# STUDIES OF COMPATIBILITY IN BETULA

by Knud E. Clausen[1]

Heterozygosity appears necessary to maintain fitness in many plant populations, and mechanisms favoring or enforcing cross pollination have evolved. Most important among the latter are dioecism and incompatibility.

While dioecism is comparatively rare in plants, incompatibility is common (Lewis 1954) and may be an important barrier to hybridization attempts. Knowledge of the amount of self-, intraspecific, and interspecific compatibility in a genus permits effective planning of a breeding program. Information on interspecific compatibility may also elucidate phylogeny and evolution within a genus.

Compatibility in the genus *Betula* is being studied at the Institute of Forest Genetics, Rhinelander, Wis. Most crosses have been made on grafts in the greenhouse, but data from some field crosses are included. An average of 5 to 6 female inflorescenes (catkins) were pollinated in each cross. The number of ovules per female catkin varies with the species but ranges from about 200 to 400. The work is continuing, and only the results of the first 3 years of tests are presented here.

## Self-Compatibility

Self-compatibility was studied in 31 individual birches belonging to nine species (table 1). No catkins or seed developed after 12 pollinations, while 6 crosses resulted in catkins containing inviable seed. Viable seed was produced from 15 selfings, but the germination percentages varied greatly. The two types of unsuccessful crosses may represent different degrees of incompatibility. The female inflorescence would be expected to abort if the pollen were completely inhibited and failed to germinate. Pollen germination, on the other hand, may permit the catkin and ovules to develop normally although the pollen tubes are inhibited before fertilization is effected.

Selfing of *Betula lenta L.* and B. *pumila* L. was unsuccessful, but must be repeated with more individuals before anything can be said about self-compatibility of these species in general. Three native species, *B. glandulosa* Michx., *B. nigra* L. and *B. populifolia* Marsh. appear to be nearly self-incompatible. Only a few crosses succeeded, and the germination was 2 percent or less in all cases (table 1).

Three out of four *B. papyrifera* Marsh. trees set seed which ranged from 6 to 11 percent germination. A certain amount of self-compatibility thus appears to be present in this species. The five trees of *B. alleghaniensis* Britt. tested so far have all been partially self-compatible but have varied considerably in the amount of filled seed produced (table 1). Variation in reaction from year to year has also been apparent. The tree which failed to set seed after selfing in 1963 had the highest germination percentage encountered so far, 45, percent, in 1964. This shows the importance of re-testing individuals which may appear self-incompatible in the first trial.

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Table 1.--Results of self-pollinations in Betula

| Species | No. of plants tested | Number of crosses with--: No seed | Inviable seed | Viable seed | Percent germination of successful crosses |
|---|---|---|---|---|---|
| B. alleghaniensis | 5 | 1 | - | 5 | 0.5-5-10-16-45 |
| B. glandulosa | 3 | 1 | - | 2 | < 1-1 |
| B. lenta | 1 | 1 | - | - | |
| B. nigra | 5 | 3 | 1 | 1 | < 1 |
| B. papyrifera | 4 | 1 | - | 3 | 6-18-11 |
| B. pendula | 10 | 4 | 5 | 2 | < 1-< 1 |
| B. populifolia | 1 | - | - | 1 | 2 |
| B. pubescens | 1 | - | - | 1 | 0.5 |
| B. pumila | 1 | 1 | - | - | |
| Total | 31 | 12 | 6 | 15 | |

Two exotic species, both European, have been included in the tests to date. Trees of *B. pendula* Roth (*B. verrucosa* Ehrh.) have mostly been self-incompatible with only 2 out of 10 trees setting seed and these had less than 1 percent germination. The single *B. pubescens* Ehrh. tree tested was nearly self-incompatible.

## Intraspecific Compatibility

Five species and 37 individual trees of *Betula* have been used in the intraspecific tests so far (table 2). In the native species, 13 of 15 attempted crosses were successful, while one cross each of *B. alleghaniensis* and *B. papyrifera* failed to set seed. In both cases the pollen used was fertile when used in other crosses, indicating that the trees considered here probably are incompatible. The two *B. papyrifera* trees are growing on a small island and could conceivably be related. The germination percentages of the successful crosses have been good to excellent in the four native species, with crosses of *B. nigra* consistently best, followed by *B. papyrifera, B. alleghaniensis* and *B. lenta* crosses (table 2).

Six of the *B. pendula* crosses failed to produce catkins, 18 set inviable seed, and 13 were successful (table 2). Most of these crosses were between "normal" trees and trees with "curly" wood or varieties with cut or colored leaves. Sterile pollen may account for the lack of seed in one cross and the inviable seed in five crosses since the pollens were nonfunctional in other combinations as well. The remaining 18 unsuccessful crosses were all made with pollen that proved fertile in other crosses and must have failed for other reasons. Misjudgment of pistillate flower receptivity and environmental influences are possible causes. Several trees are located in an area where frequent frosts in late spring and early fall could have damaged the flowers.

