different genomes could arise, possibly having selective advantages in certain habitats.

1.2(b) Hybridisation as a source of variation in birch.

Hybridisation is a possible way in which new genetic combinations in birch could have arisen and consequently could account for morphological variation. Early references to the occurrence of hybrids have been found by Tuley (1973) and Vaarama and Valanne (1973). Elwes and Henry (1909) and Gunnarsson (1925) stated that hybridisation in birch was widespread and Evans (1932) could find no trees which he would have described as typical Betula parents.

Anderson (1949) has called the complex situation involving crossing of and subsequent backcrossing to parental types, "Introgressive Hybridisation." The occurrence of such a breeding pattern would result in

a situation where the delimitation of taxa would become most difficult. Anderson considered introgression to be a widespread phenomenon and discussed techniques for elucidating situations where complex patterns of hybridisation had taken place. He noted that introgressant plants were often found in "hybridised habitats" where they appeared to have a selective advantage.

Natho (1959) considered introgression to be widespread in birch. Referring to a merging of species into one another, he quoted many instances of hybrid formation. B. carpatica he regarded as having been formed by introgression of B. pendula into B. pubescens. Natho stated that these two species in particular seemed to form hybrids and that intermediate forms were found in the wild in intermediate habitats. It was noted that birch variation was less marked when trees of the different species were growing apart. Walters (1968) agreed with this viewpoint, noting that ecological separation in Finland seemed to result in a situation where identification of birch was not difficult. Walters (1964) also noted that hybrids seemed common where natural forest had been destroyed. Vaarama and Valanne (1973) have explained patterns of variation observed in the field in terms of introgression. They have obtained evidence which suggests that introgression of B. nana into B. pubescens, where they occur sympatrically, has led to the evolution of B. tortuosa as a separate taxon. Researches of Elkington (1968) support the occurrence of hybrids between B. nana and B. pubescens in naturally disturbed areas. Evidence for possible birch hybrids comes from the work of Berrie (1952), Brittain and Grant (1967-71), Guerriero, Grant and Brittain (1970), Kenworthy, Aston and Bucknall (1972) and Dawoody (1974). Grant (1971) has called the genus Betula a "Syngameon" or one large pairing community, a situation which is paralleled in the genus Quercus.

Although introgression has considerable support as an explanation of phenomena observed in birch populations in the wild, the

complex cytological situation does not clearly support the explanation, as Vaarama and Valanne (1967) have noted. The chromosome number in B. pendula is commonly accepted as 2n=2x=28 and that in B. pubescens as 2n=4x=56 (Darlington and Wylie, 1955). There is support from studies of Skalinska et al (1959), Grant (1969) and Dawoody (1974) for a basic number of 7, not 14, in the genus Betula. An F_1 hybrid would be expected to have 42 chromosomes if B. pendula and B. pubescens were crossed and would probably have irregularities in meiosis which could lead to sterility. Johnsson (1945) and Eifler (1960) have produced such hybrids. Irregular meiosis has been observed by Helms and Jorgensen (1925), Woodworth (1929) and Dawoody (1974) but apparently is not confined to trees with chromosome number 42. Dawoody has reported that 2n=28 and 2n=56 trees also have irregular meiosis in a minority of cases. There is evidence that not all trees with a chromosome number of 42 are necessarily hybrids. Löve (1944), Johnsson (1956) and Dawoody (1974) have found trees closely resembling the diploid with this chromosome number which they have regarded as autotriploids. In this connection a difficulty over terminology noted by Briggs and Walters (1969) becomes apparent. Dawoody has suggested that plants with a chromosome number of 42 may form as a result of fusion of a B. pendula unreduced gamete and a B. pendula normal gamete and also as a result of fusion of a double-reduced gamete from B. pubescens and a normal gamete from B. pubescens. Are these to be regarded as autotriploids which implies that the gametes contain identical genomes ? Stebbin's term "segmental allopolyploid" perhaps would be more suitable.

An additional complication is that not all hybrids of B. pendula and B. pubescens would have 42 chromosomes if unreduced gametes are produced. Trees with a chromosome number 56 could theoretically arise from a cross involving B. pubescens (normal gamete) X B. pendula (unreduced gamete). Johnsson (1945) has produced such crosses under experimental conditions. Elkington (1968) and Dawoody (1974) have accepted the

hypothesis of unreduced gamete formation and in the work of Dawoody (1974) there is certain experimental evidence which supports the occurrence of such gametes. The production of hybrids with 56 chromosomes might explain the great variability of tetraploid birch observed by Jentys-Szaferowa (1959) and Walters (1968).

If hybridisation is to be seriously considered as a major cause of variation in birch then cross-pollination must take place. Despite the large body of evidence from morphological studies in favour of introgression in birch, convincing evidence of cross-pollination and subsequent fertilisation has not been obtained. Indeed no details of flowering times have been given in some studies in which introgression has been taken to be a major factor.

Jentys-Szaferowa (1938) was of the opinion that hybridisation was unlikely since flowering times of birch species did not overlap. Sarvas (1952) found a separation in flowering times of B. pubescens and B. verrucosa of not less than four days, the latter flowering first. Eifler (1956) has also noted a separation in flowering times of birch species. B. pendula being first to flower might be expected to be "purer". The reproductive system, however, is complex. Sarvas (1952) was of the opinion that female flowers could best receive pollen over a period of two to three days. Information on pollen viability has been obtained by Linskens (1964) who has found that humidity plays an important part. Alam and Grant (1971), with reference to birch pollen, have found that under laboratory conditions grains may retain viability for a considerable number of days depending on temperature. The variability of flowering times in birch species and the dependence on environment has been specifically noted by Johnsson (1945), Berrie (1952) and Natho (1959). It is difficult with the available evidence to establish what barriers may be said to be of significance in preventing cross-pollination in birch. There is no

reason to suppose that flowering times are the same throughout the whole geographical range of B. pubescens and B. pendula. In Quercus evidence for introgression has been obtained by Muller (1952), Tucker (1952) and Cousens (1965). Studies of flowering times in this genus by Cousens (1965) have shown that flowering times overlap in the north of Britain, this being connected with the shorter growing season. The effect of environment may well be of relevance in the reproduction of birch species since studies in which the existence of hybrids has been postulated (Elkington, 1968, Kenworthy, Aston and Bucknall, 1972) have been in areas with a relatively short growing season. Vaarama and Valanne (1973) have noted that hybrids have frequently been reported from north-west Finland but are more rare in the south. This may be connected with the length of the growing season.

Although there have been many references to the occurrence of birch hybrids in nature, surprisingly few successful ones have been produced under controlled conditions. The situation again resembles that in oak where Gardiner (1970) has reported a low frequency of successful artificial crosses. Johnsson (1945) found that, even under controlled conditions, few hybrid plants were produced. Only two out of seventy-three crosses involving B. verrucosa and B. pubescens produced F_1 hybrid plants. This led him to suggest the existence of sterility barriers. Eifler (1960) has suggested that in some cases trees are incompatible. Hagman (1963) has noted that retarded growth of pollen tubes occurs in cases of self-pollination of B. verrucosa and B. pubescens and also when these species are crossed. This suggests that there are both self and interspecific incompatibility reactions in birch of these species. The incompatibility seems to result from serological differences between the pollen of the species. It is interesting to note that Johnsson (1945), Eifler (1960) and Hagman (1963) have observed that the incompatibility is variable and is affected by environmental conditions. If outbreeding is favoured in birch it would explain to some extent the variation in the genus.

On the whole, studies of the reproductive biology of birch do not support a concept of widespread hybridisation. The weight of evidence available would, at the most, support hybrid formation as a rather infrequent event.

1.3 The aims and general approach in the present studies.

For a number of reasons, populations of birch from Milngavie and Dunblane were chosen for study during the present research. There is evidence from the work of England (1963), Brown and Tuley (1971), Forbes and Kenworthy (1973) and Vaarama and Valanne (1973) that environment is an important factor in birch variation. By choosing a relatively small area, large climatic differences were not likely to be a major factor in birch variation. Preliminary investigation of the Moor suggested that trees with typical morphological characteristics of both B. pendula and B. pubescens were present together with birch intermediate in these characters. As a result of restricting the study area it became easier to obtain detailed information on the habitat, including its history, which is the subject of Chapter 2.

Considerable time was devoted to the collection of cytological information. A knowledge of chromosome numbers would provide a classification of birch with the possible identification of hybrids. Furthermore, it would enable correlation of chromosome number with other areas of study. The cytology is described in Chapter 3.

Much information was available from earlier work on morphological characters of apparent usefulness in birch taxonomy although many such characters were described from studies where no correlation with cytology was made. It has been pointed out that taxonomy based on morphology suffers from a lack of reference points. In the case of birch, obtaining the morphology of "pure" parents is of major difficulty and indeed may be impossible if introgression has been widespread. An open-ended approach was adopted and instead of having pre-conceived ideas on the taxonomic position of sample trees, groupings were sought by means of scatter diagrams. Hybrid indices were considered to be of lesser value in view of uncertainty over the morphology of "pure" parents. Information

on the resemblance of trees to each other was also sought by means of computer analysis which enabled the comparison of large numbers of morphological characters simultaneously. The morphology is described in Chapter 4.

Comparison of the cytology and morphology was made. The chromosome number of morphologically intermediate trees was particularly noted. By examining the morphological data of diploid and tetraploid plants it was possible to obtain the range of variation of certain characters in birch with these chromosome numbers. In this way an assessment of the value of various criteria in the delimitation of birch taxa could be made.

In view of the disagreement in the literature on the likely occurrence of hybrids, careful observations of flowering times were made over a number of years. Because of the part-time nature of this research, it was not possible on every occasion to obtain exact dates. Considerations of time and facilities made the setting-up of controlled crosses impossible and consequently no evidence which directly supported successful fertilisation following cross-pollination was obtained. Pollen grains were measured to see if "giant grains" could be found which might be taken to support the occurrence of unreduced gametes. Fruit viability was measured in germination trials to see if any samples had a particularly low germination success rate, which might be the situation in a hybrid. The account of the studies of the reproduction of birch is given in Chapter 5.

Previous researchers have suggested that tetraploid birch are most variable and this has been connected with the hypothesis that tetraploid hybrids form as a result of fusions involving unreduced gametes. A method of distinguishing hybrid trees was sought after and in Chapter 6 details of a chromatography technique are described.

Having obtained detailed information from birch in a relatively small area at Milngavie, additional material was examined in order to provide comparative data from other sites in the British Isles. Samples were obtained from a second area and from a herbarium collection as described in Chapter 2. The taxonomic relationship of these specimens to the Milngavie ones was examined and details are given in the appropriate chapters.

CHAPTER 2.

Chapter 2.

The Birch Populations Studied.

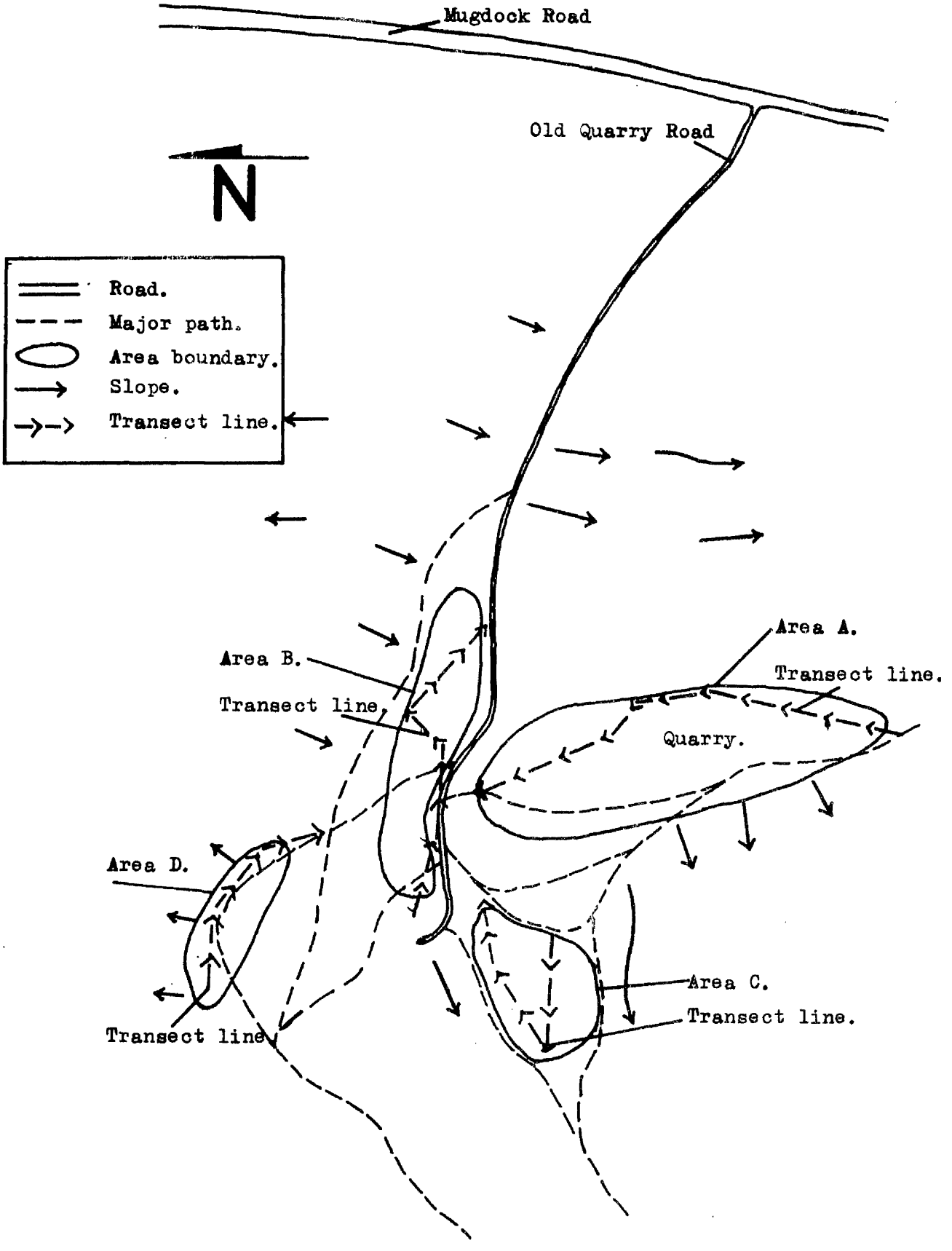
Two sites were selected for study, one at Milngavie near Glasgow and the other at Dunblane in Perthshire. Marked birch trees were sampled along transect lines and provided material for laboratory work on cytology and morphology. Observations on the reproductive biology of birch and on items of possible ecological value were made in the field. In view of the fact that the examination of the two sites yielded information on a limited area geographically, a herbarium collection from the Royal Botanic Garden, Edinburgh was studied to provide morphological data on birch from the British Isles as a whole. The collection contains specimens identified as B. pendula, B. pubescens or hybrids of these.

2.1 A description of the areas studied at Drumclog Moor, Milngavie.

The Ordnance Survey reference of the site is OS 551 761.

Geological surveys have indicated that the local rocks are sedimentary and of the Carboniferous limestone, Calciferous sandstone series. On the Moor sandstone outcrops at several points and has been quarried at one site, as shown in figure 2.1. The depth of the glacial drift deposits overlying the sandstone varies considerably. Parts of the Moor which have been closely studied in this research (see fig. 2.1) are separated from the River Allander to the south-west by steeply sloping ground. To the north and west, approximately one mile distant, is Mugdock Wood, registered as a Site of Special Scientific Interest. Within the Drumclog Moor area three broad vegetation types may be recognised. On well-drained sites one type is dominated by dwarf shrubs, especially Calluna vulgaris and another consists of bracken and various grass species. A third community with Sphagnum and Eriophorum species is found in hollows where

Figure 2.1 Drumclog Moor, Milngavie. Scale 1/2000.



the water table is high. The transition between the types of vegetation is quite abrupt in places as may be seen in figure 2.2. Here the dominance of C. vulgaris is evident in the foreground. A change to a community mainly containing Sphagnum and Eriophorum species takes place in the middle distance accompanying a marked rise in the height of the water table. The dominance of birch as a tree species may be seen beyond the very wet ground. Although some of the observed variation in types of vegetation on the Moor may result from differences in soil, drainage, aspect etc., the influence of man must be considered significant in both past and present times.

Sandstone was formerly quarried in the area. There is evidence for the foundations of a building near the quarry site which is said to be the remains of a small farm, suggesting that grazing might have been important in the past. During the Second World War parts of the Moor were ploughed and a crop of nettles raised but the crop failed in the second year and the ploughed area was invaded by heathland plants including birch. Remains of the furrows left by ploughing can still be seen on the Moor.

At the present time the Moor is a favourite picnic site for visitors from Glasgow and its suburbs. Figure 2.1 shows the network of paths which traverse the area and which are used not only by walkers but by ponies as well. Until recently vehicles had access to the Moor via the Old Quarry Road but this has now been closed to traffic. Many of the pathways show considerable erosion as a result of constant use and run-off of rainwater. The local council managing the Moor have carried out a number of drainage schemes and the ground as a whole is better drained than formerly. Every year parts of the Moor are set alight either accidentally or deliberately. On several occasions during the course of these studies the vegetation was burnt causing damage to birch trees on the fringes of burnt areas. Seedling birch are rare in certain badly burnt areas. The Moor is also a source of peat, stone and firewood for the village of Milngavie. Although only small

Figure 2.2 The start of the area A transect.

quantities of peat and stone are removed, many of the trees show signs of damage. No domestic animals graze on the Moor but deer have been seen and small herbivores are probably important in this respect.

Birch is very common. Identification using Clapham, Tutin and Warburg (1962) suggests that B.pendula and B.pubescens are present together with intermediate trees.

2.2 The sampling of the populations on Drumclog Moor.

Four areas A, B, C and D (figure 2.1) were selected for study and within each the vegetation was described. Species were identified and named according to Clapham, Tutin and Warburg (1968). Each was given an estimate of cover using the Domin scale. Table 2.1 records the vegetation of representative quadrats in the areas examined.

Soil samples were taken from the soil horizon containing birch tree roots, usually about 15 to 20 centimetres down. In practice a soil pit was dug, the profile examined and a soil sample removed from the appropriate horizon. In the laboratory roots, twigs and pebbles were removed and the remainder of the sample of soil was air-dried. For each of the air-dried samples several 12g quantities of soil were made into pastes with de-ionised water and the pH of each was measured using a pH-meter. Soil profiles revealed evidence of leaching and iron pan development. The pH values recorded in table 2.2 show that the upper horizons were highly acidic. The profile was that of a podsol and as the soil samples were taken from 15 to 20 centimetres down they were from the 'A' horizon. This horizon had two distinct strata in all areas examined on the Moor. The upper was 5 to 15 centimetres in depth and contained much humus. The lower, some 8 centimetres in depth, contained black humified organic matter and bleached sand grains. Horizon 'B' showed evidence of iron accumulation its colour being distinctly cinnamon.

Table 2.1 The vegetation associated with birch on Drumclog Moor.

Species	*Representative quadrat number. A1	A3	A5	B1	B10	C1	C5	D1
Agrostis tenuis	6		6	5	5	7	5	7
Anthoxanthum odoratum	5		6		6	6	5	5
Calluna vulgaris	5	5	5	5		4		5
Chamaenerion angustifolium					1			
Cytisus scoparius			1					
Dicranum scoparium		4						
Empetrum nigrum		5	1					
Erica tetralix		5						
Eriophorum angustifolium		1						
Festuca rubra					5	5	5	5
Juncus articulatus		1						
J.effusus		1						
Mnium hornum		3						
Molinia caerulea	6	4	6	6	5			5
Narthecium ossifragum		3						
Parmelia physodes	3	3	3	3	3	3	3	3
Polytrichum commune		4						
Potentilla erecta	3		4	4			4	4
Pteridium aquilinum	1	1	5	5				5
Rubus fruticosus			1		1			
Salix sp.			1	1	1			
Sorbus aucuparia					1			
Sphagnum spp.		4						
Vaccinium myrtillus		4	1			1	1	1
V.oxycoccus		3						

*Plants found within 1 metre quadrats were identified and given the Domin numbers shown in the table. Quadrats themselves were given the number of the birch tree(s) to which they were adjacent eg.quadrat A1 is adjacent to tree A1 at the beginning of the area A transect. The data has been presented in such a way that if no quadrat is listed eg. between A5 and B1, then the plants in quadrat A5 were found to be representative of the other quadrats between A5 and B1 with perhaps small differences in Domin numbers.

Table 2.2 pH values of soil samples from the 'A' horizon on Drumclog Moor.

Adjacent tree	pH	Adjacent tree	pH	Adjacent tree	pH
A1	3.8	B5	3.4	C14	3.2
2	"	6	"	15	"
3	"	7	"	16	"
4	"	8	"	17	"
4A	"	10	3.9	18	3.4
5	"	11	"	19	"
6	"	12	"	20	"
7	3.7	13	"	21	"
8	3.8	14	"	22	"
9	"	15	"	23	"
10	"	16	"	24	3.2
11	"	17	"	25	"
12	"	18	"	26	"
13	"	20	"	27	"
14	"	21	"	28	"
15	3.9	22	"	29	"
16	"	23	"	30	3.4
17	"	C1	3.2	31	"
18	"	2	"	32	"
19	"	3	"	33	"
20	"	4	"	D1	3.8
21	3.8	5	"	2	"
22	"	6	"	3	"
23	"	7	3.3	4	"
24	"	8	"	5	"
25	"	9	3.2	6	"
B1	3.4	10	"	7	"
2	"	11	"	8	"
3	"	12	"	9	"
4	"	13	"		

In each marked area birch were sampled at random within 3 metres to the left and right of a transect line. Trees were marked by cutting a notch or notches in the bark and then spray-painting the notch with coloured paint. Following this the position of each tree was plotted on a map and its notch/colour code noted alongside. Photographs were taken as an additional means of relocating trees and also provided data on bark and pendulousness of branches. Every tree sampled was given a code letter corresponding to the area in which it was located and a number to indicate its position in the sampling sequence along the transect line.