Five of the 13 *B. pendula* crosses listed as successful had 1 percent or less germination and thus should probably be considered almost incompatible. Four of the successful crosses had between 11 and 23 percent germination, while four ranged from 39 to 81 percent.

## Interspecific Compatibility

Seven native and two European species have been included in tests of interspecific compatibility so far (table 3). No seed was produced in 44 of the attempted crosses, 50 set inviable seed, and 48 were successful. Of the crosses producing viable seed 40 had germination percentages ranging from less than 1 to 5 percent, indicating a high degree of incompatibility in these crosses. Only 8 crosses displayed fair to good compatibility, with germination percentages between 18 and 79 percent.

Interspecific crosses with *B. nigra* as either female or male parent have been difficult to make (table 3), and only a few seedlings have resulted from the successful crosses. Relatively few crosses involving *B. lenta* have been attempted to date and only the *B. lenta × alleghaniensis* cross had high germination. However, all seedlings raised from this cross died within a few months. Whether this represents true "hybrid break-down" or had other causes is unknown. *Betula alleghaniensis* has crossed well with *B. papyrifera* in both directions but poorly with other species. Of the *B. pendula* crosses only *B. pendula × papyrifera* and its reciprocal have been very successful. Most crosses with *B. populifolia* have produced few viable seeds and small seedling populations. Except for one combination where it was used as the male parent *B. pubescens* has performed similarly. Successful combinations involving *B. papyrifera* have already been mentioned. As the female parent *B. glandulosa* has been successfully crossed with *B. pubescens*

Table 2.--Results of intraspecific Betula crosses

| Species | Number of crosses with-: No seed | Inviable seed | Viable seed | Percent germination of successful crosses |
|---|---|---|---|---|
| B. alleghaniensis | 1 | -- | 3 | 31-47-51 |
| B. lenta | - | -- | 1 | 36 |
| B. nigra | - | -- | 5 | 63-77-96-96-100 |
| B. papyrifera | 1 | -- | 4 | 21-65-67-95 |
| B. pendula 1/ | 6 | 18 | 13 | From < 1 to 81 |
| Total | 8 | 18 | 26 | |

1/ Including 4 varieties.

only, and in a few attempts as the male parent it has crossed with difficulty. Crosses with *B. pumila* have largely been unsuccessful, but the attempts are too few to be meaningful.

## Discussion

The present study shows that self-incompatibility is common in species of *Betula,* but the degree of incompatibility varies with the individual plants. The results agree with previous work in Europe. Selfing of *B. pendula* produced an average of 2.6 percent filled seed in Finland (Hagman 1963) and from 0 to 60 percent viable seed in Germany (Stern 1963c). Selfed Finnish *B. pubescens* yielded 0.25 percent filled seed (Hagman 1963).

Self-fertile trees are probably undesirable in a breeding program or seed orchard. On the other hand, the high degree of self-compatibility present in certain trees makes it possible to use selfing as a means of approaching homozygosity.

The results of the intraspecific crosses show that while most *Betula* trees can be crossed without difficulty, some incompatibility occurs. Apparent incompatibility in the *B. pendula* crosses reported here was unusually high, possibly in part due to inbreeding. Three parent trees in four of these crosses came from the same Swedish seedlot and could conceivably be half-sibs. Similarly, seven other trees which proved incompatible or nearly when intercrossed are of a single Finnish origin. That crosses between different seed sources of *B. pendula* can be successful has been shown by Vaclav (1961), who used pollen from the U.S.S.R., Poland, Germany, France, and Belgium on Czecho-Slovakian trees with excellent results.

Although comparatively little work has been done on intraspecific compatibility in birch, the results obtained so far should emphasize the importance of testing all trees to be used in basic studies, breeding programs, or seed orchards.

Reproductive isolation of species is caused by a number of different mechanisms, including incompatibility. That reproductive isolation between species of *Betula is* not complete is evident both from the number of natural interspecific hybrids reported in the literature and from the results of artificial hybridization.