Leaves, required for measurement and subsequent comparison, were taken on dwarf vegetative shoots which, according to Jentys-Szaferowa (1949-51), are least variable. Since some trees were growing in more shade than others, branches were always taken from the south-facing crown using a long pruning-pole. Twigs collected in this way were pressed for later measurement. Male and female catkins were taken from several positions on each tree and were stored in an air-dry state in polythene bags.

The careful labelling of birch allowed the collection of data on flowering times and seed production over a number of years and made possible the relocation of trees at any time for the collection of more specimens.

The transect in area A (figure 2.1) passed alongside a piece of waterlogged ground. Birch trees A1 to A4A were actually growing on the edge of this ground. These trees were of interest in view of some uncertainty in the literature on the subject of birch tolerance of wet ground. Clapham, Tutin and Warburg (1962) were of the opinion that B. pubescens was more tolerant of wet areas than B. pendula and Forbes and Kenworthy (1973) appeared to find quite the reverse. Trees further along the transect were situated in the disused sandstone quarry and were of particular interest in view of the fact that they had colonised disturbed

ground. There is evidence in the literature that intermediate birch tend to establish themselves in disturbed ground (Natho 1959, Walters 1964). The assessment of the age of trees was most difficult since in a majority of cases growth was not confined to one stem, perhaps as a result of damage when they were younger. Based on comparisons with birch of known age growing elsewhere, all sample trees in area A were estimated at older than thirty years. Figure 2.2 is a photograph taken at the beginning of the area A transect. The marker shows the position of very wet ground with birch growing on its edge. To the right of centre, the higher ground leading to the quarry may be seen.

Trees in area B were, on the whole, younger than those in area A and were thought to be post-war. The transect passed through ground showing evidence of furrows produced as a result of ploughing during the Second World War. Again this type of area was of interest in view of its disturbed history.

Birch in area C appeared to be of similar age to those in area A. The growth of birch was particularly dense here. Plants C19 to C33 on the transect were situated along the edge of heathland which was burned on at least two occasions during the present studies. Figure 2.3 is a photograph of birch at the start of the transect, the large trees in the foreground being C1 and C2.

Area D contained the oldest birch trees sampled, the large trees right of centre in figure 2.4 being D1 and D2.

Figure 2.3 The start of the area C transect.

Figure 2.4 The start of the area D transect.

2.3 A description of the areas studied at Wanderwrang Wood, Dunblane.

The Ordnance Survey reference of the site is NN 773 003.

Geological surveys have indicated that the local rocks are of the lower Old Red sandstone. A sketch map of the area is given in figure 2.5. Records show that Wanderwrang Wood in 1907 was predominantly large and medium-sized beech, oak and Scot's pine with forty-year old spruce. There were, at that time, patches of Douglas fir and birch, the latter produced by natural seeding. During the First World War the timber was felled and pine planted in its place. These conifers were subsequently felled and in 1944 replanting with spruce and pine took place in the northern half of the Wood. The other half was left rough and birch established itself. The older trees sampled in the present studies were thus more than thirty years old and had grown from seed. In the last four years most of these trees have been felled for firewood but already there are established seedlings to the west of the track shown in figure 2.5.

2.4 The sampling of the population in Wanderwrang Wood.

The vegetation was named using the species list given by Clapham, Tutin and Warburg (1968). Cover was estimated using the Domin scale. Table 2.3 is a record of the vegetation in representative quadrats.

Soil was sampled using methods described in Chapter 2.2. Samples were found to have an acid pH, the range of values obtained being 3.4 to 4.2. Soil profiles showed less marked stratification than Milngavie ones but evidence of leaching was noticeable.

Trees were marked and coded in the same way as those sampled at Drumclog Moor. Vegetative and reproductive material was collected and field observations such as flowering times were made.

Figure 2.5 Wanderwrang Wood, Dunblane. Scale 1/400.

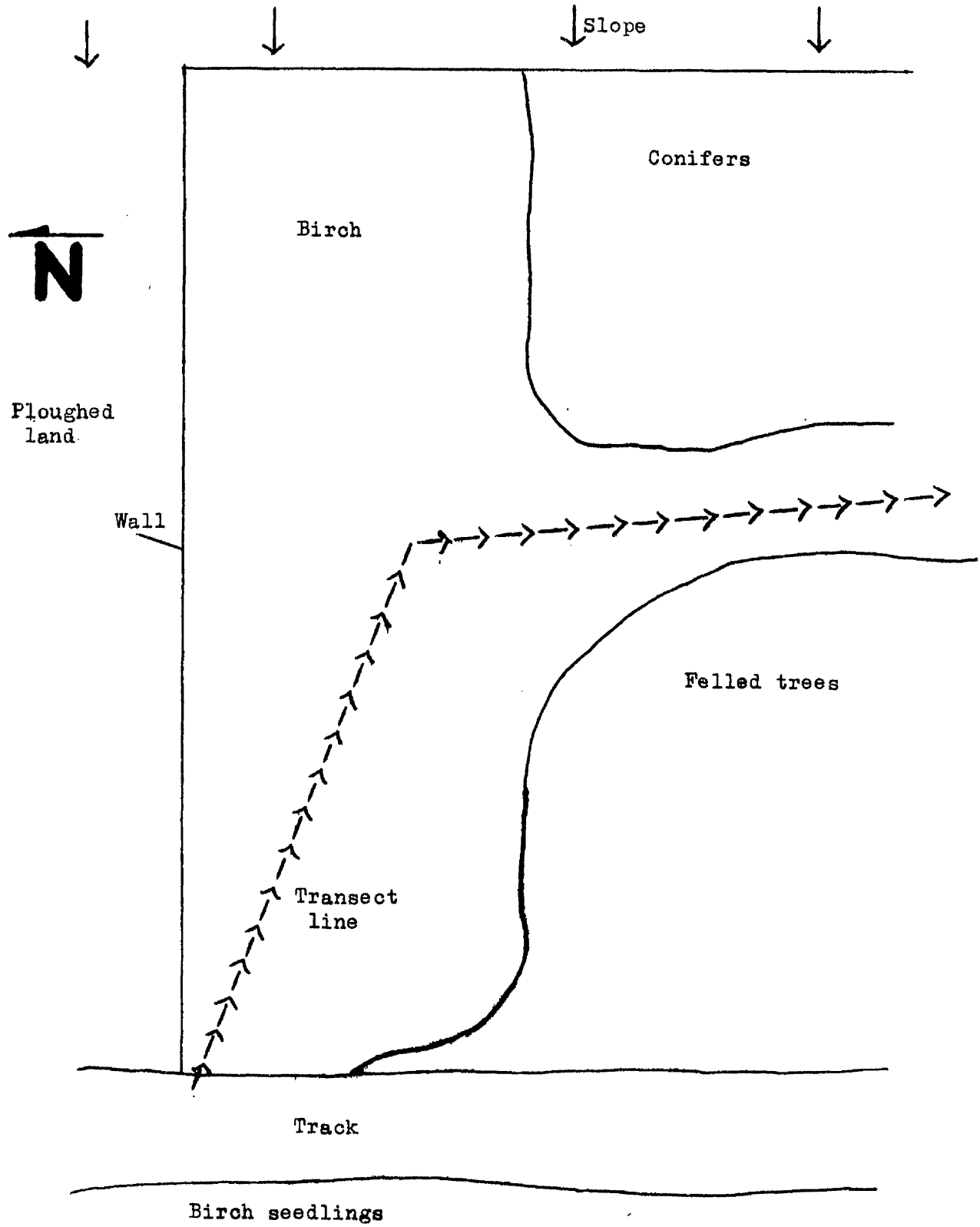


Table 2.3 The vegetation associated with birch in Wanderwrang Wood.

Species	*Representative quadrat number. E1	E3	E7	E10	E12	E15
Cirsium arvense	1	1				
Crataegus monogyna	1					
Deschampsia caespitosa	6	6	8	8	8	8
Dryopteris felix-mas		1				
Hypnum cupressiforme	3	3			3	
Lonicera periclymenum	1				1	1
Mnium hornum	3	3	3			3
Oxalis acetosella	3	4				
Parmelia physodes	3	3	3			
Peltigera sp.		1				
Ranunculus repens	4	4	4			1
Rhytidiadelphus squarrosus		3	3			
Rubus fruticosus	3					
Sambucus nigra	1					
Stellaria media						
Urtica dioica	1					

*The table has been prepared in the same way as table 2.1.

2.5 Conclusions.

Birch material has been collected for morphological examination. In the case of the Drumclog Moor and Wanderwrang Wood populations the habitats from which the materials were collected have been studied allowing the correlation of morphology and habitat to be examined. The inclusion of the herbarium collection permits comparison of the morphological variation of local birch populations with that in trees from the whole of the British Isles.

Information on breeding behaviour has been gathered from the two local populations examined in the present studies.

CHAPTER 3.

Chapter 3.

The Cytology of Birch.

3.1 Introduction.

Chromosome numbers for the genus Betula have been obtained from studies of meiosis and mitosis. Darlington and Wylie (1955) and Bolkhovskikh, Grif, Matvejeva and Zakharyeva (1969) have listed sources of chromosome numbers for B. pendula and B. pubescens. A table of chromosome numbers from references known to the present author is shown below.

Author(s)	Observed Chromosome Number		
	28	42	56
Helms, Jorgensen (1925)	x		x
Woodworth (1929)	x		x
Tischler (1934)	x		x
Johnsson (1940,44,45)	x	x	x
Löve (1944,54)	x	x	x
Delay (1947)	x		
Berrie (1952)	x	x	x
Skalinska et al (1959)	x		x
Eifler (1960)	x	x	x
Gadella, Kliphuis(1966)	x		x
Kenworthy et al (1972)	x	x	x
Dawoody (1974)	x	x	x

Uneven chromosome numbers have been recorded in studies made by Berrie (1952), Kenworthy, Aston and Bucknall (1972) and Dawoody (1974).

Darlington and Wylie (1955) regarded 14 as the basic chromosome number in the genus Betula. Thus B. pendula would be regarded as diploid 2n=2x=28 and B. pubescens as tetraploid 2n=4x=56. Skalinska, Czapik, Piotrowicz et al (1959), however, considered x in the genus to be 7, not 14. Dawoody (1974) has produced evidence of sub-grouping during meiosis into 7 bivalents which would be consistent with a basic number of 7. Studies of birch in Canada made by Grant (1969) give some further support for this basic number. Using a cytophotometry technique, the absorption of root-tip chromosomes, stained by a Feulgen method, was

measured. Trees with a chromosome number of 84 had an amount of D.N.A. expected to occur in nuclei with 63 chromosomes which the author suggested would be more consistent with a basic number of 7.

The cytology of birch is difficult. Chromosomes are small and because polyploidy is common in the genus, numbers are fairly large. The use of root-tips from parent Betula is most difficult since it is often the case that several plants are growing close together in the field and establishment of root connections is no easy task. Root-tips for cytology can be obtained from seedlings but the method may be criticised on the grounds that chromosome numbers may not be the same as in parent plants. This could arise through the formation of unreduced gametes. Furthermore, there is the possibility that hybrids would be sterile and would not be detected since no seed were produced. Difficulties could be overcome by rooting cuttings. Skalinska et al (1959) and Dawoody (1974) have had success with squashes of leaf material.

In the present study both seedlings and cuttings have been used to provide root material. The chromosome numbers of trees were particularly required to enable a classification of birch and correlation of cytology with morphology.

3.2(a) Chromosome studies of root-tip material.

Seed collected in the field were germinated on moist Whatman seed-test circles in petri-dishes. The dishes were placed in a warm laboratory under continuous light from fluorescent tubes. Young plants were removed from the dishes at about the ten-day stage, before the root-hairs became inseparable from the paper. Terminal 5mm pieces of root-tip were immersed in saturated aqueous solution of 1-bromo-naphthalene, at room temperature, for four hours. The method has been used by Darlington and La Cour (1960) and Marchant (1968). Root-tips were then transferred without washing to 1:3,ethanoic acid:ethanol in a freezer for at least twenty four hours. Davies (1952) has suggested that material may be kept for at least six months in this manner. Material not immediately required for examination was stored in 70% ethanol in a freezer, as recommended by Marchant (1968) and McAllister (1972). Root-tips were hydrolysed in 1N hydrochloric acid for six minutes at 60°C in a water bath and the terminal 2mm was mounted on a slide in lacto-propionic orcein. The staining method was that of Dyer (1963) who found it particularly useful for plants with small or many chromosomes. Stain was used undiluted. Root-tip material was tapped out in the stain and,after removal of large pieces,was squashed between slide and cover-slip. A Zeiss microscope equipped with phase contrast was used to examine cells. A minimum of three cells from each preparation had their chromosome number recorded and several root-tips from each sample were prepared. The magnification necessary was between x800 and x1600. If several counts of 28 or 56 were obtained from a sample tree, then it seems likely that the parental tree could be regarded as diploid or tetraploid respectively. This point is discussed in Chapter 7.

3.2(b) The results of chromosome studies of root-tip material.

The small size of chromosomes and the occurrence of

dark-stained bodies in the cytoplasm made it difficult to obtain large numbers of unambiguous preparations. Consequently in table 3.1 uncertainty is indicated by a $\pm$ following the given chromosome number. Where only one number is quoted it was repeatedly obtained. From the table it can be seen that chromosome numbers of 28 and 56 were frequently recorded from trees which, according to Clapham, Tutin and Warburg (1962), could be regarded as B. pendula and B. pubescens respectively as a result of having such numbers. Cells with uneven chromosome numbers were observed but these occurred in ambiguous preparations and as such there is no persuasive evidence from these studies for aneuploids. In the cases of sample numbers A18, B14 and C4 many repeated attempts to obtain unambiguous preparations yielded no success. It is not clear why this should have been the case. No cells in material examined had a chromosome number of 42.

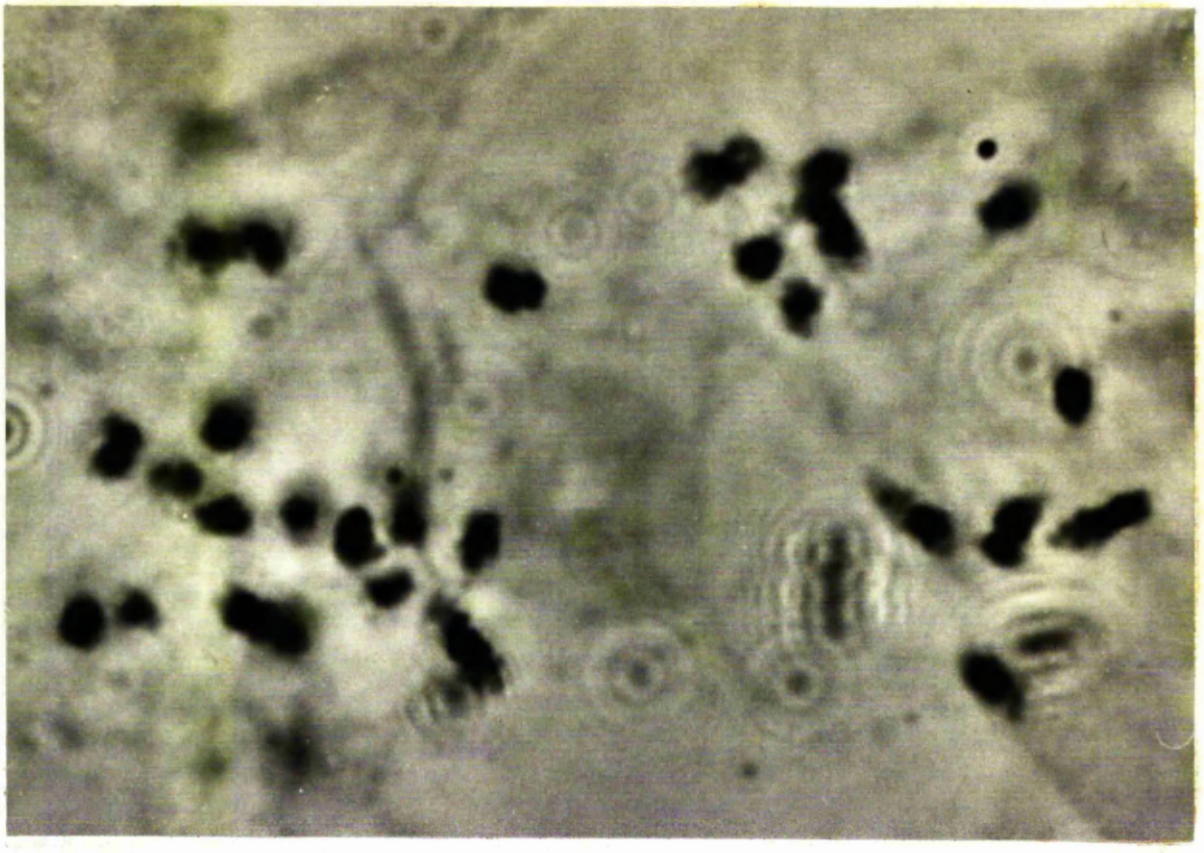
Figures 3.1 and 3.2 are photographs of a diploid and a tetraploid type respectively with accompanying interpretative drawings.

Table 3.1 The chromosome number of progeny from Milngavie seed.

Tree	Chromosome no.	Tree	Chromosome no.	Tree	Chromosome no.
A1	56	B5	$58^{\pm}$	C14	28
2	$57^{\pm}1$	6	$55^{\pm}$	15	56
3	28	7	$57^{\pm}$	16	28
4	28	8	$51^{\pm}, 48^{\pm}$	17	28
4A	28	10	$56, 57^{\pm}1, 56$	18	28
5	56	11	$55^{\pm}1$	19	56
6	$56, 56, 55^{\pm}1$	12	$57^{\pm}1$	20	$55^{\pm}1$
7	56	13	$56, 58^{\pm}, 56$	21	56
8	56	14	$23^{\pm}$	22	$55^{\pm}1$
9	$28, 28, 29^{\pm}1$	15	28	23	$56, 56, 55^{\pm}1$
10	28	16	56	24	56
11	28	17	56	25	28
12	56	18	$55^{\pm}1$	26	56
13	$31^{\pm}, 29^{\pm}, 28$	20	56	27	56
14	28	21	$26^{\pm}$	28	$55^{\pm}1$
15	$29^{\pm}, 27^{\pm}, 28$	22	56	29	$57^{\pm}1$
16	28	23	56	30	56
17	56	C1	$28, 28, 29^{\pm}$	31	$29^{\pm}, 27^{\pm}, 28$
18	?	2	28	32	56
19	28	3	$57^{\pm}1$	33	$57^{\pm}1$
20	$53^{\pm}, 55^{\pm}$	4	?	D1	56
21	$56^{\pm}1$	5	56	2	56
22	56	6	56	3	56
23	56	7	56	4	$55^{\pm}1$
24	56	8	$56^{\pm}$	5	56
25	56	9	no germination	6	56
B1	$55^{\pm}1$	10	$56, 56, 55^{\pm}1$	7	56
2	$29^{\pm}, 26^{\pm}, 27^{\pm}1$	11	$56, 56, 57^{\pm}1$	8	56
3	$27^{\pm}1, 28, 28$	12	28	9	56
4	56	13	$56, 55^{\pm}1, 56$		

Making allowance for the difficulty experienced in obtaining unambiguous preparations it seems likely that the preparations studied came from diploid or tetraploid plants. The tentative nature of the cytology is indicated in later chapters by regarding samples as "likely" diploid or tetraploid. This also takes account of the possibility that the chromosome number of progeny need not reflect that of the parental tree from which seed were collected.

Figure 3.1 A diploid example, Tree C18.



1μm

Interpretative drawing.