Table 3.--Number of attempted and successful interspecific Betula crosses and best results obtained [1]

| Female Series [2] | Female Species [3] & ploidy | Male: Costatae nig 2X | Costatae len 2X | Costatae all 6X | Albae pen 2X | Albae ppf 2X | Albae pub 4X | Albae pap 4-6X | Nanae gld 2X | Nanae pum 4X |
|---|---|---|---|---|---|---|---|---|---|---|
| Costatae | nig 2X | | 1/0<br>0 | 5/1<br>1 | 7/5<br>2.5 | | 1/0<br>0 | 8/0<br>0 | | |
| | len 2X | 2/0<br>f | | 1/1<br>41 [4] | 1/0<br>f | | 1/0<br>f | 2/0<br>0 | | |
| | all 6X | 9/1<br>1 | 2/0<br>0 | | 4/0<br>0 | | 1/1<br>1 | 8/4<br>79 | | |
| Albae | pen 2X | 6/1<br>< 1 | | 3/1<br>< 1 | | 2/0<br>0 | 3/0<br>0 | 7/4<br>18 | | 1/0<br>0 |
| | ppf 2X | 1/1<br>< 1 | | 1/1<br>< 1 | 1/1<br>3 | | 1/1<br>1 | 1/1<br>3 | 1/1<br><1 | 1/1<br>3 |
| | pub 4X | 1/1<br>1 | | 1/1<br>< 1 | 1/1<br>< 1 | 1/0<br>0 | | 1/1<br>1 | 2/1<br>1 | 1/1<br>< 1 |
| | pap 4-6X | 7/5<br>1 | 3/1<br>< 1 | 7/5<br>23 | 4/3<br>58 | 1/0<br>f | 1/0<br>0 | | 1/0<br>f | 1/0<br>f |
| Nanae | gld 2X | 4/0<br>0 | | 3/0<br>0 | 3/0<br>0 | 3/0<br>0 | 3/2<br>60 | 3/0<br>f | | 1/0<br>f |
| | pum 4X | 2/0<br>f | | 1/0<br>f | 1/0<br>f | 1/0<br>f | 1/0<br>f | 1/0<br>f | | |

1/ In each box of the data columns, the first figure shows the number of attempts, the second shows number of crosses producing viable seed, and the third (beneath the second) shows highest percent germination (f = no seed).

2/ According to Winkler (1904).

3/ Key to Species: nig = nigra; len = lenta; all = alleghaniensis; pen = pendula; ppf = populifolia; pub = pubescens; pap = papyrifera; gld = glandulosa; pum = pumila

4/ Progeny inviable.

Variation in germination percentages occurred even within apparently successful species combinations in table 3. This can be attributed mainly to differences in compatibility between the individual plants used. European work with *B. pendula x pubescens* and its reciprocal cross has also demonstrated large differences due to individual trees (Eifler 1960; Vaclav 1961; Hagman 1963; Stern 1963a).

The demonstrated compatibility between *B. pendula* and *B. papyrifera* agrees with findings by Johnsson (1945) in Sweden, Klaehn (1952) in Germany, and Vaclav (1961) in Czechoslovakia. The last author reported better compatibility between *B. pendula* and *B. alleghaniensis* than found so far in this study. That *B. pubescens* crosses with other species only with difficulty, particularly when used as the female parent, has also been the experience in Europe (Johnsson 1945; Klaehn 1952; Vaclav 1956; Eifler 1960).

What are the possible causes of incompatibility and how can it be overcome? Incompatibility in *B. pendula* and *B. pubescens is* expressed by retarded growth of the pollen tubes in the styles (Hagman 1963). Tube growth inhibition is apparently due to an enzymatic reaction and seems to be specific, i.e., only self pollen or pollen of another species is inhibited. Thus, when Hagman (1963) pollinated with a mixture of self pollen and other pollen of B. *pendula,* the presence of self pollen did not reduce the seed set. Similar results were obtained when a mixture of pollen from two species was used (Vaclav 1961; Hagman 1963). Since enzymatic reactions are temperature-sensitive, changes in temperature may partially overcome the incompatibility. Low temperatures during pollen germination increased seed set after selfing of *B. pendula* and *B. pubescens* ( Hagman 1963; Stern 1963c). High temperatures, on the other hand, have been effective in other plants (Townsend 1965; Kwack 1965 ). Pollination at late stages of female flower development has increased self-compatibility in *B. pendula* and *B. pubescens* and has also been effective in crosses between these species (Hagman 1963 ).

The incompatibility reaction in many plants is controlled by a single gene, S, which usually has many alleles. Stern ( 1963c) found evidence that this gene may be present in *B. pendula,* and it may occur in other species also.

In the gametophytic system of incompatibility the effect of the S gene may be overcome by polyploidy. Doubling the number of chromosomes results in the pollen carrying two different S alleles and may lead to complete self-compatibility (Lewis 1956). Irradiation offers another solution to overcoming incompatibilities. Fruit set in *Prunus avium L.* has been increased by treating the pollen mother cells with X-rays (Lewis, 1954). Apparently the irradiation caused the S gene to mutate to a self-compatibility allele. This treatment may also break crossing barriers between certain species, since the two incompatibility systems appear to be related and may be controlled by the same gene (Lewis 1956; Stern 1963b).

Within-series crosses might be expected to exhibit higher compatibility than between-series crosses. There is some evidence in the literature (Johnsson 1945; Klaehn 1952) and in table 3 that this may be true, particularly in crosses within the series *Albae.* However, it is also apparent in table 3 that certain between-series crosses can be as successful as crosses within series. The poor crossability shown by *B. nigra* may indicate that this species is only distantly related to the other species considered here. The fact that it also differs from the other native species in other respects, e.g. natural range, habitat, time of seed maturation, agrees with this suggestion.

European work and the present study have shown that there are often large differences between reciprocal crosses (table 3). This may in part depend on the individuals used. For example, the result of crossing one *B. alleghaniensis* as female parent with a certain *B. papyrifera* was 79 percent germination, while the reciprocal cross gave less than 1 percent germination. But the same *B. papyrifera* produced 13 percent viable seed when crossed as female with another *B. alleghaniensis* tree. Similarly, one *B. glandulosa* (female) crossed with a certain *B. pubescens* ( male) gave 60 percent germination while the reciprocal cross produced no viable seed. Only 1 percent sound seed resulted from a cross of the same *B. pubescens* with a different *B. glandulosa.*

Differences between species in chromosome numbers may complicate interspecific compatibility patterns in *Betula.* In *Solanum,* crosses between parents of similar ploidy show increased compatibility with increasing ploidy, and crosses between parents of dissimilar ploidy are most successful when the male parent has the higher chromosome number (Marks 1965). Insufficient information is available for *Betula* to determine whether compatibility increases with increasing ploidy levels. There are, however, indications that crosses succeed more readily when made in the direction low ploidy female x high ploidy male. European workers have consistently found the cross *B. pendula* (2X) *x pubescens (4X)* easier to make than the reciprocal cross (Johnsson 1945; Eifler 1958, 1960; Hagman 1963; Stern 1963a). In this study there are two combinations in support of this hypothesis and one against. The crosses *B. glandulosa* (2X) x *pubescens* (4X) and *B. lenta (2X)* x *alleghaniensis* (6X) were compatible while the reciprocals were practically incompatible. On the other hand, *B. papyrifera* (4-6X) *x pendula* (2X) has always been more successful than the reciprocal cross (table 3). Although the results of crossing *B. alleghaniensis* with *B. papyrifera* vary according to the direction of the cross (table 3), they will not be considered in this connection due to uncertainty about the chromosome numbers of the *B. papyrifera* parents.

Most of the progenies raised from the interspecific crosses have been measured and scored for various characteristics and will be compared with appropriate control progenies in order to determine hybridity of the material. Analysis of the data is in progress.

Apomixis in *Betula* has been suggested by previous work in which seedlings were obtained from unpollinated controls in *B. alleghaniensis, B. lenta, B. papyrifera,* and B. *populifolia*[2], and B. *pendula* (Vaclav 1961). No such evidence was found in this study, although normal- appearing but inviable seed was produced from some of the unpollinated controls used throughout the study.


## Summary

Results after 3 years of tests show that self-compatibility in *Betula* usually is very low. Of the 31 individuals belonging to 9 species tested so far, most have been self-incompatible or have had 1 percent or less germination. However, occasional trees had higher degrees of self-compatibility, indicating some variation between trees in this characteristic. The best germination percentages have been 45 percent in B. *alleghaniensis* and 11 percent in B. *papyrifera.*

Intraspecific tests have varied from complete failure to 100 percent germination, depending on the individuals used in the crosses. Most of the crosses with native species were successful. *Betula nigra* crosses were consistently best, followed by *B. papyrifera,* and B. *alleghaniensis.* The results of the *B. pendula* crosses were highly variable, and only part of the failures could be ascribed to pollen inviability, technical problems, or unfavorable environment.

The results of the interspecific crosses varied greatly with only one-third of 142 attempts setting viable seed. *Betula nigra* appears difficult to cross with other species, while B. *alleghaniensis* has crossed well with B. *papyrifera* but poorly with other species. Other successful crosses include B. *papyrifera* × *pendula,* its reciprocal, and B. *glandulosa* × *pubescens.*

Crosses between series of the genus appear to succeed as readily as within-series crosses. There are often large differences between reciprocal crosses. This may partly depend on the individual parents and partly be due to differences between the species in chromosome numbers. Interspecific crosses between species of dissimilar ploidy seem to be more successful when the male parent has the higher chromosome number.

No evidence of apomixis in *Betula* was found in this study.


[2] *E. J. Schreiner, 1961. Personal communication concerning work done at the Northeastern Forest Experiment Station, Upper Darby, Penn., in 1939-1940.*

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