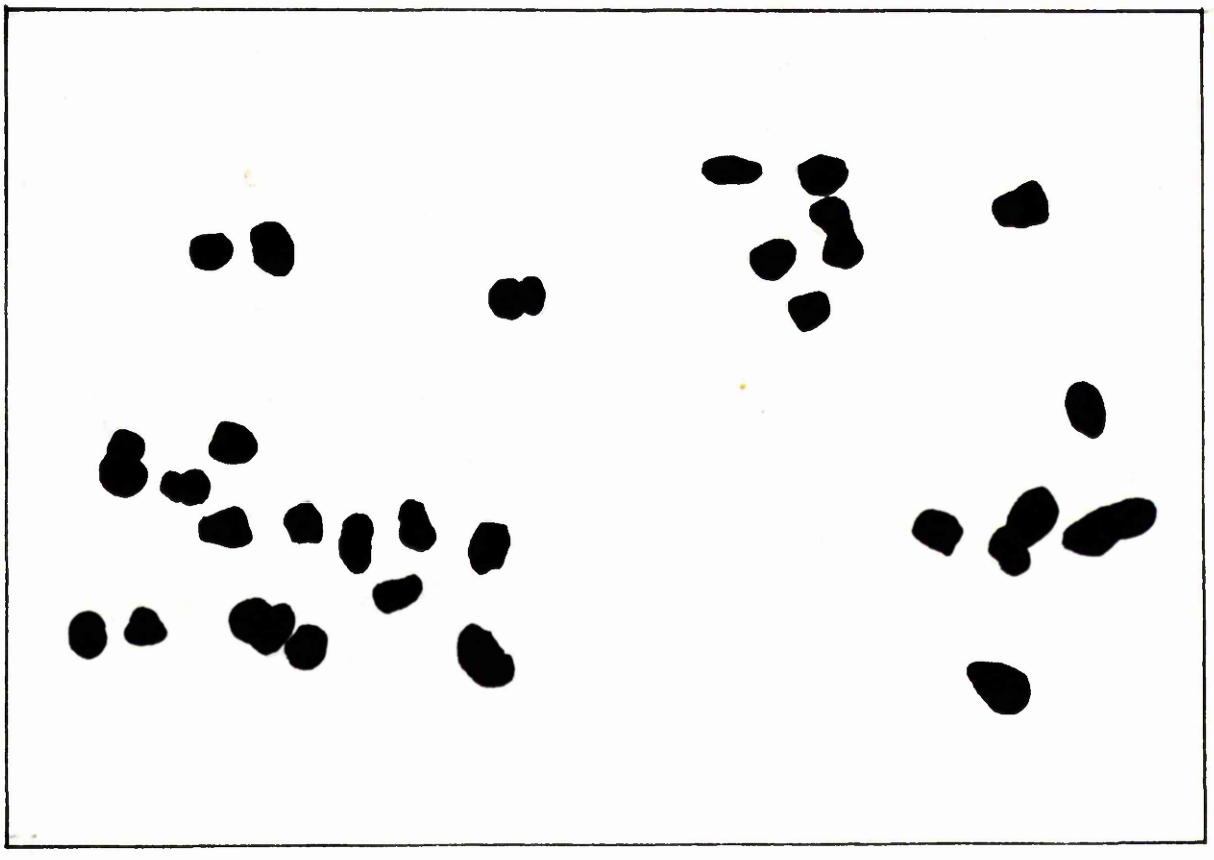
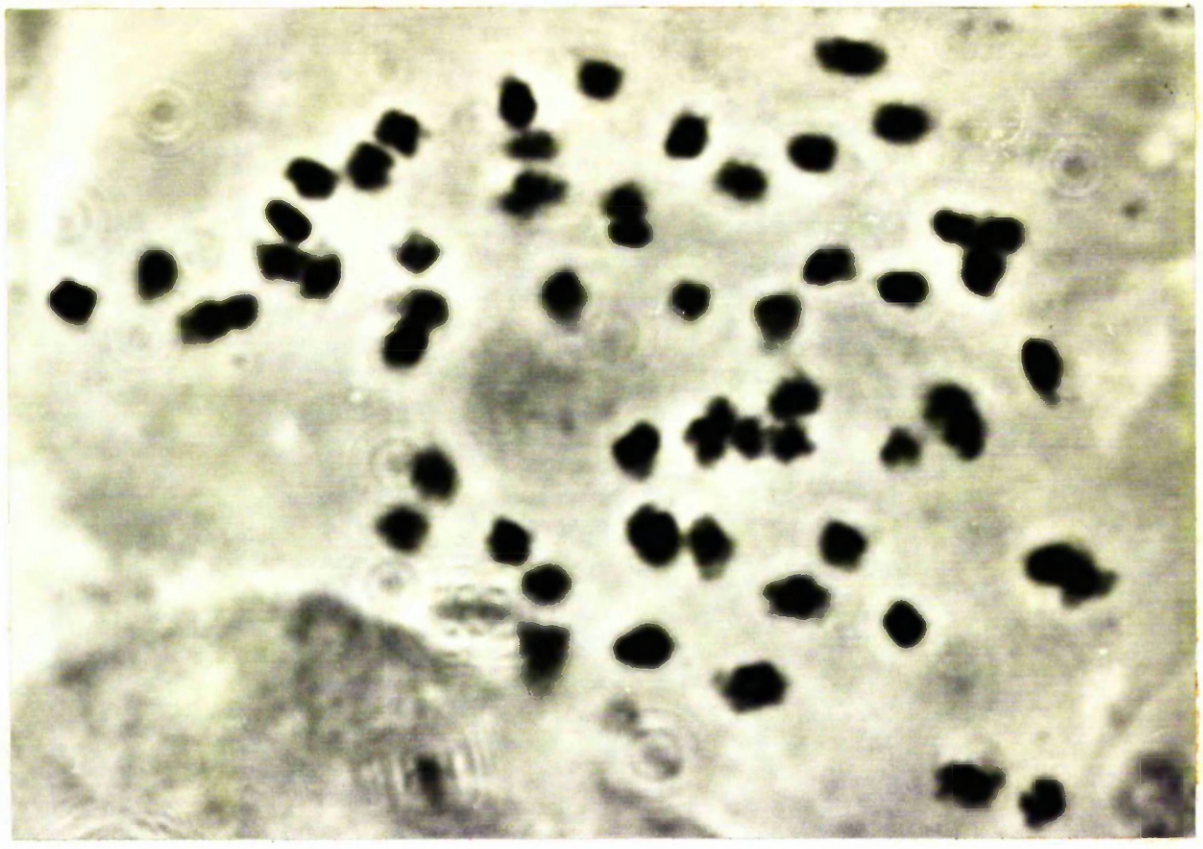
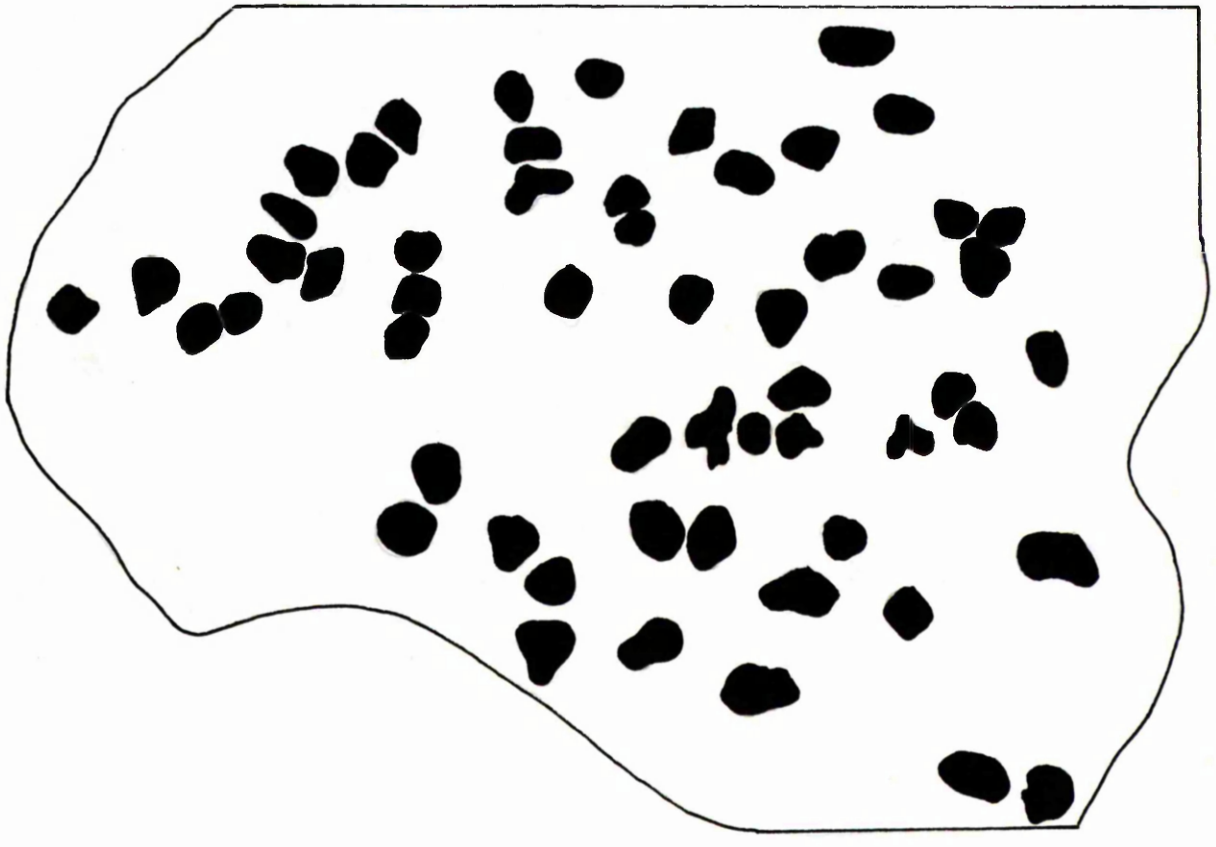


Figure 3.2 A tetraploid example, Tree C30.



1μm

Interpretative drawing.

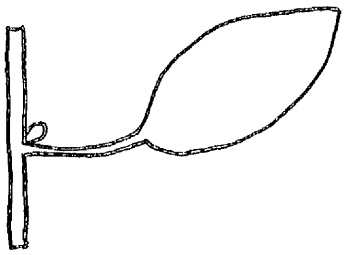


3.3(a) The culture of cuttings and cytology of root-tips.

Cuttings were taken from vegetative shoots and both woody and non-woody branches were used for comparison, the latter type representing the current season's growth. Collections were made between May and August.

In the experiments a cutting comprised a piece of twig cut off between two successive internodes so that a node with associated leaves and buds remained as shown in figure 3.3.

Figure 3.3



Such cuttings were placed in the various culture media in pots or beakers which had been blacked out. Fifty percent of each treatment were dipped in either hormone rooting powder or Indole-butyric acid at a concentration of 1000 parts per million. The solvent for the I.B.A. was 50% alcohol. (I.B.A. was suggested by John Pelham in a personal communication. He has had success with this method at the Institute of Tree Biology in Edinburgh). The plants, when fully prepared, were given a sixteen hour photoperiod, light being provided by fluorescent tubes. It was found to be most important to maintain the cuttings in a humid atmosphere and this was conveniently achieved by enclosing the pots or beakers in polythene bags. Culture solutions were changed weekly.

The following culture media were tried and in each case both hormone and non-hormone-treated cuttings were used.

1) Tap water alone ie. a control.

2) Media recommended by Ingestad (1970, 1971) for the growth of birch

seedlings. The composition of the stock solutions is shown in table 3.2 which is reproduced from the original papers.

Table 3.2

Compound	Concentration in solution (g per litre).	
	B.	C.
HNO_3		1.6
NH_4NO_3	106.2	
KNO_3	37.2	
KH_2PO_4	28.6	
K_2SO_4	22.2	
$Ca(NO_3)_2$		14.3
$Mg(NO_3)_2$		26.0
$Fe_2(SO_4)_3$		2.50
$MnSO_4$		0.55
H_3BO_3		0.57
$CuCl_2$		0.032
$ZnSO_4$		0.036
Na_2MoO_4		0.007

In practice only B plus C combinations were tried. In view of the fact that solutions were changed weekly the Ingestad media for pH adjustment were not considered necessary.

3) Vitafeed solutions with a high nitrogen and potash content and also containing chelated trace elements. The elements Magnesium, Manganese, Iron, Zinc, Copper, Boron and Molybdenum were all present in the medium. A dilution of 1:200 v/v was made up as a stock solution according to the data sheet provided by Vitax Ltd.

4) Moist Sphagnum was wrapped around the cut surface of the cutting and a square of polythene was tied round the moss to keep it moist.

5) Solutions were made up from tablets supplied by Messrs. Griffin and George. The tablets are based on a Hoagland formula, the concentrations of the various compounds being shown in table 3.3.

Table 3.3

Compound	Concentration in solution (g per litre).
$NH_4H_2PO_4$	0.50000
$Ca(NO_3)_2$	0.50000
KNO_3	0.50000
$MgSO_4$	0.50000
H_3BO_3	0.01140
$MnCl_2 \cdot 4H_2O$	0.00725
$ZnSO_4 \cdot 7H_2O$	0.00080
$CuSO_4 \cdot 5H_2O$	0.00032
$HMoO_4 \cdot H_2O$	0.00008

6) Peralite watered with tap water.

3.3(b) The results of the culture experiments and the cytology of roots.

Only cuttings taken in early May produced callus and roots in any of the treatments. Cuttings taken at later times produced callus but no roots. The production of callus proceeded with or without hormone treatment but roots only appeared on certain plants dipped in hormone. Two treatments led to successful root production. These were 1) I.B.A.-dipped cuttings grown in water culture (tap water), 2) Hormone rooting powder-dipped cuttings grown in peralite and watered with tap water. In both cases roots were evident within four weeks. There was no detectable difference in the ability of woody and non-woody shoots to produce roots. Several cuttings were lost due to fungal infection and it is possible that the refinement of sterilising against infection with eg. hypochlorite could improve the success rate. Certainly in cases where cuttings had been surface-sterilised, fungal infection appeared to be less marked.

The fact that cuttings taken after May produced no roots and showed no bud development suggests that a dormancy effect may be involved.

Table 3.4 summarises the results of the root-culture experiments.

Table 3.4 Results of the root-culture experiments.

		Cuttings collected -			
		Early May	June	July	August
I.B.A. or rooting powder.	Tap water	Roots	X	X	X
	Ingestad			X	X
	Vitafeed			X	X
	Sphagnum	X	X	X	X
	Hoagland	X	X	X	X
	Peralite	Roots	X	X	X
No hormone.	Tap water	X	X	X	X
	Ingestad			X	X
	Vitafeed			X	X
	Sphagnum	X	X	X	X
	Hoagland	X	X	X	X
	Peralite	X	X	X	X

A cross denotes that a treatment was tried but no roots were produced. "Roots" indicates successful treatments.

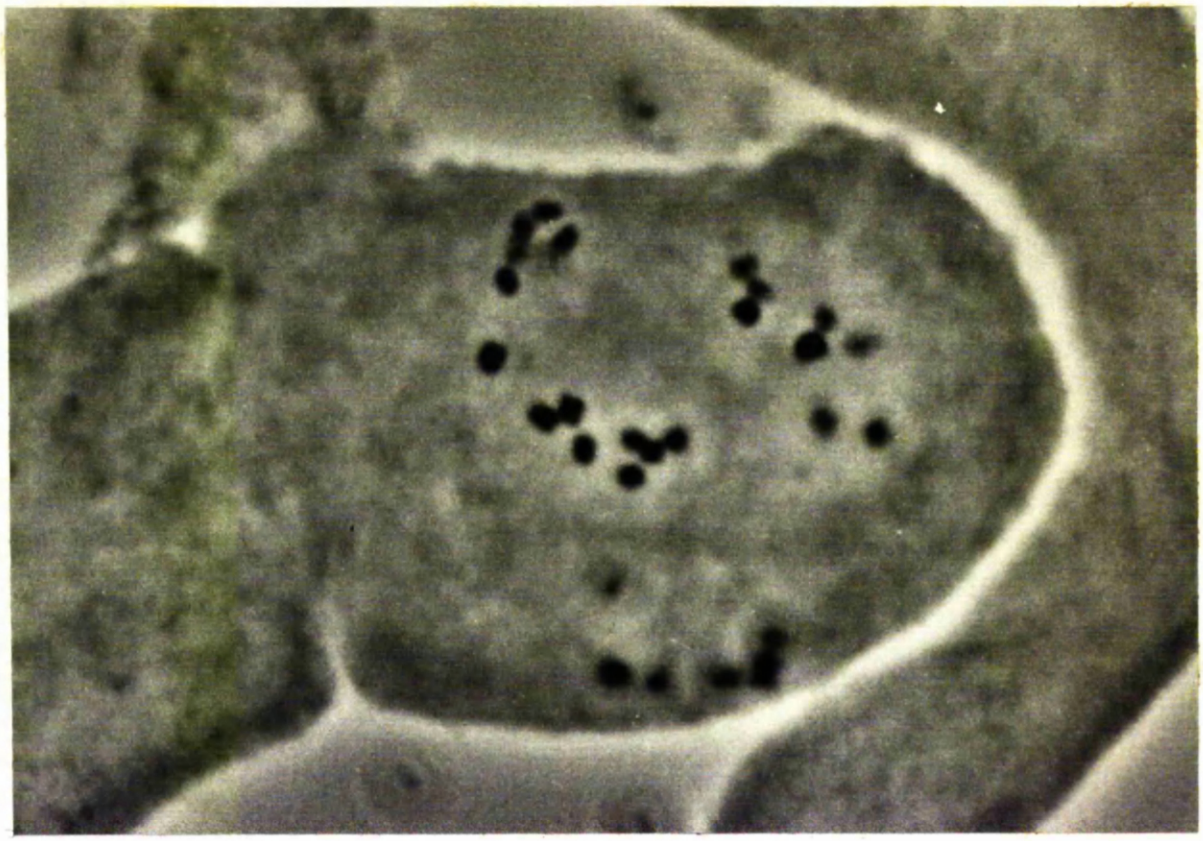
Attempts over a period of three years to produce rooted cuttings for cytology proved to be unsuccessful in a majority of cases. The success in relatively simple media containing tap water is perhaps surprising when balanced media such as Hoagland did not encourage rooting. It should be noted, however, that both the Ingestad medium and Vitafeed were used only in cases when cuttings were taken late and the failure to produce roots does not necessarily reflect on the suitability of the solutions as media. It was disappointing to have rather less cytological preparations from this part of the work than planned for comparison with those obtained from seedlings. In the few successful cases it was found that the chromosome number obtained from Milngavie rooted cuttings was the same as that obtained from the root-tips of germinated seeds, for a given plant.

Cuttings were taken from trees at Drumclog Moor which produced no seed during the present studies. In this way sterile hybrids could be detected. Some of the cuttings rooted successfully using methods

already described. During examination of squash preparations from one of the rooted cuttings from a non-seed-bearing tree an interesting situation was discovered. Two cells which were close together in a root-tip were found to have different chromosome numbers. The cells are shown in figures 3.4 and 3.5. In figure 3.4 one chromosome is slightly out of focus in the photograph and is indicated in the interpretative drawing by a dotted line. This cell has a chromosome number of 28. Although the cell in figure 3.5 does not have an unambiguous chromosome number it contains more than 50 chromosomes. It is perhaps significant that only hormone-treated cuttings successfully rooted and hence provided cytological preparations since there is evidence in the literature (Sharma and Sharma 1965) that hormones induce changes in chromosomes. This raises interesting problems as to possible effects of hormones on root cells of birch.

In the studies of rooted cuttings from non-seed-bearing birch no chromosome numbers of 42 were found.

Figure 3.4 Root-tip cell counted as having 28 chromosomes.



1μm

Interpretative drawing. The dotted line shows a chromosome just out of focus in the photograph.

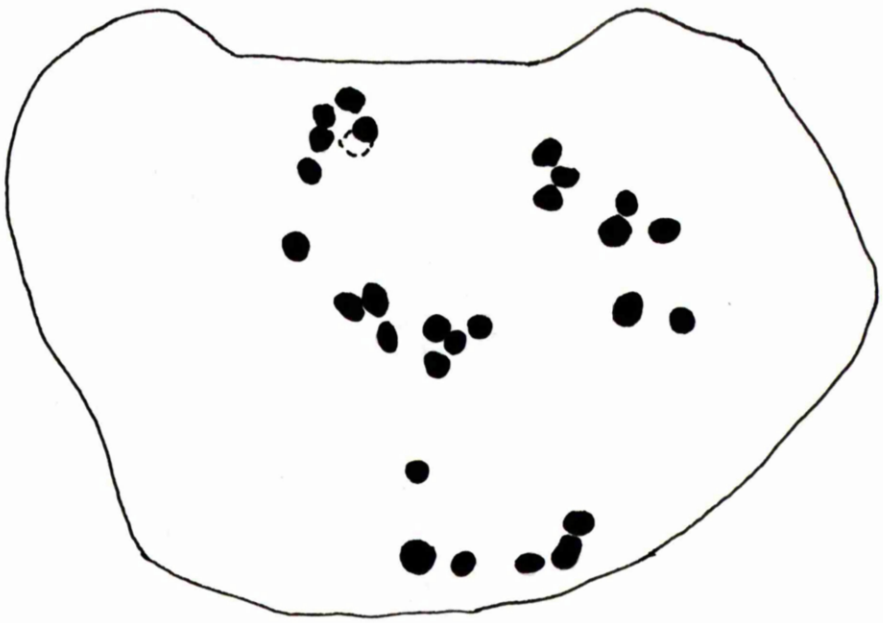
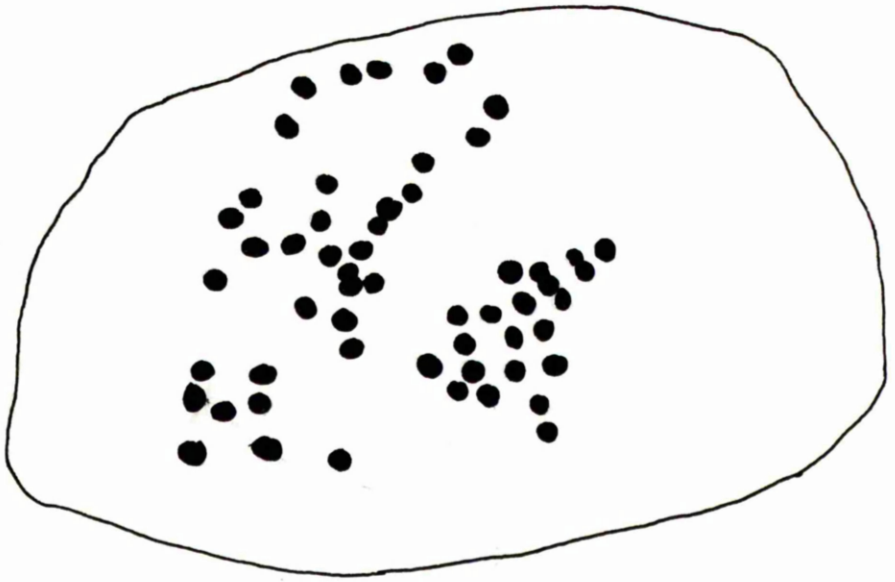


Figure 3.5 Root-tip cell, from same preparation as Fig. 3.4, counted as having more than 50 chromosomes.

1μm

Interpretative drawing in which 56 chromosomes are shown. The chromosome number cannot in this case be regarded as unambiguous.



3.4 Conclusions of the cytology.

Root-tip-squash preparations of good quality may be prepared by the method described. In many cases, however, the small size and large number of chromosomes make accurate counting extremely difficult. Consequently, the present studies do not provide persuasive evidence for aneuploids. Preparations from which chromosome numbers other than 28 or 56 were obtained could be described as ambiguous.

No cells with a chromosome number of 42 were observed which suggests that no F_1 progeny, formed by hybridisation involving haploid gametes of B. pendula and B. pubescens, were in the samples. Progeny with the tetraploid chromosome number could have formed from fusion of unreduced gametes but, in the absence of chromosome counts from parental material, no supporting evidence has been obtained. In those cases where the chromosome number of both progeny and parent was known they were the same but as already pointed out in this Chapter the success rate in rooting experiments was disappointingly low.

Cytology provided information which enabled the classification of trees as likely diploid or tetraploid. In view of the ambiguous nature of some preparations and the possibility that progeny might have a different chromosome number from parents, trees could not be described as certain diploids or tetraploids in many cases.

The information obtained in the cytology allowed examination of the morphological variation in likely diploids and tetraploids and an evaluation of different methods for morphological classification of birch. These studies are the subject of Chapter 4.

CHAPTER 4.

Chapter 4.

The Morphology of Birch.

4.1 Introduction.

In Chapter 1 it was stated that the phenotypic variation in birch led to taxonomic difficulty and to differences in opinion as to the interpretation of the variation.

A large number of characters, of apparent taxonomic value in the genus Betula, are quoted in the literature.

Jentys-Szaferowa (1949-51, 1952, 1955, 1959) listed sixteen leaf characters which she used to distinguish species collectively called B. alba L. These are noted in table 4.1.

Table 4.1 Characters studied by Jentys-Szaferowa.

1) Petiole length.
2) Blade length.
3) Blade width.
4) Number of pairs of lateral nerves.
5) Distance of first tooth from blade base.
6) Number of teeth between tips of second and third nerves.
7) Distance between tips of second and third nerves.
8) Ratio of blade length to petiole length.
9) Ratio of blade length to blade width.
10) Mean distance between nerves.
11) Ratio of blade length to distance of first tooth.
12) Position of broadest part.
13) Angle of second nerve.
14) Base angle.
15) Apical angle.
16) Number of leaves on a shoot.

Natho (1959) used the criteria listed in table 4.2 to distinguish birch taxa.

Table 4.2-Characters used by Natho to distinguish birch taxa.

1) Warts on young twigs.
2) Pubescence of young twigs.
3) Pubescence of petiole.
4) Pubescence of leaves.
5) Contrast of nerves on the underside of leaves.
6) Texture of leaf eg. tough.
7) Leaf apex.
8) Leaf dentition.
9) Leaf shape.
10) Pubescence of bud scales.
11) Resinous leaf buds.
12) Bark colour.
13) Crown of tree.
14) Female catkins pendulous.
15) Catkin length.
16) Height.
17) Petiole length.
18) Waxy warts on leaves.
19) Number of nerve pairs.
20) Number of layers of palisade cells.
21) Epidermis/ stomata cells.

Fruit characters have been examined using a method similar to the one devised by Jentys-Szaferowa (1959). Bialobrzeska and Truchanowiczowna (1960) listed the characters in table 4.3 as having taxonomic value.

Table 4.3-Fruit characters used by Bialobrzeska and Truchanowiczowna.

1) Length of achene.
2) Width of achene at broadest.
3) Distance between tips of wings.
4) Angle of top and bottom of achene.
5) Length of broadest part of achene from base.
6) Position of wings in relation to achene.
7) Ratio of length to width.
8) Ratio of width wing to width achene.
9) Angles of wings.

The characters listed in tables 4.1 to 4.3 have been used in the taxonomy of birch in general, not purely for tree birch. Clapham, Tutin and Warburg (1962) described the characters of British tree birch as shown in table 4.4.

Table 4.4 The descriptions of B. pendula and B. pubescens given by Clapham, Tutin and Warburg (1962).

B. pendula	B. pubescens
1) Bark smooth, silvery white, peeling, changing to black and fissured into rectangular bosses at base.	1) Bark smooth, less silvery often brownish, sometimes grooved at base, no bosses.
2) Branches more or less pendulous.	2) Branches not pendulous, usually.
3) Twigs glabrous, brown, with pale warts.	3) Twigs more or less pubescent or glabrous, with or without brown resinous warts.
4) Leaves 2.5cm, glabrous, ovate-deltate, acuminate, truncate or broadly cuneate at base, doubly serrate with prominent primary teeth curved towards the apex.	4) Leaves 1.5-5.5cm, very variable in shape, sub-acute or acute, rounded or cuneate at base, coarsely and sometimes irregularly serrate, teeth not curved towards apex, pubescent.
5) Scales of fruit with short broad cuneate base, lateral lobes broad spreading and curving down, middle lobe deltate, blunt.	5) Scales cuneate at base, lateral lobes rounded or nearly square, more or less spreading or ascending, middle lobe long or short, narrow, oblong or triangular-lanceolate.
6) Wings of fruit 2-3 times as broad as achene, upper edge surpassing stigmas.	6) Wings 1-1.5 times as broad as achene, upper edge not surpassing stigmas.
7) 2n=28.	7) 2n=56.

In nature, however, these characters are not always distinct and birch trees with an appearance intermediate between B. pendula and B. pubescens are often found, as noted in Chapter 1.

The range of variation in tree birch of known chromosome number is not clear and as a result it is difficult to state the taxonomic position of "intermediates".

In the literature it has been suggested that B. pubescens is more variable than B. pendula (Jentys-Szaferowa 1950). It must be noted, however, that in several studies morphological criteria have been used to identify species, the specific ranges in variation of these criteria have then been calculated, and these same species have been used as a comparative taxonomic unit. Having previously used certain characters to identify trees as either B. pendula or B. pubescens, it is difficult to see how the range of variation of these characters may be objectively obtained.

It is not clear, therefore, what the limits of variation are for tree birch using morphological data alone. The situation theoretically may be one involving two taxa which are equally variable and which are distinct from one another and from their hybrids. Another interpretation may explain the same situation as one where a taxon varies little, the other taxon is most variable, and trees otherwise thought to be hybrids are included in the range of variation of the more variable taxon.

4.2(a) Materials and methods.

The herbarium collection was examined to provide information on the variation within tree birch found in the British Isles, and on the taxonomic treatment given to the specimens.

Material from Drumclog Moor, Milngavie and from Dunblane, collected as described in Chapter 2, was examined. A range of characters was selected as detailed later in this chapter and measurements were made. The information collected was incorporated in scatter diagrams and was analysed by a computer, both methods seeking to identify groups of trees with similar characters. Since leaf and fruit characters were used both separately and together to form groupings it was possible to compare the classifications thus obtained. The naming of groups as"B. pendula" type, "B. pubescens" type, or"intermediate" was a tentative classification only and was included to compare the taxonomic position of trees classified on morphology or cytology evidence alone with that obtained using all criteria.

Characters selected for examination were as follows-

1) Bark and branch characters.

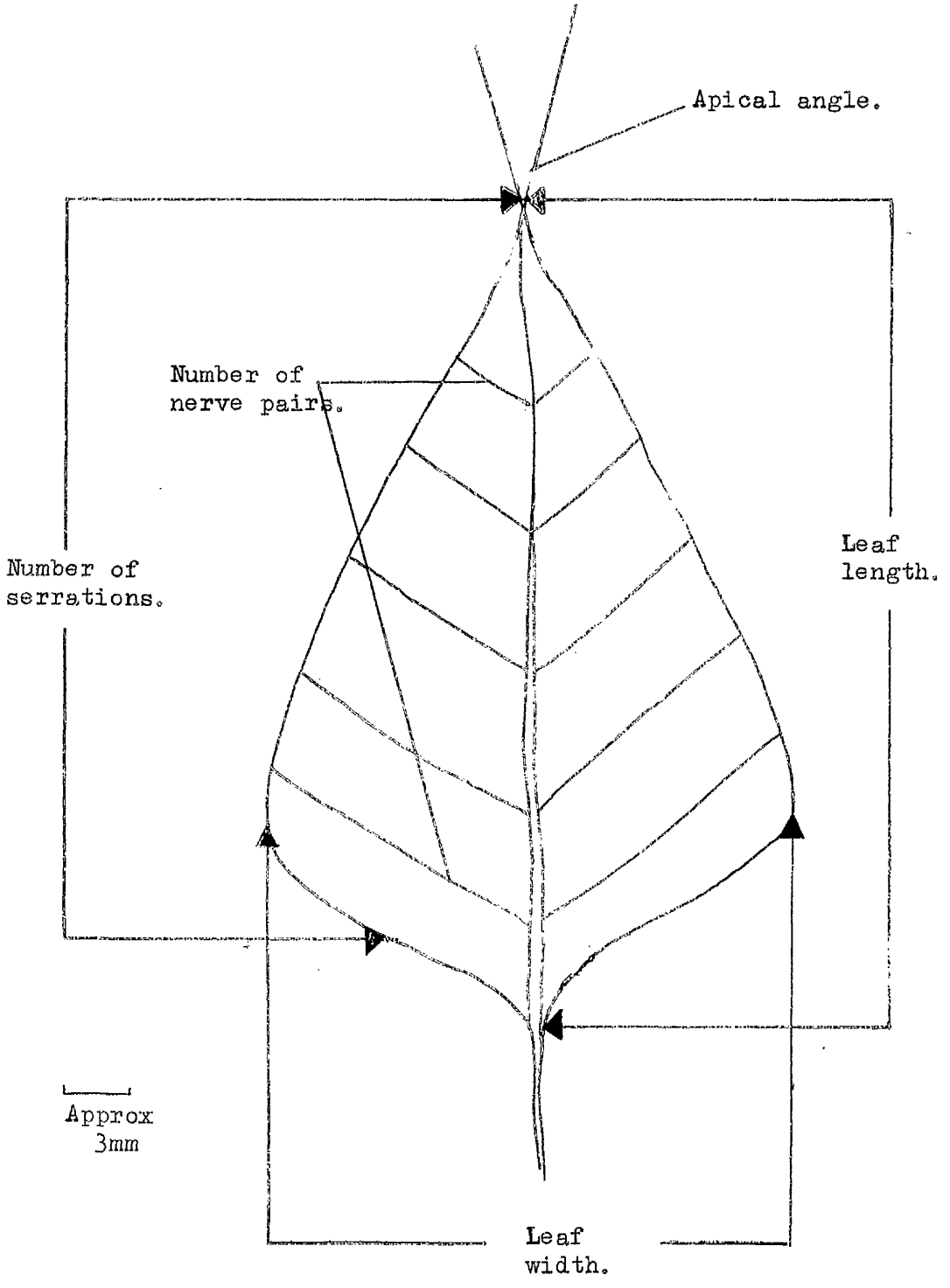
The colour of bark and the presence or absence of black rectangular bosses were noted in the field. The presence or absence of pendulous branches was recorded.

2) Leaf characters.

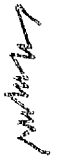
Leaf measurements were taken from dwarf vegetative shoots which had been growing near the crown of trees. The specimens were pressed in all cases.

For each tree fifteen leaves, taken at random, were examined. The type and number of serrations, leaf length, leaf width (at broadest), ratio of leaf length to leaf width and the apical angle were noted. Figure 4.1 illustrates the characters studied.

Figure 4.1 Leaf characters.



Doubly serrate.



Irregularly serrate.

3) Fruit and catkin characters.

Fruits were taken at random from bulk samples and in view of their size they were laid out between two strips of sellotape which held them flat and in place. With the aid of a dissecting microscope, fifteen bracts and fruits were measured on each strip. Characters examined were the width of one wing at its broadest part, the width of the achene excluding wings, the length of the achene, the pubescence at the stigmas, the length of the stigmas in relation to the wings, and the bract length. The ratio of length achene to width achene and the ratio of wing width to achene width(excluding wings) were calculated.

The lengths of fifteen catkins per tree, sampled at random, were measured.

Figure 4.2 illustrates the characters examined.

A full summary of the characters selected for study is given in table 4.5.

Figure 4.2 Fruit and catkin characters.

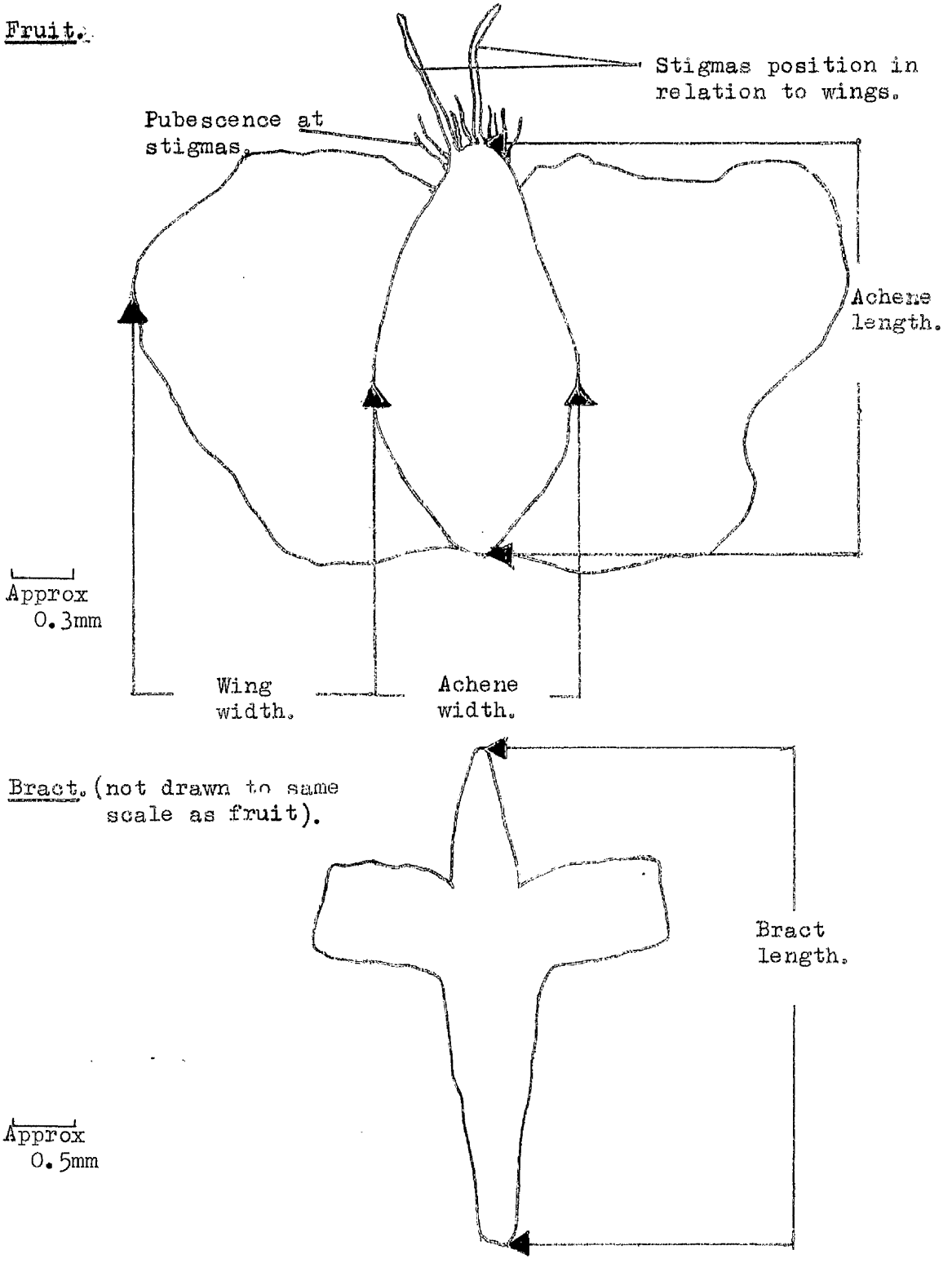


Table 4.5 The characters measured and the ratios calculated.

Bark	Colour-Brown, silver etc. Smooth, fissured etc. Change at base to black rectangular bosses or not.
Branches	Pendulous or not.
Leaves	Number of serrations on one side. Doubly or irregularly serrate. Number of serrations between nerves two and three. Leaf length in cm. Leaf width (at broadest) in cm. Apical angle °. Calculated ratio of leaf length to leaf width.
Fruit	Width of one wing (at broadest) in mm. Width of achene (at broadest) in mm. Length of achene in mm. Stigmas surpassing wings or not. Pubescence at stigmas. Calculated ratio of achene length to achene width. Calculated ratio of wing width to achene width.
Catkin	Length of catkin in cm. Length of bract in mm.

Character means were calculated for every tree from fifteen measurements made. These values were plotted on scatter diagrams and groupings were sought with particular note being given to "morphological intermediates". Groups were thus formed graphically, not on the basis of an identification of a tree as one species or another.

4.2(b) Results.

1) Bark and leaf measurements.

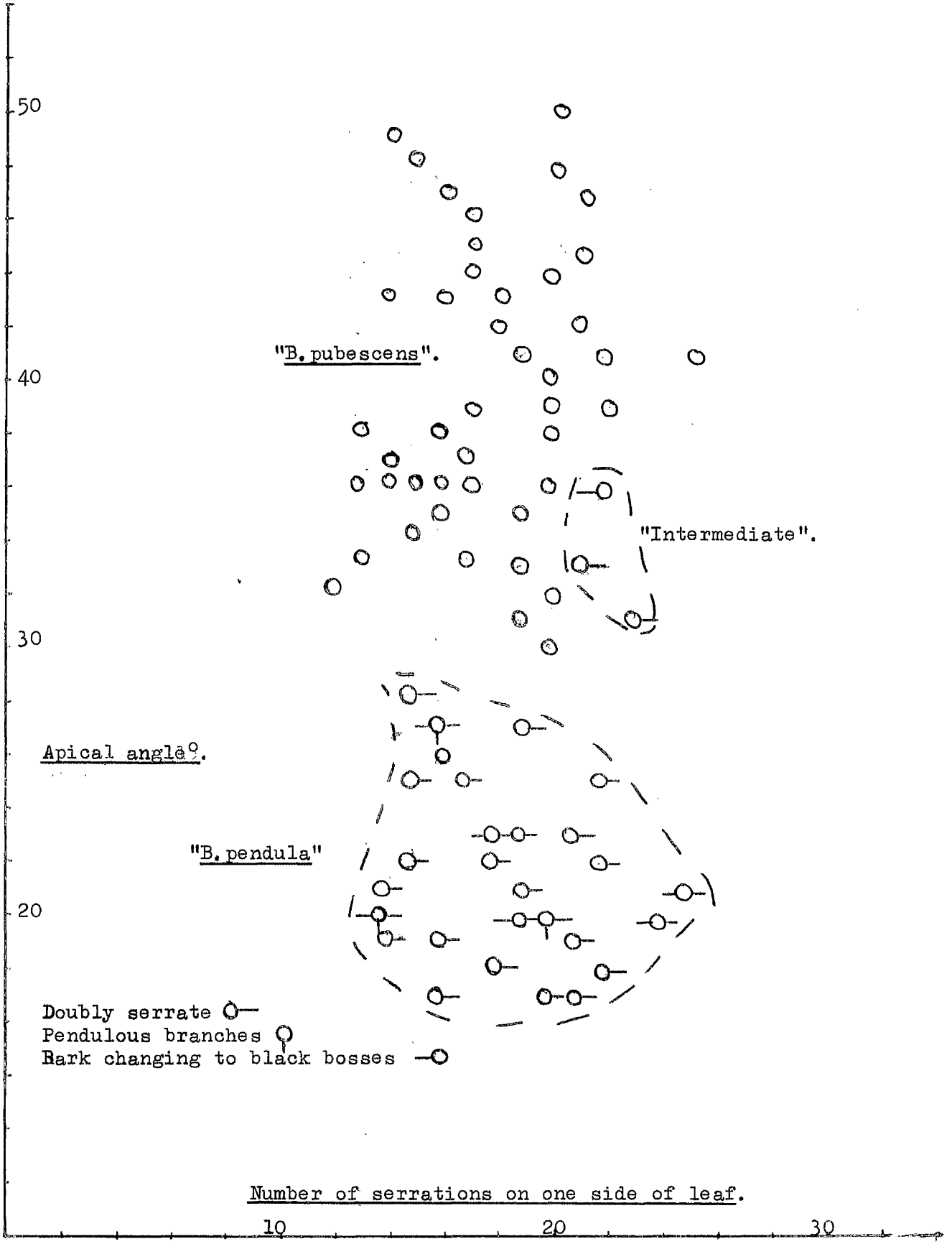
A scatter diagram of measurements from Milngavie samples is shown in figure 4.3, bark, branch and leaf characters being included. If the diagram is examined without preconceived ideas about groupings, the following points emerge. The use of apical angle of leaves on one axis separates out the group circled between about 20° and 30° within which trees tend to have doubly-serrate leaves. Only trees in this group have pendulous branches, but the character is not widespread. Individuals with bark changing to black bosses at the base are found in this group, with one exception. The range of number of serrations on leaves is about the same for this group of trees as for the other individuals graphed. One tree does not have bark type and leaf serrations like the others in the group, which in general have attributes of B. pendula as described by Clapham, Tutin and Warburg (1962). These trees are henceforth referred to as the "B. pendula" group, the use of inverted commas denoting a tentative classification made by the present author.

The trees outwith the "B. pendula" group have characters associated with B. pubescens (Clapham, Tutin and Warburg 1962), with three exceptions, and are henceforth referred to as the "B. pubescens" group.

Three trees circled have leaf serrations and bark type normally found in B. pendula according to Clapham, Tutin and Warburg (1962), but have leaf apical angles closer to those of the "B. pubescens" group. In view of the fact that these trees are morphologically intermediate for the characters studied, they are tentatively described as "intermediates".

It is interesting to compare the leaf morphology of trees which are classified in the herbarium collection with figure 4.3 in order to find the ranges of variation in the taxonomic groupings formed in two different ways. The naming of specimens from the collection is that used by

Figure 4.3 Milngavie samples-tentative groupings.



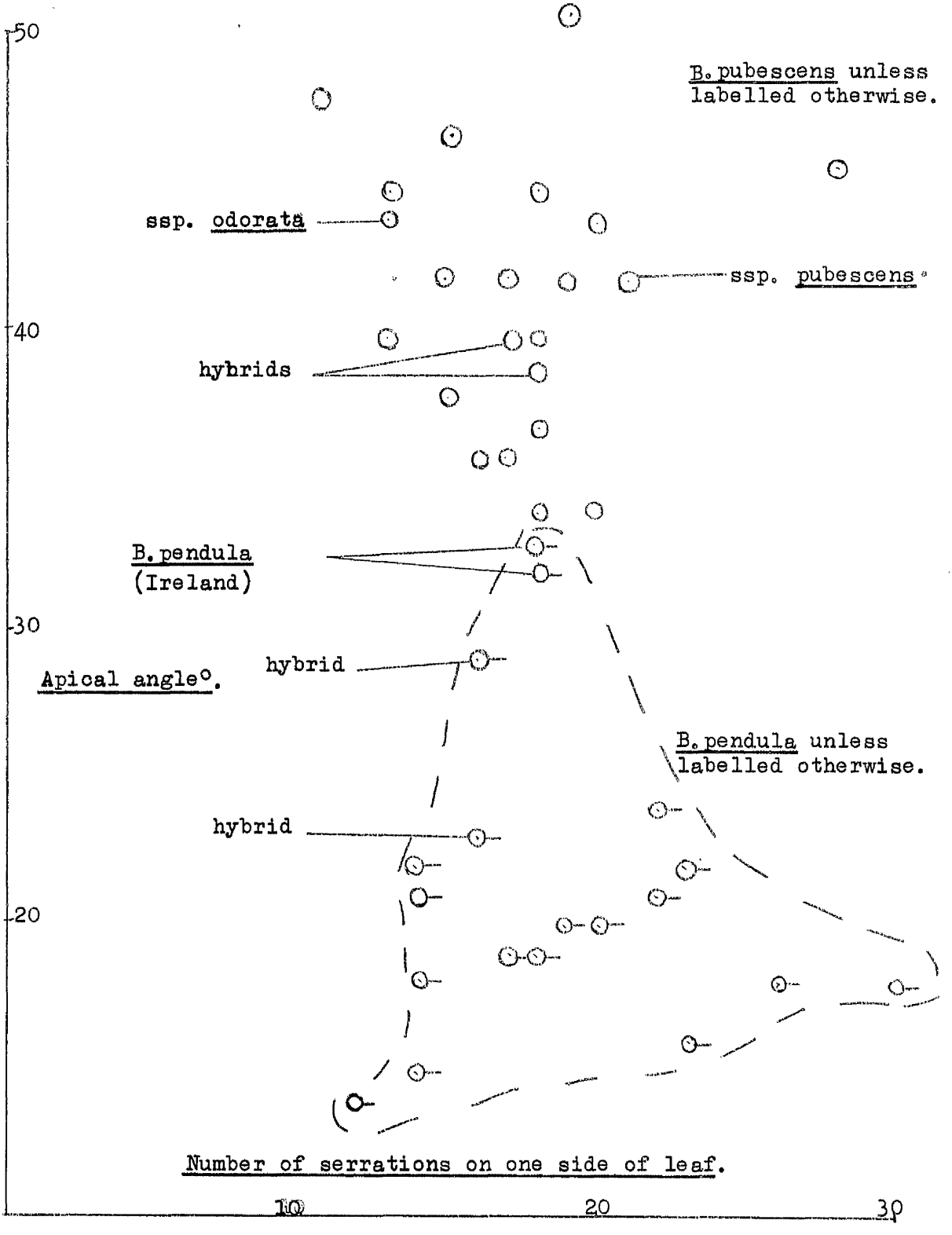
the Royal Botanic Garden, Edinburgh.

Figure 4.4 is a scatter diagram of leaf measurements from some of the herbarium specimens which shows their range of variation. In the case of specimens labelled B. pendula, the apical angles are typically smaller than those from Milngavie leaves, but most fall within the angle range of the " B. pendula" group from that sample. No data on bark or branch characters could be obtained in most cases from herbarium material. The position of the Irish herbarium specimens labelled B. pendula shows that their leaves have rather large apical angles. The diagram also indicates that generally B. pubescens herbarium specimens are distinct from B. pendula ones, based on measurements of leaf characters. Specimens labelled ssp. odorata and ssp. pubescens fall within the range of variation of B. pubescens samples from the herbarium collection. The hybrids from the collection fall within either of the two groups B. pendula or B. pubescens and do not occupy an intermediate position as might be expected.

Although sufficient information is not available to draw comparable scatter diagrams from the data of Jentys-Szaferowa (1951) and England (1963), if apical angles are compared, their distribution from England's research on B. pendula and B. pubescens closely resembles that obtained from the scatter diagrams of both the herbarium material and the Milngavie samples. In the case of the European means quoted by Jentys-Szaferowa (1951), the value for B. pendula of 20° would fall within the ranges found in the present work, but the value for B. pubescens of 30° would be lower and outwith the ranges of samples of trees resembling that species.

Figure 4.4 Leaves from the herbarium collection.

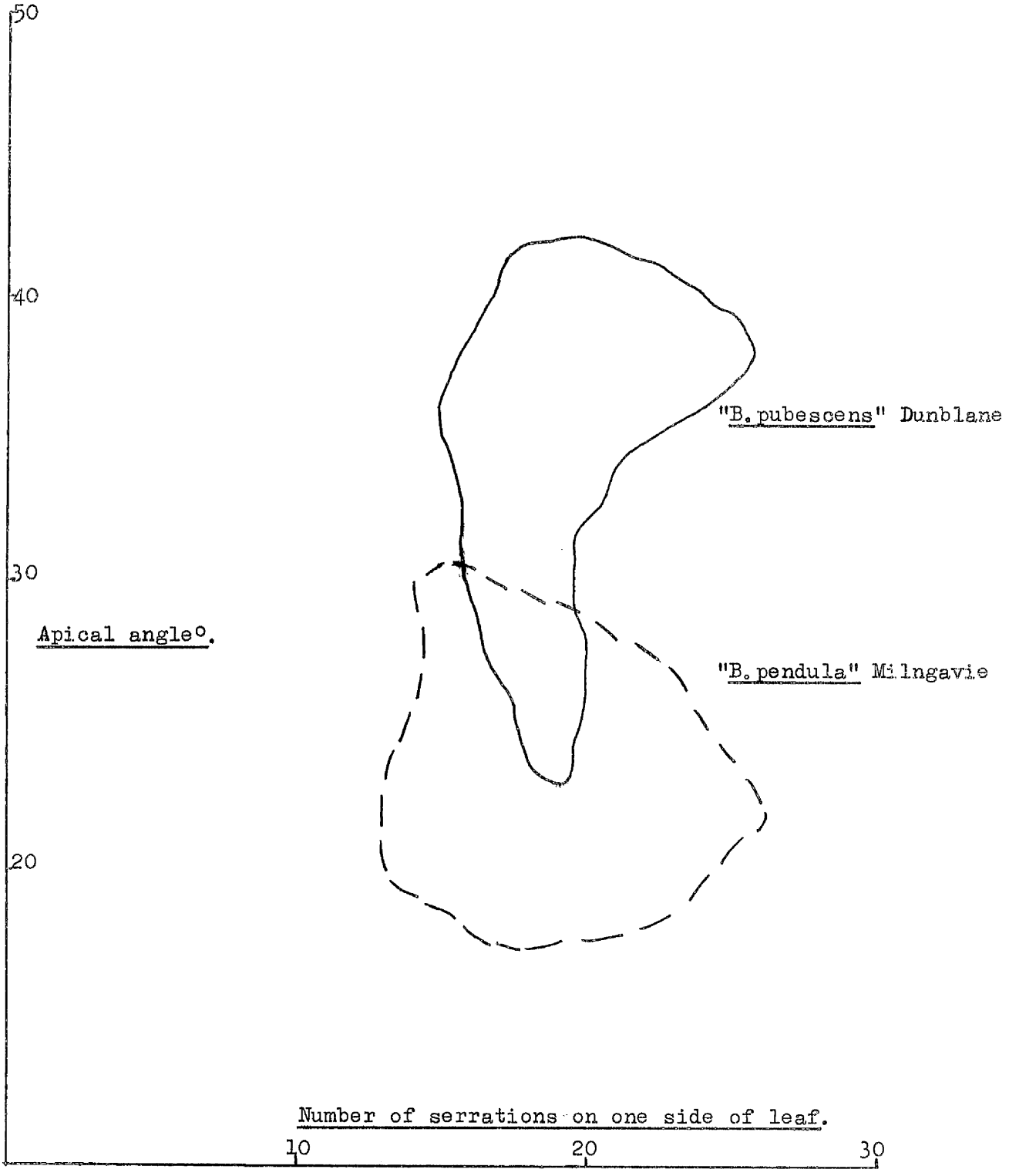
All names are taken from the herbarium specimens.



A scatter diagram based on bark, branch and leaf characters of Dunblane samples was prepared in the same way as for Milngavie ones. The distribution of points and comparison with the descriptions given by Clapham, Tutin and Warburg (1962) suggested the tentative naming of these as "B. pubescens" types. The distribution of measurements is compared with those obtained from Milngavie samples in figure 4.5. For simplicity the individual points are omitted and only the distribution of Milngavie "B. pendula" types is shown.

The apparent overlap shown in figure 4.5 results from the fact that Dunblane "B. pubescens" types tended to have smaller apical angles than those from Milngavie. Such a situation is open to different interpretations. It is possible to attribute the overlap to the variability of "B. pubescens", or alternatively the situation could be taken to result from introgression of "B. pubescens" by "B. pendula".

Figure4.5 Comparison of leaf measurements from Milgavie and Dunblane samples.



2) Catkin and fruit measurements.

A scatter diagram of measurements of Milngavie samples is shown in figure 4.6. Trees which have little or no pubescence on their fruits and have a high ratio of wing width to achene width are grouped. These trees also have stigmas not surpassing the wings of the fruit. The group has attributes of B. pendula as described by Clapham, Tutin and Warburg (1962), and as such is henceforth tentatively called the "B. pendula" group. There is, however, no sharp boundary between this group and the other which has attributes of B. pubescens, as described by Clapham, Tutin and Warburg (1962). Quite a number of trees in the latter group produced fruits which had similar width wing to width achene ratios and catkin lengths to the "B. pendula" group, but had stigmas surpassing wings and fruits which were markedly pubescent.

Two plants produced fruits which had stigmas not surpassing wings, but which had markedly pubescent areas.

For Milngavie trees table 4.6 was composed in order to compare the classifications obtained using leaf and fruit criteria separately. The first column lists those trees classified as "B. pendula" from the leaf scatter diagram and the second column those outwith the group which had certain characters associated with B. pendula according to Clapham, Tutin and Warburg. To simplify matters most tree numbers are omitted from the "B. pubescens" column since they are the remaining trees not mentioned in the other two columns.

The second set of columns similarly deals with the groups formed on the basis of fruit measurements.

The third set of columns is for later reference.

Figure 4.6 Milngavie sample fruit measurements.

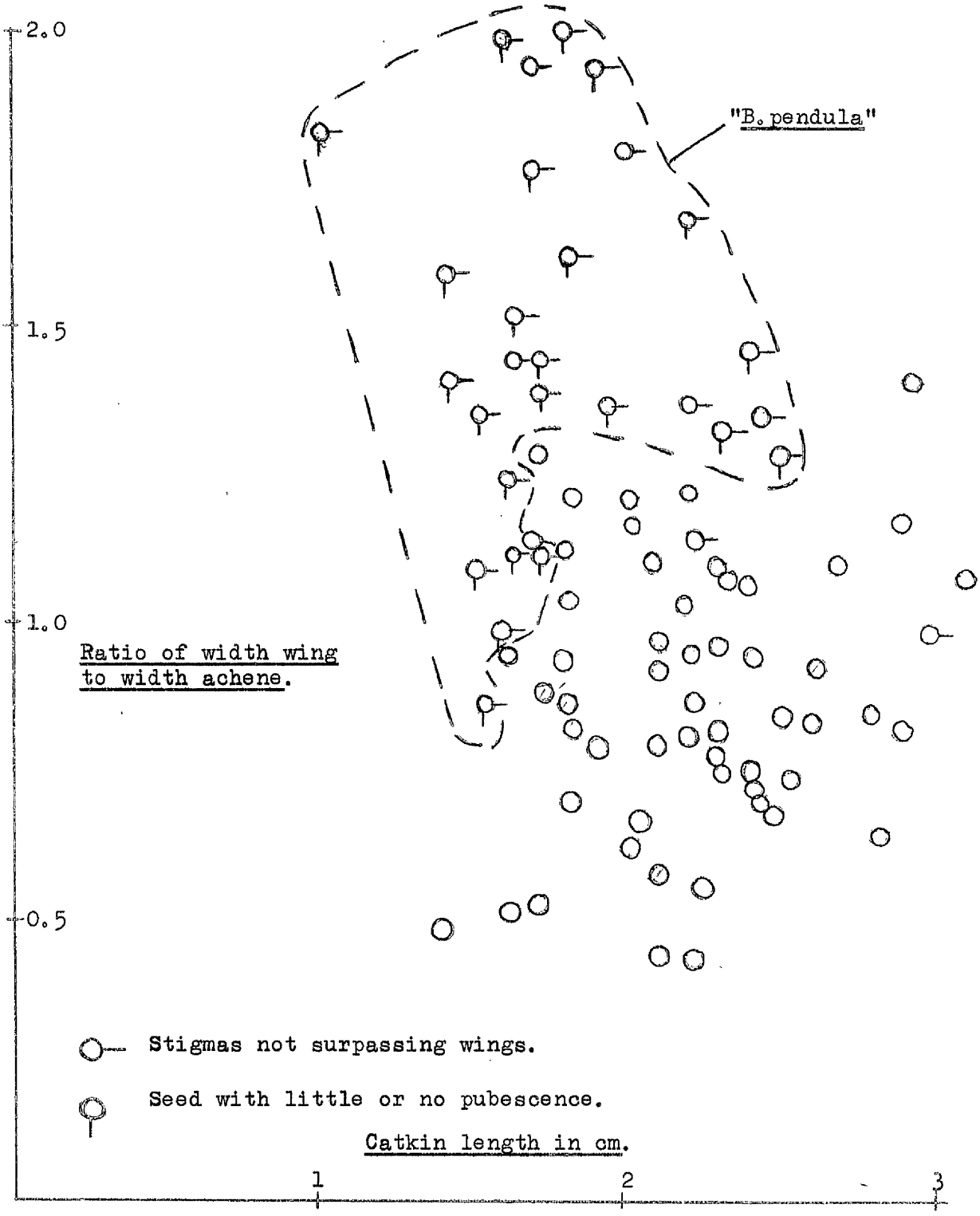


Table 4.6 Comparison of groups formed using different morphological criteria.

Leaf characters			Fruit characters			Both leaf and fruit		
"B. pen."	"Inter."	"B. pub."	"B. pen."	"Inter."	"B. pub."	"B. pen."	"Inter."	"B. pub."
	A3		A3				A3	
A4			A4			A4		
	A4A		A4A			A4A		
A9			A9			A9		
A10			A10			A10		
A11			A11			A11		
A14			A14			A14		
A15			A15			A15		
A16			A16			A16		
		A17		A17			A17	
A18			A18			A18		
A19			A19			A19		
		A24	A24				A24	
B2			B2			B2		
B3					B3		B3	
B4					B4		B4	
B7					B7		B7	
B14			B14			B14		
B15			B15			B15		
B20			B20			B20		
B21			B21			B21		
B23			B23			B23		
C1			C1			C1		
C2			C2			C2		
		C6		C6			C6	
	C8				C8		C8	
C12			C12			C12		
C14			C14			C14		
C16			C16			C16		
C17			C17			C17		
C18			C18			C18		
C20			C20			C20		
C22					C22		C22	
C25			C25			C25		
		C26	C26				C26	
C31			C31			C31		

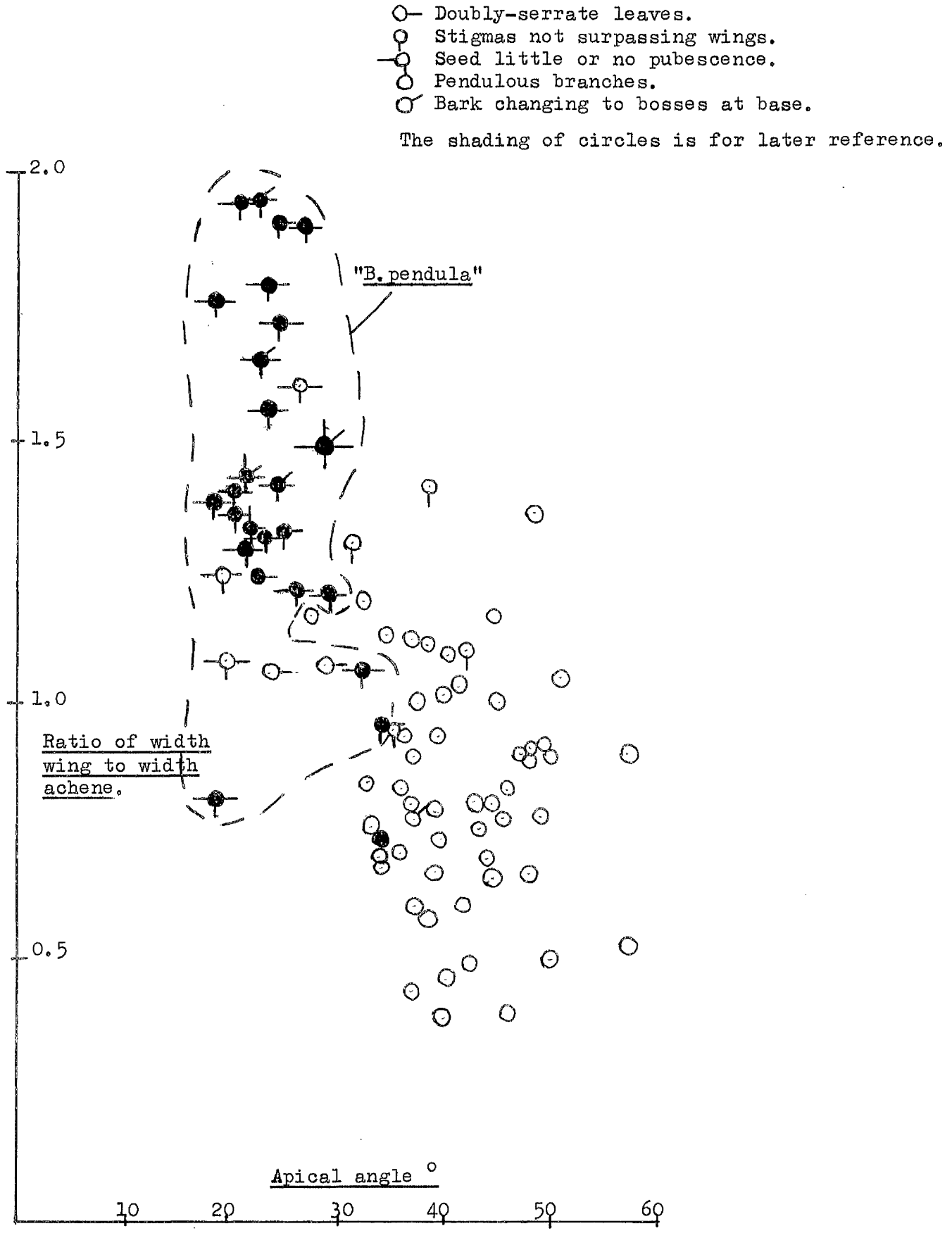
To simplify the table the majority of the "B. pubescens" tree numbers are omitted. They are in fact the remaining trees not listed in any column in the table.

Table 4.6 shows that the majority of trees classified as "B. pendula" on the basis of leaf criteria were also grouped together using fruit characters. Trees A4A and A3 which were placed outside the group formed on the basis of leaf measurements, but which had doubly-serrate leaves, were within the group formed on the basis of fruit measurements. Tree C8 which had bark like B. pendula (Clapham, Tutin and Warburg 1962), but which was placed outside the tentative "B. pendula" grouping from leaf measurements, was placed in the "B. pubescens" group on the basis of fruit measurements. Trees B3, B4, B7 and C22 classified as "B. pendula" on leaf characters, grouped with "B. pubescens"on fruit characters. In the case of A24 and C26 the reverse was the case.

The comparison made suggests that the use of only vegetative characters may lead to a different classification of trees from that made on the basis of reproductive ones, but not in a majority of cases.

An additional scatter diagram was drawn using both leaf and fruit characters of Milngavie trees. This(figure 4.7)gives the information in the third set of columns in table 4.6. Not surprisingly, since the criteria were essentially the same as previously used, the majority of the "B. pendula" group remained unchanged. Trees, which on leaf measurements had appeared in one group and on fruit measurements the other, appeared as "intermediates" having characters of both B. pendula and B. pubescens (Clapham, Tutin and Warburg 1962). For later reference these trees are A3, A17, A24, B3, B4, B7, C6, C8, C22 and C26.

Figure 4.7 Milngavie leaf and fruit measurements.



4.3(a) The use of computer analysis in the morphology.

With the help of Dr. H. Dickinson of the Department of Computing, University of Glasgow, the morphological data were subjected to computer analysis to investigate what groupings could be obtained by such a method. The programs made use of Cluster Analysis which is discussed by Sokal and Sneath (1963). Statistical similarity between trees was measured by comparing characters of each one with all others thus giving a "similarity co-efficient".

On the advice of Dr. Dickinson, Clustan programs (Wishart 1972) were selected. The options used were Hierar-Ward's method and Reloc. Hierar commences by considering each individual as a cluster then combines statistically similar clusters. Program Reloc classifies individuals in a number of clusters then the individual's similarity is compared with all clusters and is relocated within the group with which it has greatest similarity.

The data computed are listed in table 4.7.

As recommended by Sokal and Sneath (1963) logically correlated characters were not used, hence the omission of ratios such as length to width of leaf. In order to permit examination of discrete data such as bark colour, each colour state was given a numerical value for computing purposes. It has been argued that discrete and continuously variable data should not be used to calculate distance co-efficients, but Sokal and Sneath do endorse the use of such a method. In the present study, groupings formed with and without discrete data were found to be identical with only one exception.

4.3(b) Results of computer analysis.

1) Program Hierar.

The results of Hierar-Ward's method, using the sixteen

Table 4.7 The morphological data used in cluster analysis.

Character number.	Character.
1	Width of fruit wing.
2	Width of achene.
3	Length of achene.
4	Bract length.
5	Catkin length.
6	Leaf width.
7	Number of leaf serrations.
8	Leaf length.
9	Apical angle.
10	Number of serrations between nerves two and three.
11	Pendulous or non-pendulous branches.
12	Serrations on leaves double or irregular.
13	Stigmas surpassing wings or not.
14	Hairy or non-hairy fruits.
15	Bark colour.
16	Presence or absence of black bosses on trunk.

variables shown in table 4.7 are incorporated into a dendrogram, figure 4.8. The vertical scale has been modified to allow representation of all data on one page. Since trees were all given a number for computing purposes, table 4.8 is included to show the relationship between these numbers and the tree codes used previously.

Using the dendrogram and table 4.8 together, the three clusters formed when clusters 3 and 4 were fused at similarity co-efficient 12.13 were found to contain the trees listed in table 4.9.

Table 4.8 Correlation of tree and computer numbering systems.

Tree number	Computer	Tree number	Computer	Tree number	Computer
A1	1	E5	31	C14	61
2	2	6	32	15	62
3	3	7	33	16	63
4	4	8	34	17	64
4A	5	10	35	18	65
5	6	11	36	19	66
6	7	12	37	20	67
7	8	13	38	21	68
8	9	14	39	22	69
9	10	15	40	23	70
10	11	16	41	24	71
11	12	17	42	25	72
12	13	18	43	26	73
13	14	20	44	27	74
14	15	21	45	28	75
15	16	22	46	29	76
16	17	23	47	30	77
17	18	C1	48	31	78
18	19	2	49	32	79
19	20	3	50	33	80
20	21	4	51	D1	81
21	22	5	52	2	82
22	23	6	53	3	83
23	24	7	54	4	84
24	25	8	55	5	85
25	26	9	56	6	86
B1	27	10	57	7	87
2	28	11	58	8	88
3	29	12	59	9	89
4	30	13	60		

Table 4.9 Three clusters formed using program Hierar.

Cluster 1		Cluster 2		Cluster 3
A1	C6	A3	C26	A9
2	7	4	31	14
5	8	4A		C12
6	9	10		14
7	10	11		
8	11	15		
12	13	16		
13	15	18		
17	19	19		
21	21	20		
22	23	24		
23	24	B2		
25	27	3		
B1	28	4		
5	29	7		
6	30	14		
8	32	15		
10	33	20		
11	D1	21		
12	2	23		
13	3	C1		
16	4	2		
17	5	16		
18	6	17		
22	7	18		
C3	8	20		
4	9	22		
5		25		

Computer analysis indicated the characters with lowest variation within clusters which could therefore be regarded as "good diagnostics".

For Cluster 1 these characters were 1) leaves not doubly-serrate, 2) no black bosses at the base of the trunk, 3) non-pendulous branches, 4) hairy at the base of the stigmas on the fruit. Trees in Cluster 1 differed markedly from the population as a whole in having 1) irregular leaf serrations, 2) stigmas surpassing wings of fruit, 3) hairs at base of stigmas, 4) large apical angles on leaves.

For Cluster 2 the character varying least within the group was non-pendulous branches. Characters of this Cluster which differed markedly from the population as a whole were 1) doubly-serrate leaves, 2) short catkins, 3) number of serrations between nerves two and three greater, 4) smaller apical angle, 5) broad wings on fruits, 6) stigmas not surpassing wings on fruit, 7) non-hairy fruits.

Cluster 3 trees were all pendulous, with doubly-serrate leaves, stigmas not surpassing wings and small apical angles. They differed from the rest of the population in these characters and in having bark with black bosses, non-hairy fruits, narrow achenes, wide wings on fruits and long bracts.

Cluster 1 trees obviously have attributes of B. pubescens and both Clusters 2 and 3 attributes of B. pendula, as described by Clapham, Tutin and Warburg (1962).

Clusters 2 and 3 differed from each other in that trees in the latter group had pendulous branches, longer bracts, bosses at base of trunk and broader wings on fruits. Otherwise they were very similar.

2) Program Reloc.

The clusters formed using Reloc are listed in table 4.10. The same variables were used as in program Hierar.

For Cluster 1 the main diagnostic characters appeared to be 1) irregular serrations on leaves, 2) hairy fruits, 3) large leaf apical angles, 4) stigmas longer than wings of fruit. The main characters distinguishing Cluster 2 were 1) doubly-serrate leaves, 2) non-hairy fruits, 3) small apical angles, 4) stigmas shorter than wings. Cluster 3 was distinguished on the basis of the same characters as in program Hierar.

4.4 A comparison of the groupings formed by the different methods used in the morphology.

Table 4.6 was prepared from scatter diagrams and three groups were postulated 1) those having characters of B. pendula, 2) those having characters of B. pubescens, 3) those having characters of both. The descriptions given by Clapham, Tutin and Warburg (1962) were used as a basis for assigning trees to the three groups. A total of ten trees was placed in the "intermediate" group.

Table 4.9 was made up of three groups formed by computer analysis (program Hierar). Cluster 2 was found to contain trees tentatively called "B. pendula". If tables 4.6 and 4.9 are compared, (using the data in table 4.6 from the third set of columns), the following points emerge. Trees A4, A4A, A10, A11, A15, A16, A18, A19, B2, B14, B15, B20, B21, B23, C1, C2, C16, C17, C18, C20, C25 and C31 were classified as "B. pendula" from scatter diagrams and from computer analysis (Hierar). Cluster 3 of table 4.9, which separated from Cluster 2 on the basis of branch, bract, bark and fruit wing characters, but which nevertheless had trees with B. pendula characters

Table 4.10 Three cluster formed using program Reloc.

Cluster 1		Cluster 2	Cluster 3
A1	C5	A3	A9
2	6	4	14
5	7	4A	C12
6	8	10	14
7	9	11	
8	10	15	
12	11	16	
13	13	18	
17	15	19	
20	19	B2	
21	21	3	
22	22	14	
23	23	15	
24	24	20	
25	26	21	
B1	27	23	
4	28	C1	
5	29	2	
6	30	16	
7	32	17	
8	33	18	
10	D1	20	
11	2	25	
12	3	31	
13	4		
16	5		
17	6		
18	7		
22	8		
C3	9		
4			

according to Clapham, Tutin and Warburg (1962), contained another four of the "B. pendula" group in table 4.6. These trees were numbers A9, A14, C12 and C14. Trees in table 4.6 occupying an "intermediate" position were placed by the computer in clusters with either attributes of B. pendula or B. pubescens. Of the trees in question A3, A24, B3, B4, B7, C22 and C26 were placed in Cluster 2 ("B. pendula") and A17, C6 and C8 in Cluster 1 ("B. pubescens"). Cluster 2, however, included tree A20 which was classified as "B. pubescens" in table 4.6.

In summary, the scatter diagram figure 4.7, constructed using seven variables, tended to show up "intermediates" but the computer analysis, using the total sixteen variables, suggested that two groups "B. pendula" and "B. pubescens" were present. The inclusion of A20 in the "B. pendula" group formed by computer analysis and the lack of a cluster of "intermediates" were the two disagreements with the classification based on scatter diagrams. After relocation of trees using computer program Reloc, six moved from one cluster to another. The trees in question were A20, A24, B4, B7, C22 and C26. Significantly one of these was A20 which moved from the "B. pendula" cluster to the "B. pubescens" one. Thus table 4.10 agrees more closely with table 4.6 than does table 4.9. The other trees relocated were in all cases "intermediates" of table 4.6.

4.5 The correlation of morphology and cytology.

To clarify the taxonomic position of trees, chromosome numbers were correlated with the morphological groupings formed. In figure 4.7 shading of circles indicates probable diploids. The "B. pendula" group delineated on morphological grounds is seen to contain six trees with the tetraploid chromosome number and furthermore one tree outwith the group has the diploid number. The trees are respectively B4, B7, B20, B23, C20 and C22 (tetraploids) and A13 (diploid). Program Hierar produced the same classification of these trees in as much as the six tetraploid trees were again grouped with diploid ones in Cluster 2 and the diploid A13 was included with tetraploids in Cluster 1. Program Reloc, on the other hand, placed only four trees A13, B20, B23 and C20, based on their morphology, in the "wrong" group as suggested by the cytological evidence.

As stated in Chapter 3, no "intermediate" chromosome numbers of 2n=42 were obtained and thus no "intermediate" trees could be regarded as F_1 hybrids formed by fusion of normal gametes of B. pendula and B. pubescens. Trees "misclassified" were in the majority of cases tetraploid, although their morphology suggested them as diploids. Since the possibility of unreduced gametes forming such trees arises, this matter is considered further in the following chapters.

4.6 Conclusions of the morphology.

Scatter diagrams have been studied as the basis of a tentative classification of birch.

Using leaf, bark and branch characters, a group of trees was distinguished as having attributes of B. pendula (Clapham, Tutin and Warburg 1962). This group was called the "B. pendula" one by the present author. Similarly a "B. pubescens" group and an "intermediate" one were recognised.

Birch specimens taken from the herbarium collection, when examined in the same way, fell into two groups on a scatter diagram. The range of variation in these specimens was similar to that observed in Milngavie samples. Herbarium specimens, labelled hybrids, did not appear to be outwith the ranges of variation of labelled B. pubescens specimens and B. pendula ones. Using the methods of the present author the hybrids in the collection would not have been tentatively classified as "intermediates".

The distribution of Dunblane samples overlapped that of Milngavie "B. pendula" ones on scatter diagrams despite the fact that all trees from the Dunblane site had leaf, branch and bark attributes of B. pubescens (Clapham, Tutin and Warburg 1962). This raises a problem as to the explanation of such overlap. As stated in Chapter 4.2 (b), the Dunblane samples could simply reflect a wide range of variation in leaf apical angle in "B. pubescens" or they could be products of introgression. In such a situation chromosome numbers would have been of great interest and value but as noted in Chapter 2 birch on the Dunblane site were felled before any source of root material was obtained.

The use of measurements of fruit characters in taxonomy has been studied and results compared with those obtained from examination of vegetative characters. In a majority of cases the present author found that the taxonomic groups formed by the different methods contained the same trees. Different classifications of some trees, however, were obtained

depending on the choice of characters. For this reason, a knowledge of chromosome numbers is extremely important. Six trees which were regarded morphologically as being likely "B. pendula" were in fact tetraploids and one likely "B. pubescens" was in fact diploid.

It is of interest that the computer analysis using program Reloc gave the morphological classification closest to the cytological one. It may be the case that the use of scatter diagrams over-emphasised the intermediacy of trees. Certainly the cytological evidence does not support the occurrence of any F_1 hybrids in the samples examined.

The knowledge of chromosome numbers of trees which have been studied morphologically enables the calculation of the ranges of variation in the diploid and tetraploid birch examined (bearing in mind the limitations of the cytology noted in Chapter 3). Tables 4.11 and 4.12 show respectively the data obtained from diploids and tetraploids.

Table 4.11 The range of variation in diploids.

	Range	Mean	1xStandard Error
Number of leaf serrations on one side.	14-25	19	±0.7
Number of serrations between nerves 2 and 3.	4-6	5	±0.12
Leaf length.	3.0-5.6cm.	3.9cm.	±0.12
Leaf width.	2.4-4.9cm.	3.1cm.	±0.12
Apical angle of leaf.	19°-35°	25°	±0.9
Ratio of leaf length to leaf width.	1.1-1.5	1.3	±0.02
Width of wing of fruit.	1.0-2.0mm.	1.6mm.	±0.06
Width of achene.	0.8-1.3mm.	1.0mm.	±0.03
Length of achene.	1.8-2.5mm.	2.1mm.	±0.03
Ratio of achene length to achene width.	1.5-2.6	2.0	±0.07
Ratio of wing width to achene width.	0.7-2.0	1.5	±0.06
Catkin length.	1.0-2.4cm.	1.8cm.	±0.07
Bract length.	3.4-5.8mm.	4.5mm.	±0.13

Discrete variables (expressed as a % of the trees sampled).

32% had silver bark.
23% " black bosses at base of trunk.
12% " pendulous branches.
100% " doubly-serrate leaves.
92% " stigmas not surpassing wings.
80% " little or no pubescence at stigmas.

Table 4.12 The range of variation in tetraploids.

	Range	Mean	1xStandard Error
Number of leaf serrations on one side	12-25	18	±0.37
Number of serrations between nerves 2 and 3.	3-5	4	±0.07
Leaf length.	2.3-4.4cm.	3.4cm.	±0.05
Leaf width.	1.8-3.4cm.	2.6cm.	±0.04
Apical angle of leaf.	20°-58°	41°	±1.0
Ratio of leaf length to leaf width.	1.1-1.6	1.3	±0.02
Width of wing of fruit.	0.5-1.7mm.	1.0mm.	±0.06
Width of achene.	1.0-1.6mm.	1.2mm.	±0.02
Length of achene.	1.5-2.3mm.	1.9mm.	±0.03
Ratio of acene length to achene width.	1.3-2.2	1.7	±0.06
Ratio of wing width to achene width.	0.4-1.6	0.8	±0.04
Catkin length.	1.4-3.1cm.	2.2cm.	±0.06
Bract length.	3.0-4.9mm.	3.9mm.	±0.07

Discrete variables (expressed as a % of the trees sampled).

97% had silver-brown bark.
98% " no black bosses at base of trunk.
100% " ascending ie. non-pendulous branches.
95% " irregularly serrate leaves.
89% " stigmas surpassing wings.
95% " marked pubescence at stigmas.

The continuous variables were examined statistically in order to find out if differences between diploid and tetraploid trees could be regarded as significant. The results in table 4.13 are based upon analyses of variance of means.

Two variables do not differ significantly, even at the 5% level. Table 4.11 illustrates the point that several discrete variables, normally associated with the diploid, are found in rather a small percentage of the samples.

The morphology and cytology do not provide an explanation of the origin of tetraploid trees examined. Although apparent F_1 hybrids were not found, trees particularly resembling the diploid in their morphology, but having the tetraploid chromosome number did occur in the samples. These, in theory, could have arisen as a result of fusions involving unreduced gametes.

In Chapter 5 the reproductive biology of tree birch is discussed. In particular, evidence is sought in support of the hypothesis that unreduced gametes may fuse to form tetraploids.

Table 4.13 The statistical significance of differences between mean values for diploid and tetraploid.

Number of serrations on one side of leaf.	Not significant at the 5% level.
Number of serrations between nerves 2 and 3.	Significant at the 0.1% level.
Leaf length.	" " " 1% "
Leaf width.	" " " 0.1% "
Apical angle of leaf.	" " " " "
Width of wing of fruit.	" " " " "
Width of achene.	" " " " "
Length of achene.	" " " " "
Catkin length.	" " " " "
Bract length.	" " " " "
Ratio of leaf length to leaf width.	Not significant at the 5% level.
Ratio of wing width to achene width.	Significant at the 0.1% level.
Ratio of achene length to achene width.	" " " " "

CHAPTER 5.

Chapter 5.

Studies of the Breeding System.

5.1(a) Flowering times-Introduction.

Literature on the subject of flowering times in birch was reviewed in Chapter 1.

Convincing evidence of widespread cross-pollination has not been obtained and indeed details of flowering times have not been investigated at all in many studies.

The work of Jentys-Szaferowa(1938), Sarvas(1952) and Eifler (1956) suggested a separation between the flowering times of B. pendula and B. pubescens.

Berrie(1952) and Natho(1959) noted, however, that the times of flowering were variable and that the process occurred over a number of days. Berrie(1952) in his studies of B. pendula and B. pubescens found considerable differences in the flowering times of trees of the same species growing close together. Cousens(1965) has made the suggestion that length of growing season is an important factor in the determination of flowering times and that where the season is relatively short overlap may be a more likely occurrence.

The research work of Linskens(1964) and Alam and Grant (1971) has provided information on pollen viability which appears to be dependent on environmental conditions.

5.1(b) The flowering and seeding of birch studied at Milngavie and Dunblane.

During September and October 1971 seeds were being dispersed at Milngavie. Male catkins were apparently present at that time. (Berrie 1952, studied meiosis in male catkins in mid-August). The development of the male catkins was followed during the early months of 1972. Pollen was

apparently dispersed in May of that year, but on that occasion the exact dates of flowering were not obtained. Stigmas in female catkins appeared to wither about mid-June. Seeds were ripe and being dispersed from late August onwards in 1972.

Having obtained a limited amount of information on flowering times in 1972, the development of pollen in male catkins was again followed in 1973. During February-March, green and presumably unripe pollen grains were easily seen using a dissecting microscope. In mid-April the pollen appeared to be turning yellow in flowers of dissected male catkins. On May 1st, 1973 male catkins on some trees were open and the powdery pollen was clearly being dispersed. Table 5.1 shows the stage of flowering of Milngavie trees on the 5th and 6th of May 1973.

The table shows that both likely diploid and tetraploid trees had ripe pollen and open female flowers at the same time. Some individuals had both male and female flowers open simultaneously, and in one case a hermaphrodite catkin was found. It appeared that a greater percentage of the diploid trees had neither male nor female flowers open at the time of examination, as compared with tetraploid plants. The word "open" is used to describe flowers, particularly female, rather than "ripe" for the reason that it is difficult to establish when a female stigma is ready to receive pollen. Even in cases where microscopically examined female flowers had pollen on stigmas, it was not known if they had been successfully fertilised by that pollen.

Examination on May 20th, 1973 of Dunblane birch revealed that both male and female catkins, on trees bearing morphological resemblance to B. pendula and B. pubescens as described by Clapham, Tutin and Warburg (1962) had open flowers. Flowering appeared to have ended on these trees by May 31st.

In September 1973 it was particularly notable that trees at Milngavie, which previously had been prolific seed producers, had very few

Table 5.1-Flowering of male and female catkins at Milngavie.

A cross denotes the presence of apparently ripe pollen in open male catkins, and open female flowers. D indicates likely diploid and T likely tetraploid.

Tree.	Male.	Female.	DorT	Tree.	Male.	Female.	DorT	Tree.	Male.	Female.	DorT
A1	X	X	T	B2		X	D	C8	X	X	T
2	X	X	T	3	X	X	D	9	X	X	?
3			D	4		X	T	10			T
4		X	D	5	X	X	T	11	X	X	T
4A			D	6	X		T	12	X	X	D
5	X	X	T	7	X	X	T	13	X	X	T
6	X		T	8	X	X	T	14			D
7	X		T	10	X		T	15	X	X	T
8	X	X	T	11	X	X	T	16			D
9	X	X	D	12	X	X	T	17			D
10	X		D	13	X	X	T	18			D
11	X	X	D	14	X		D	19	X	X	T
12		X	T	15	X	X	D	20	X	X	T
13	X	X	D	16	X		T	21			T
14	X		D	17	X	X	T	22			T
15	X	X	D	18	X	X	T	23			T
16	X	X	D	20			T	24	X		T
17	X	X	T	21	X	X	D	25			D
18	X	X	?	22		X	T	26			T
19	X	X	D	23	X	X	T	27			T
20			T	C1			D	28			T
21	X	X	T	2			D	29	X		T
22		X	T	3	X	X	T	30	X	X	T
23	X		T	4			?	31	X	X	D
24	X	X	T	5			T	32	X	X	T
25	X	X	T	6			T	33	X	X	T
B1		X	T	7	X	X	T				

seed-bearing catkins. Similar observations on the variability of seed production have been made by Berrie (1952) and Kinnaird (1968).

In 1974 pollen dispersal took place from May 2nd at Dunblane and from May 5th at Milngavie. On the latter site only about 23% of the diploid trees had open male flowers as compared with about 60% of tetraploid ones.

In summary, evidence has been obtained that flowering times of likely diploid and tetraploid birch overlap in two geographical areas examined. There appeared to be little or no difference in the range of flowering times at the two sites studied. The present study provides evidence that trees with attributes of B. pendula as described by Clapham, Tutin and Warburg (1962) tend to flower later than those with attributes of B. pubescens which disagrees with the observation made by Sarvas (1952) that the diploid flowered first. As already stated, however, it is not certain that the opening of flowers indicates ripeness to pollinate or to be pollinated. Furthermore there is the possibility that sterility barriers exist (Hagman 1963) which would prevent fertilisation even if cross-pollination took place.

5.2(a) Pollen studies-Introduction.

Jentys-Szaferowa (1959) observed that B. pubescens was morphologically more variable than B. pendula. Walters (1968) used the term "tetraploid aggregate" to describe B. pubescens. A possible source of variation is the formation of tetraploid birch by hybridisation involving unreduced gametes. Elkington (1968) suggested this as a mechanism of hybrid formation between B. nana and B. pubescens. Theoretically, B. pendula could produce diploid pollen 2x=28 (where x represents the gametic number of the diploid) capable of fertilising normal gametes of B. pubescens 2x=28, assuming compatibility of such gametes. In this way a tetraploid birch 2n=4x=56 could arise. If this were the case, the occurrence of "giant" pollen grains in male flowers of B. pendula would be consistent with the cross-pollination theory involving such gametes but would not in itself support the formation of a hybrid.

Pollen studies, however, are difficult due to the influence of environmental factors on pollen size. Schoch-Bodmer (1940) found variations in pollen size which appeared to result from nutritional effects, water supply differences and from the position of flowers on the inflorescence in Lythrum salicaria. In this case differences of 6 micrometres were observed depending on flower position. Mikkelson (1949) stated that pollen size in Pelargonium seemed to decrease with increase in temperature but because of the important influence of nutrition, firm conclusions on the effect of temperature could not be drawn. Water deficiency and temperature were quoted as factors affecting pollen size by Linskens (1964). The evidence in the literature suggests that pollen measurements as an indication of chromosome number must be used with caution.

Studies of Betula pollen have been made. Erdtman (1943),

Erdtman, Berglund and Praglowski (1961) and Erdtman, Praglowski and Nilsson (1963) have examined pollen from B. pendula and B. pubescens. In the earliest of these studies equatorial diameters were measured and the range of values for B. pubescens was found to be 20-28.6 micrometers. The range for B. pendula was 18.6-24.3 micrometers. Pollen grains were furthermore described as having three, sometimes four pores in both species.

There is evidence that the use of pollen studies in birch taxonomy is difficult. Erdtman (1969) concluded that there were many difficulties in distinguishing species on the basis of pollen grain measurements. Birks (1968), in a study of B. nana pollen, found that B. nana could not be distinguished from B. pendula or B. pubescens on size alone. On the basis of size frequency curves B. pendula and B. pubescens were indistinguishable.

Against the background of difficulties, the present examination of pollen was undertaken to find out the range of pollen sizes from trees under study and to look for relatively large sizes in the distribution which might be considered "giant" grains,

5.2.(b) The materials and methods used in the examination of pollen by means of light microscopy.

Ripe male catkins were taken at random from trees during May 1973 and air-dried. Clausen (1960) found no significant size differences in pollen taken from various positions on a birch tree or from within a catkin. Samples were mounted in silicone oil and after a short time, which was kept constant for each sample, equatorial diameters were measured with the aid of a Zeiss microscope. Although the method was kept as simple as possible, every precaution was taken to give all samples the same treatment. The mean equatorial diameter for each pollen grain was calculated. Fifty such grains were measured from every tree sample, all being known likely

diploids or tetraploids.

5.2.(c) Results of pollen studies.

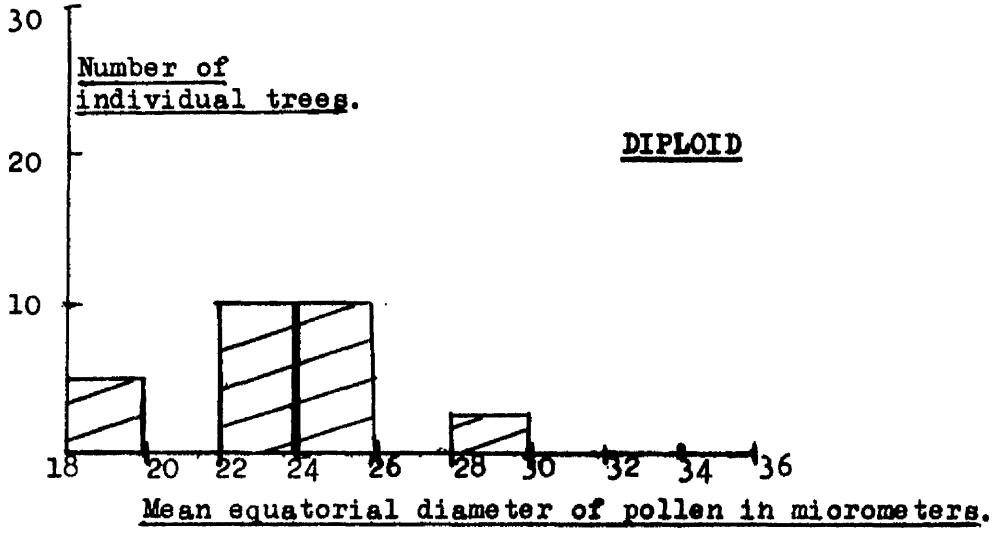
The range of measurements of equatorial diameters of pollen from likely diploid parents was found to be 19.4-32.3 micrometers and from likely tetraploid ones was 22.6-32.3 micrometers. The mean values obtained were respectively 22.6 and 26.6 micrometres. The standard error of the mean was $\pm$ 0.7 for the diploid and $\pm$ 0.3 for the tetraploid.

Figure 5.1 shows graphically the range in mean values obtained from trees of both types.

One diploid tree, A11, had one pollen grain with a diameter as large as the largest tetraploid grain, that is 32.3 micrometers. In view of difficulties associated with pollen measurement and the overlap in the ranges of mean values obtained, it would be unwise to speculate about the existence of "giant" pollen grains on the basis of one exceptional example. The possibility cannot be ruled out nevertheless.

In all cases examined the pollen grains had three pores. Figures 5.2 and 5.3 show examples of the pollen studied. No tests of pollen viability were made in the present research work but the photographs of typical pollen grains resemble ones presented by Dawoody (1974) which he described as "aborted grains".

Figure 5.1-The range of values of pollen sizes in likely diploids and tetraploids.



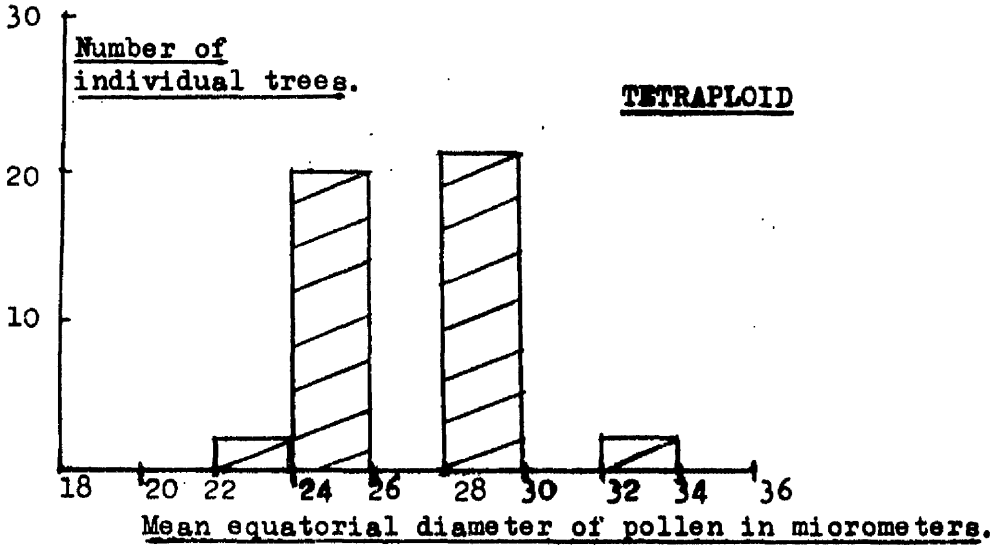


Figure 5.2 Pollen from a diploid tree.

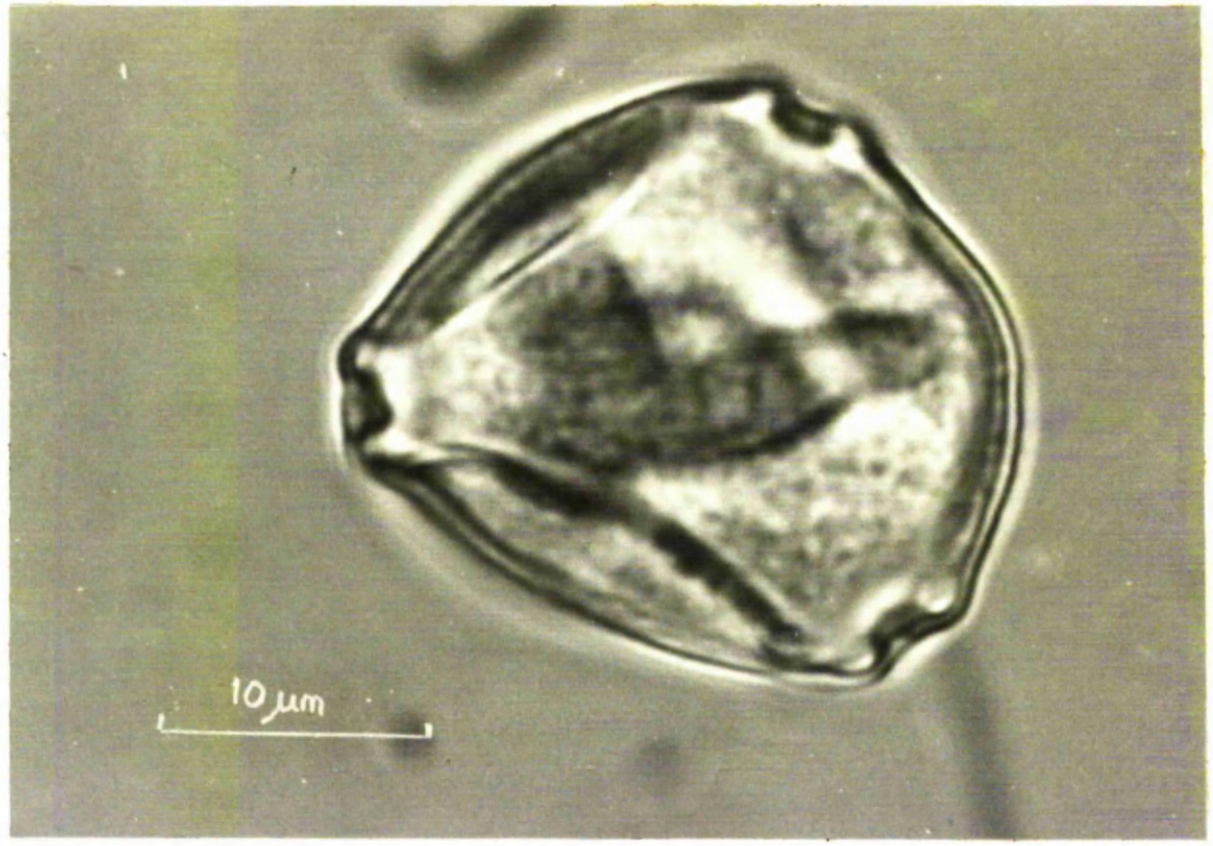
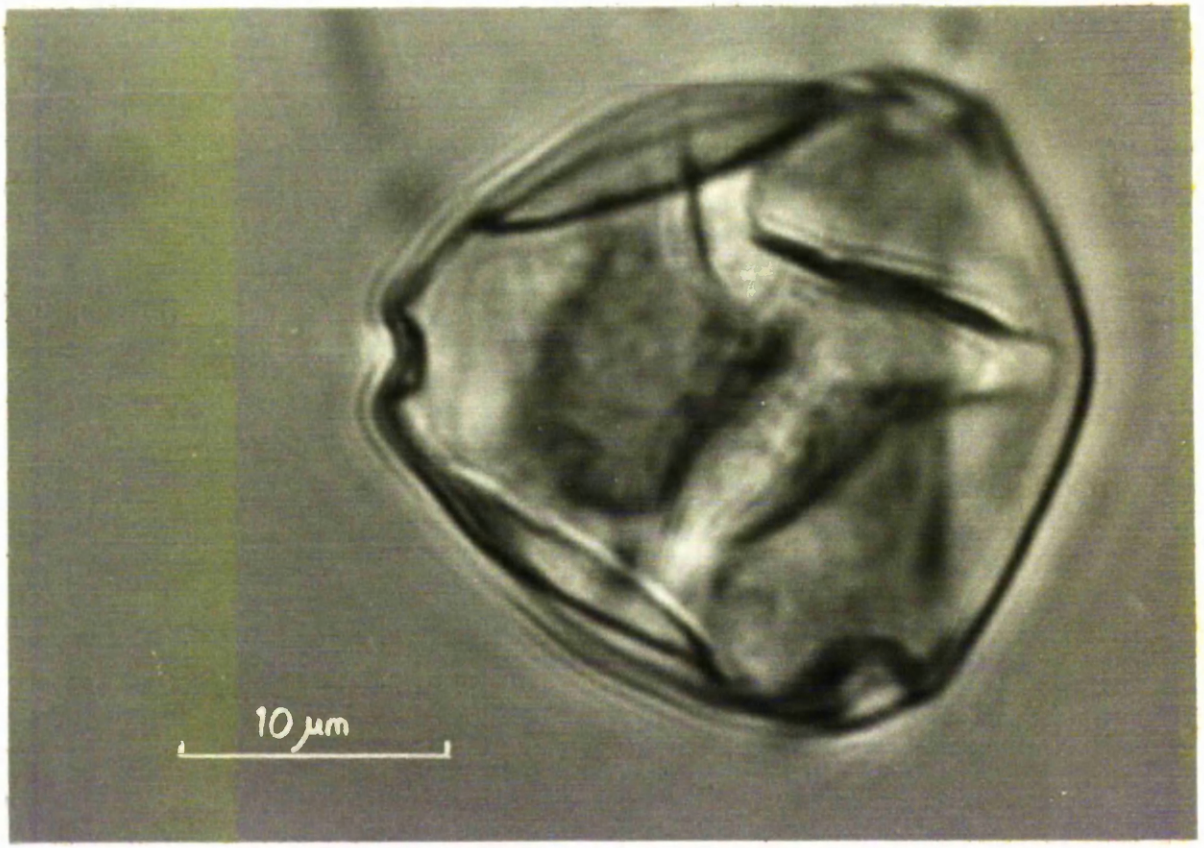


Figure 5.3 Pollen from a tetraploid tree.



5.3(a) Seed germination trials-Introduction and Method.

Birch are known to be sensitive to daylength (Wareing and Phillips 1970). Long days are necessary for flower initiation and also to break seed dormancy. The latter may also be broken by 1) slitting seed coats, 2) maintaining seeds in a high concentration of oxygen, 3) chilling, 4) gibberellic acid. Valanne (1973) has studied Betula seed germination.

In the present study air-dried seeds, which had been stored in polythene bags and kept in a cool place, were placed on moist Whatman seed test circles in petri-dishes. The dishes were put under continuous light from fluorescent tubes set up in a warm laboratory. For each tree studied, three dishes, each containing twenty-five seeds, were set up as described and laid out in a random arrangement. The number of seeds which had germinated was noted after eighteen days.

Seed germination was closely examined in order to find out if any trees produced exceptionally few or even no viable seed, which might be a feature of a hybrid.

5.3(b) Results of the germination trials.

The results are shown in table 5.2. From this table it can be seen that the percentage germination of seeds from likely diploid trees was generally lower than those from tetraploids. Only one tree, C9, produced seed which did not germinate at all. Seed viability declined with increase in storage time. Although, in the main, trial seeds collected in autumn were germinated in the following season, seed from these batches were still found to be capable of growing in the next but one season. Some seeds collected in August-September 1971 still had 40-50% viability in summer 1974.

Table 5.2-Total germination as a % of the total number of seeds planted.

D indicates likely diploid and T likely tetraploid.

Tree	%	Chromosome Number	Tree	%	Chromosome Number	Tree	%	Chromosome Number
A1	80	T	B5	84	T	C14	12	D
2	80	T	6	96	T	15	76	T
3	80	D	7	80	T	16	44	D
4	48	D	8	76	T	17	36	D
4A	8	D	10	84	T	18	48	D
5	8	T	11	96	T	19	8	T
6	40	T	12	84	T	20	40	T
7	80	T	13	56	T	21	76	T
8	8	T	14	8	D	22	64	T
9	8	D	15	76	D	23	72	T
10	28	D	16	8	T	24	64	T
11	16	D	17	76	T	25	8	D
12	48	T	18	48	T	26	88	T
13	8	D	20	46	T	27	80	T
14	40	D	21	48	D	28	78	T
15	8	D	22	88	T	29	76	T
16	48	D	23	40	T	30	76	T
17	46	T	C1	6	D	31	84	D
18	8	?	2	54	D	32	38	T
19	8	D	3	48	T	33	2	T
20	16	T	4	46	?	D1	76	T
21	40	T	5	40	T	2	48	T
22	24	T	6	96	T	3	78	T
23	16	T	7	92	T	4	46	T
24	76	T	8	48	T	5	88	T
25	80	T	9	0	?	6	64	T
B1	82	T	10	84	T	7	76	T
2	81	D	11	64	T	8	80	T
3	8	D	12	42	D	9	80	T
4	96	T	13	78	T			

5.4 Conclusions from the studies of the breeding system.

The occurrence of cross-pollination has been found to be a distinct possibility in two geographical locations. Diploid and tetraploid trees certainly had male and female catkins open at the same time. The present study, however, provides no information on the fertilisation following cross-pollination, which would result in hybrid formation.

Pollen studies did not yield conclusive evidence on the formation of"giant grains", since a considerable overlap in size ranges was found. In one case an atypical size was recorded for a likely diploid which equalled the equatorial diameter of the largest tetraploid grain examined.

The results of germination trials showed that there was a wide range in seed viability, with diploids tending to produce more inviable seed.

In Chapter 4 the possibility that unreduced gametes could form hybrids was stated and it was particularly noted that the majority of trees whose morphological data led to "misclassification",as compared with chromosome number, were tetraploid. Since 2n=4x=56 would be the resulting chromosome number of crosses involving unreduced B.pendula gametes and normal B.pubescens ones, the similarities of tetraploids to diploids observed in the morphology is interesting. The overlap in flowering times would be consistent with the gene flow involved, and the possibility of "giant" pollen grain production cannot be ruled out. Tetraploids in the"misclassified" category were B4, B7, B20, B23, C20 and C22. From table 5.2 it can be seen that seed from these trees were 40%-90% viable and none fell into the lowest germination category. Tree C9, which produced inviable seeds, was a "B.pubescens" type morphologically, and no evidence suggested it as an "intermediate".

Since the evidence from pollen studies was inconclusive regarding the occurrence of unreduced gametes, information on chromatography was sought which could enable the detection of hybrids formed by the fusion of such gametes. This is the subject of Chapter 6.

CHAPTER 6.

Chapter 6.

Chromatographic Studies of Birch Extracts.

6.1 Introduction

In the last decade or so biochemical data have been increasingly used in taxonomic work. Techniques have included electrophoresis, D.N.A. hybridisation, serology and chromatography, and their use has been described by Hawkes (1968).

Chromatography has been widely used in studies of secondary plant metabolites in particular. Examples are known of apparently simple gene systems controlling the syntheses of such compounds (Turner 1966), as distinct from the more complex genetic control of some morphological variables The fact that biochemical differences may reflect genetic ones has been well illustrated in the case of Antirrhinum, where single genes apparently control biochemical effects in pigment synthesis (Harborne 1965).

Alston and Turner (1959) examined extracts of Baptisia spp. and found interesting combinations of parental compounds in hybrids. Dedio, Kaltsikes and Larter (1969) and Kaltsikes and Dedio (1970) obtained evidence from chromatographic studies for extensive hybridisation and introgression in Triticum spp. and Aegilops spp. respectively. Other such studies include the work of Smith and Levin (1963) on Asplenium, Stebbins et al (1963) on Viola, Widen and Britton (1969) on Dryopteris dilitata, Grant and Whetter (1966) on Lotus and Grant and Whetter (1970) on Avena. Of particular interest is the research work of Grant (1971) and Koshy et al (1972) on Canadian birch, and that of Kenworthy et al (1972) on B. pubescens and B. nana in this country.

Such studies have provided evidence that chromatography may be easily applied, and may give reproducible results helpful in discriminating between parental and hybrid types in situations where introgression is suspected of having taken place.

Certain inherent difficulties in chromatography work have been found including 1) compounds may undergo changes during drying processes, (Reinhold and Liwschitz 1968), 2) chlorophylls from leaf extracts may undergo extensive changes when chromatographed on silica gel, (Reinhold and Liwschitz 1968), 3) the total absence of a compound is difficult to demonstrate, (Harborne 1968).

The present study is an attempt to find evidence of differences between chromatographic patterns obtained from leaf extracts of plants with different chromosome numbers. It does not involve the extensive chemical analysis of such extracts in order to characterise them.

6.2(a) The materials and methods used for thin-layer chromatography.

Fresh leaves were collected at Milngavie and Dunblane in July of 1972, 1973 and 1974. They were air-dried at 45°C for twenty-four hours in an oven and extracted in a cool, dark place with 1% hydrochloric acid in methanol for varying lengths of time. Extraction was originally carried out for forty-eight hours, but it was subsequently found that no qualitative difference could be detected when the time of extraction was varied between eighteen and seventy-two hours.

The extracting solvent was added at the rate of $5cm^3$ for each 0.4g of dry leaf material. Extracts were found to keep for at least fourteen days in a cool, dark place, without deterioration.

Apparently the position from which leaves are taken is not critical as it was found that extracts of leaves from different parts of the same tree produced identical chromatographic patterns. Leaves stored in an air-dry condition for twenty-four months, when extracted, produced the same pattern as leaves from the same batch which had not been stored but which had been used immediately following collection.

Acid/Methanol extracts were applied using a microsyringe in quantities of 5 microlitres to glass plates coated with Kieselgel G (Merck product), the thickness of the layer being 0.010 inches. Plates were activated at 110°C before use.

A convenient method of dealing with large numbers of samples was found to be one-dimensional multiple pass, as suggested by Grant (1971). The solvents used were 1) Cyclohexane : Ethyl ethanoate, 1:1 and 2) Methanol : Chloroform, 30:70. Eluent 1 was allowed to run to the ten centimetre mark on the plate twice, and eluent 2 to the five centimetre mark twice.

Standard dye solution containing N,N-Dimethyl amino azo benzene (Yellow), Sudan red G (Red) and Indophenol (Blue) was applied to each plate to provide reference points.

After chromatograms had been run they were sprayed with 5% sulphuric acid in ethanol as a locating reagent, and the plates were dried at 110°C. They were then examined in ultra-violet light of wavelengths 254 nanometres and 360nm provided by Camag equipment.

Plates were also scanned with a Joyce-Loebl Chromoscan in order to obtain a measure of the fluorescence of spots in ultra-violet light.

6.2(b) Results

Figure 6.1 is a composite diagram showing the position, colour in U.V. light and total number of spots observed in the present studies.

Upon examination it was found that spots 1-9 appeared on all chromatograms. Since spots 10-15 were not found on all plates, tables 6.1, 6.2 and 6.3 were drawn up to show the distribution of these spots in the trees examined at Milngavie.

Figure 6.1-Composite diagram

Colour Key-

1) Black
2) Black
3) Green-Brown
4) Green-Brown
5) Green
6) Red
7) White
8) White
9) Orange-Red
10) Blue
11) Blue
12) White
13) White
14) White
15) White

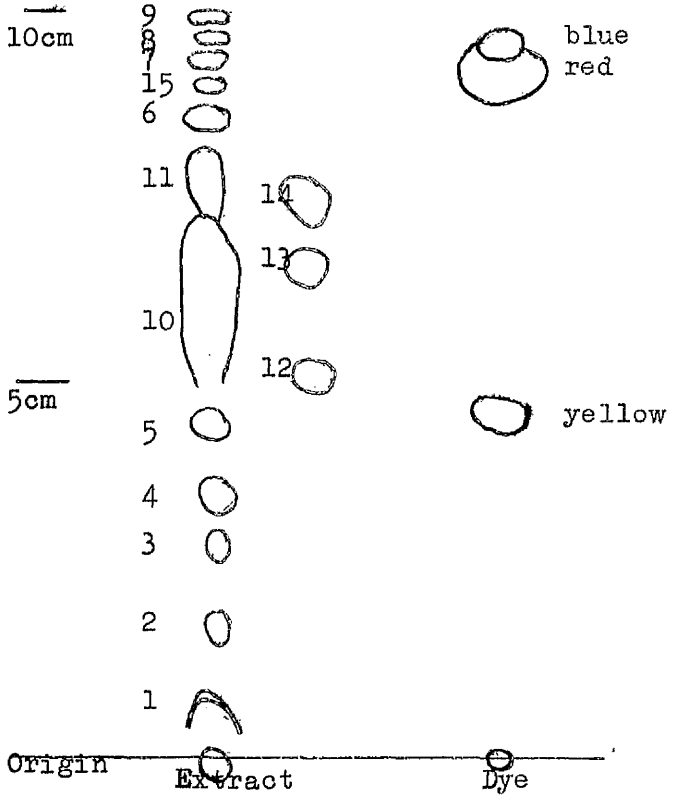


Table 6.1-Distribution of spots 10-15 (X denotes presence).

Tree number	Spot 10	11	12	13	14	15
Area A1	X	X				
A2	X	X				
3			X	X	X	
4			X	X		
4A			X	X		
5	X	X				
6	X	X				
7	X	X				
8	X	X				
9			X	X	X	
10				X	X	X
11				X	X	
12	X	X				
13			X	X	X	
14			X	X	X	
15				X		
16			X	X	X	
17	X	X				
18			X	X	X	
19				X	X	X
20	X	X				
21	X	X				
22	X	X				
23	X	X				
24	X	X				
25	X	X				

Table 6.2-Distribution of spots 10-15 (X denotes presence).

Tree number	Spot 10	11	12	13	14	15
Area B1	X	X				
B2			X	X	X	X
3			X	X	X	
4	X	X				
5	X	X				
6	X	X				
7	X	X				
8	X	X				
10	X	X				X
11	X	X				
12	X	X				
13	X	X				X
14				X	X	
15			X	X	X	
16	X	X				
17	X	X				
18	X	X				
20			X	X	X	
21			X	X	X	
22	X	X				
23	X	X				
Area D1	X	X				
D2	X	X				
3	X	X				
4	X	X				
5	X	X				
6	X	X				
7	X	X				
8	X	X				
9	X	X				

Table 6.3-Distribution of spots 10-15 (X denotes presence).

Tree number	Spot 10	11	12	13	14	15
Area C1				X		X
C2				X	X	X
3	X	X				
4	X	X				
5	X	X				
6	X	X				
7	X	X				
8	X	X				
9	X	X				X
10	X	X				X
11	X	X				
12			X	X	X	
13	X	X				
14			X	X	X	
15	X	X				X
16				X	X	
17			X	X		
18			X	X		
19	X	X				
20			X	X	X	X
21	X	X				X
22	X	X				X
23	X	X				X
24	X	X				X
25			X	X		
26	X					
27	X	X				
28	X	X				
29	X	X				
30	X	X				X
31				X	X	X
32	X	X				
33	X	X				

From the tables it can be seen that there are two groups of plants 1) those clearly having spots 10 and 11 (in one case a plant had 10 and not 11), and in some cases 15.

2) those clearly having spots in the range 12-14, and in some cases 15.

Since spot 15 appears in both groups it is not regarded at this stage as being of major significance in distinguishing them.

Chromatograms of Dunblane samples were in all cases of the group one type.

The possibility that spots in the range 10-14, which appeared critical, could mask each other was investigated. Several extracts of group one type were co-chromatographed with extracts of group two type. In all cases both blue and white spots could be distinguished. The result of such an experiment is shown in figure 6.2.

Figure 6.2-The chromatographic pattern of combined extracts compared with the same extracts run separately.

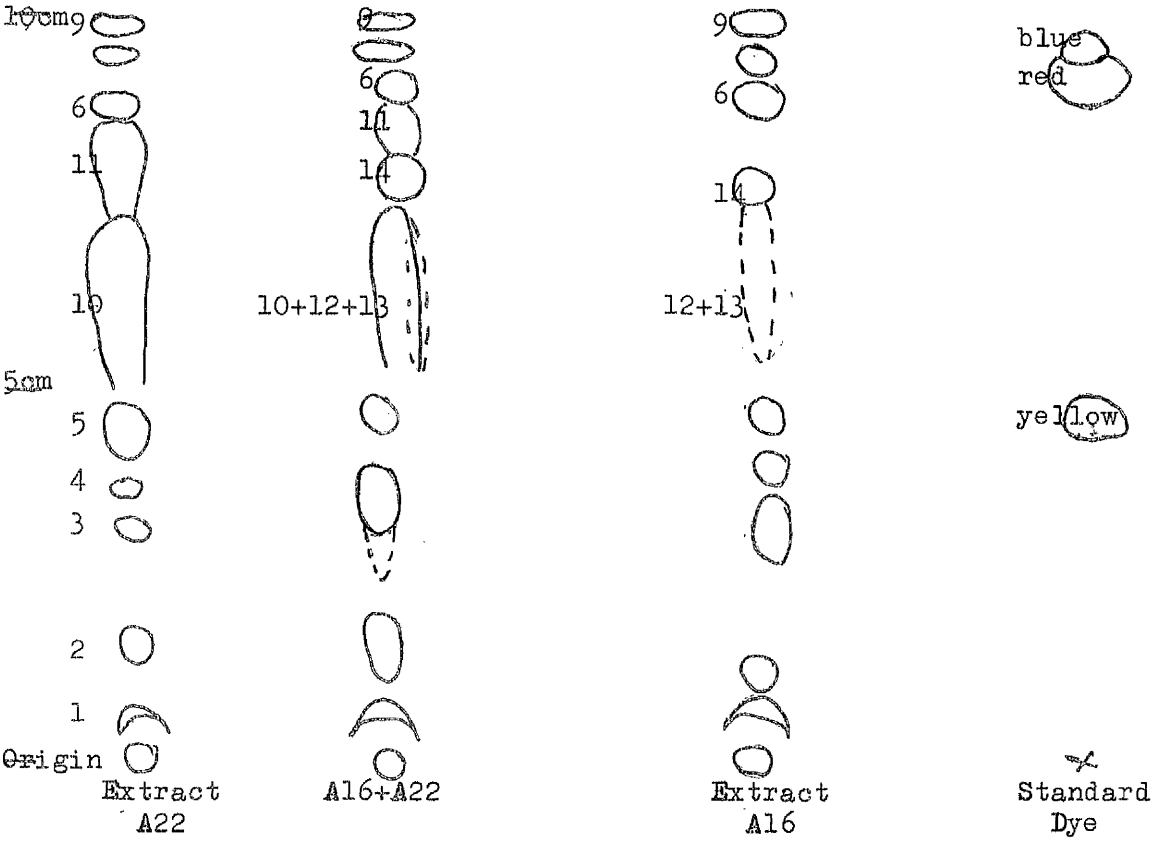


Table 6.4 shows a correlation of chromatographic pattern with chromosome number. To simplify matters trees having spots 10 and 11 are called Type 1 and those having spots in the group 12 to 14, Type 2. From the table it can be seen that Type 1 trees are likely tetraploids and Type 2 are likely diploids with only two exceptions, namely B20 and C20.

Figure 6.3 shows the pattern typically obtained from leaf extracts of the diploid type and the fluorescence of spots.

Figure 6.4 shows the patterns obtained from leaf extracts of the tetraploid type and the fluorescence of spots. In both cases a Joyce-Loebl Chromoscan was used to measure fluorescence.

Table 6.4-Correlation of chromatography and cytology.

Tree	Type	Chromosome Number	Tree	Type	Chromosome Number	Tree	Type	Chromosome Number
A1	1	Tetraploid	B5	1	T	C14	2	D
2	1	T	6	1	T	15	1	T
3	2	Diploid	7	1	T	16	2	D
4	2	D	8	1	T	17	2	D
4A	2	D	10	1	T	18	2	D
5	1	T	11	1	T	19	1	T
6	1	T	12	1	T	20	2	T
7	1	T	13	1	T	21	1	T
8	1	T	14	2	D	22	1	T
9	2	D	15	2	D	23	1	T
10	2	D	16	1	T	24	1	T
11	2	D	17	1	T	25	2	D
12	1	T	18	1	T	26	1	T
13	2	D	20	2	T	27	1	T
14	2	D	21	2	D	28	1	T
15	2	D	22	1	T	29	1	T
16	2	D	23	1	T	30	1	T
17	1	T	C1	2	D	31	2	D
18	2	?	2	2	D	32	1	T
19	2	D	3	1	T	33	1	T
20	1	T	4	1	?	D1	1	T
21	1	T	5	1	T	2	1	T
22	1	T	6	1	T	3	1	T
23	1	T	7	1	T	4	1	T
24	1	T	8	1	T	5	1	T
25	1	T	9	1	?	6	1	T
B1	1	T	10	1	T	7	1	T
2	2	D	11	1	T	8	1	T
3	2	D	12	2	D	9	1	T
4	1	T	13	1	T			

Figure 6.3-The typical chromatographic patterns of the diploid and the fluorescence of spots in ultra-violet light.

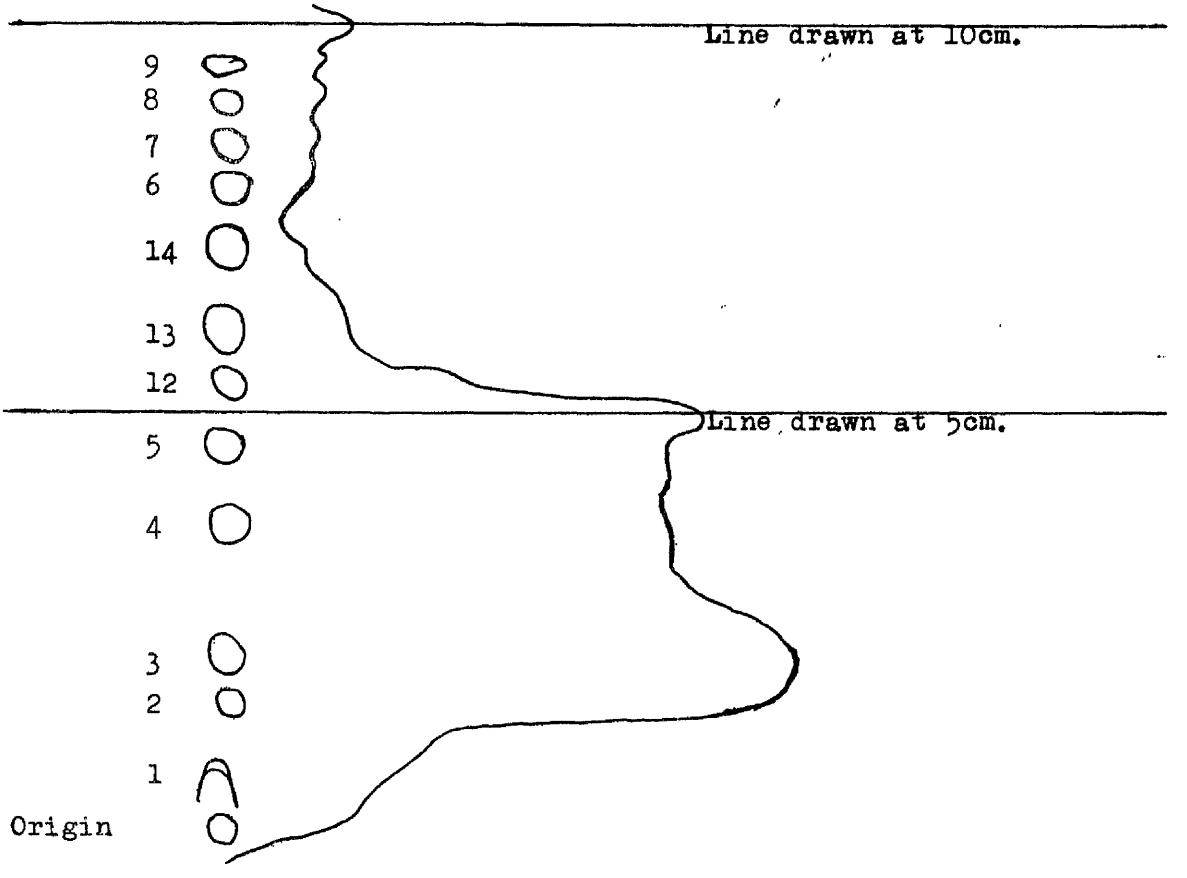
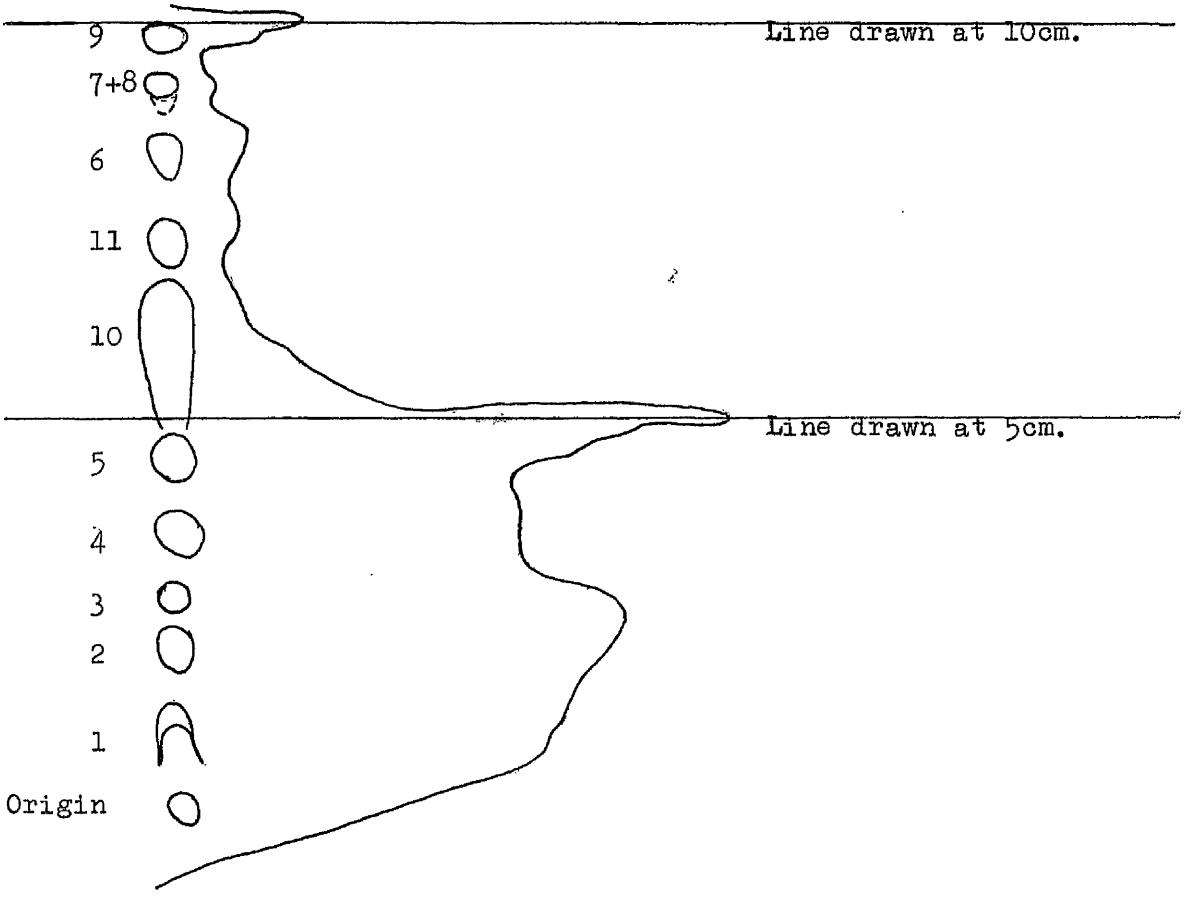


Figure 6.4-The typical chromatographic patterns of the tetraploid and the fluorescence of spots in ultra-violet light.



6.3 Conclusions of the chromatography studies.

A technique has been developed that enables the examination of leaf extracts in large numbers. The chromatographic patterns obtained are reproducible and, since within the limits investigated extracts appear to be stable, the technique is fairly simple. Extracts so far examined have been crude but given time samples could be purified prior to examination. There is an overall similarity between the patterns obtained from diploid and tetraploid plants but some consistent differences have been observed between leaf extracts. This has enabled tentative identification of a tree from its chromatographic pattern, in the majority of cases so far examined, as being either diploid or tetraploid. Significantly, two trees which were likely tetraploids had characteristic spots of the diploid and both of these, B20 and C20, were trees misclassified on morphological grounds.

It would appear possible that further investigation and perhaps refinement of methods described in this thesis would lead to a situation in which chromatography could be used in the taxonomy of birch. In view of difficulties associated with the identification of hybrids produced by the fusion of unreduced gametes, such a technique would be most valuable.

CHAPTER 7.

Chapter 7.

Conclusions.

In the previous Chapters several methods for the study of variation in Betula have been presented and discussed. The present Chapter draws together general conclusions from the researches. Areas where further investigation would be important are indicated.

Chromosome numbers in birch.

Betula is a difficult genus cytologically. In the present studies it has been found that the small size of chromosomes and the occurrence of dark staining bodies make the determination of chromosome numbers difficult, particularly at the tetraploid level. A cautious interpretation of the cytological results has been adopted. The problems of inferring the likely chromosome number of a plant from its progeny have been discussed in Chapter 3. Root-tips have been obtained from cuttings treated with hormones and in one squash preparation adjacent cells were found to have different numbers of chromosomes. This may be an effect of the hormone (Sharma and Sharma, 1965). On the other hand, the work of Dawoody (1974) suggests that it is not uncommon to observe different chromosome numbers in cytological preparations from birch. The evidence stresses the point that several cells should be counted before deciding on the chromosome number of a given plant. It seems likely that 2n=2x=28 and 2n=4x=56 plants have been found in samples but there is no evidence for triploids from the present studies. Berrie (1952) has recorded uneven chromosome numbers but does not consider this evidence for aneuploids persuasive in view of the difficulties associated with birch cytology. The present author agrees with this point of view based on his own work. Further research is required to elucidate the cytology of the genus Betula.

The status of "intermediate" plants.

In the present studies trees with attributes of B. pendula and B. pubescens have been examined. It has been observed that both species display considerable morphological variation. Furthermore, trees with characteristics of both species have been found and tentatively called "intermediates" by the present author. It must be borne in mind that the described variation in these studies reflects the situation in the field and the role of environment and other factors is likely to be of great importance. Cultivation tests would be important in the study of birch variation. Evidence from the cytology and chromatography suggests that two groups are present in the samples. One group has a chromosome number 2n=28 and the other 2n=56 and each has a characteristic chromatographic pattern. The work of Dawoody (1974) and others cited in the text provides evidence for unreduced gamete formation. If such gametes do form and successfully produce progeny then plants with a chromosome number of 2n=56 could have arisen in two ways, 1) from diploid ancestors (unknown but possibly B. pendula involved) and 2) from fusions involving unreduced gametes, eg. B. pendula (unreduced gametes) X B. pubescens (normal reduced gametes). Karyotype studies of meiosis are extremely difficult and consequently genomic relationships are hard to analyse. However, chromatography is fairly simple to apply and some useful information has been obtained by this method. It might be expected that two chromatographic patterns would be obtained from tetraploids depending on their parentage. The present studies appear to support this. Tetraploids with the chromatographic pattern normally found in the diploid have been examined. The occurrence of such plants favours the hypothesis of unreduced gamete formation. In the localities studied the two major taxa flower at the same time and there is evidence from the studies of Dawoody (1974) that giant pollen grains, presumably having the unreduced chromosome number, are produced. A great deal of research is

needed to discover if hybridisation is possible and with what consequences.

Ecological considerations.

A group of trees sampled from the edge of a small area on Drumclog Moor with a high water table were diploids with characteristics of B. pendula. Clapham, Tutin and Warburg (1962) were of the opinion that B. pubescens was the more tolerant of wet areas. In view of observations from the present studies and those of Forbes and Kenworthy (1973) it would appear that the situation is complex. It may be that ecotypic differentiation is important in birch populations. An investigation of population variation including cultivation and tolerance tests would be interesting. Anderson and others have shown that hybridisation may be associated with habitat disturbance which breaks down ecological barriers. This has happened in the genus Quercus (Muller 1952). Drumclog Moor is an example of a disturbed habitat in which birch display complexity. Evidence for triploids has not been obtained from this site but this does not guarantee that such plants are not produced in the area. There is also the possibility already discussed that hybrids have formed by means of fusions of unreduced gametes and have been included in the tetraploid group.

The taxonomy of birch.

In the present studies of a limited number of plants the range of morphological variation in diploid and tetraploid birch has been examined. Since no cultivation tests were carried out, however, it cannot be stated whether the variation has a genetic basis. The occurrence of plants with morphological characters of B. pendula and B. pubescens and the fact that chromosome numbers of 28 and 56 have been obtained suggest that the two species are present in the samples. If, however, birch with a chromosome number 56 include hybrids then the assignment of a specific

name to all tetraploid plants could be regarded as misleading. In this connection the views of Gilmour (1961) and Gilmour and Walters (1963) are of interest. These authors have pointed out that a general classification should serve a range of purposes. It is suggested that special classifications within genera may be made without alterations in nomenclature. Applying these views to birch, there seems no reason why a special classification may not be adopted within the group generally named "B. pubescens". For example such tetraploids may be regarded as a cytodeme which indicates that a group of plants with the same chromosome number are to be found growing in nature. This would allow the possibility that some trees within the group may be hybrids. Similar methods of classification could be applied to other genera in which hybridisation appears to have taken place such as Salix, Populus, Quercus etc. (Stace 1975).

In view of the fact that the present studies are of plants in a limited range it would not be appropriate to suggest a taxonomic treatment for birch although a knowledge of local populations would be an important contribution to the overall picture. The decision must be the job of a taxonomist who has worked with material drawn from the entire range.

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