Marlewood Research and Development Paper
Number 38

[illegible] AND [illegible]
[illegible] RIBES, L. [illegible]
[illegible] AND A PROPOSED METHOD
FOR THEIR STUDY

A. C. [illegible]

Introduction

The taxonomist studying hybridization of the Silver and Pubescent birch, Betula pendula Roth and B. pubescens Ehrh., is faced with a more complex problem than one studying, for example, hybridization between European and Japanese larch. Despite a certain amount of debate, it was widely believed that the formation of hybrids between these two birches was a frequent occurrence. The discovery by Helms and Jorgensen (1925) that the problem is complicated by polyploidy may have prompted later experimenters to take a closer look at the situation, but did not lead to an immediate abandonment of the earlier theory. Helms and Jorgensen observed that B. pendula is a diploid species with 2n = 28 chromosomes and B. pubescens is a tetraploid with 2n = 56 chromosomes. At the same time, they found a triploid which could be regarded as an F_1 cross between the two species. The event which introduced a distinct note of caution in the further acceptance of a theory of unhibited hybridization was the publication of the results of Johnsson's experiments and conclusions (Johnsson 1945). His attempts to cross the two species artificially met with comparatively little success. His research is also supported independently by K. Stern (1956 pers. comm.) and Behrndt (1952). Behrndt's experiments are older than Johnsson's but the vicissitudes of war interfered with the publication of his work. In the same decade, the observations made by Jentys-Szaferowa (1938), on the small overlap of the flowering periods of both species, did not assist in the maintenance of the older belief, and attempts were made by a number of research workers to reconcile the situation. Johnsson (1945) and Lindquist (1947) suggested that the two species might overlap in certain characteristics, particularly in old age. Behrndt (1952), on the strength of his own experience, adopted a different standpoint; "It was found that amongst the progeny of 62 single stems from Austria, Hesse, Mark Brandenburg, East Prussia, Finland, there was not one example which presented any difficulties in defining to which species it belonged: despite careful examination, we found no case in which a single plant of a progeny belonged partly to B. pubescens and partly to B. verrucosa. All were found to belong distinctly to either one or the other species. Intermediate forms were never found. It was apparent that the range of variation in both species was much less in younger trees than in trees of timber dimensions, and it is very probable that Regel, Morganthaler and others, would not have arrived at their all-embracing inter-fertility theory if they had not limited their examinations exclusively to trees of timber age".

More recent research has prevented us from adopting a view at either extreme. Eifler (1956, 1958 and 1961) observed that certain individuals of both species, albeit under artificial conditions, would inter-cross much more readily than others to produce considerable numbers of triploid progeny. She also discovered that the cross was more successful when B. pendula was employed as the female parent - a phenomen noted similarly by Hagman (1963). Some individuals had chromosome numbers varying slightly above or slightly below the triploid value of 42; others again had 28 chromosomes, the same number as in the diploid species. The latter type were especially prone to attack by leaf rust and seldom survived. Most important of all is her remark that the triploid was not as sterile as had generally been believed.

The presence of partly fertile triploids of hybrid origin, assuming their recurrence under natural conditions, would provide one means for further gene exchanges between the two species. There are other possibilities and Johnsson (1945), although of the opinion that the event would occur only rarely suggests that the pollen of B. pendula may contain unreduced grains i.e. individual grains with a full diploid complement. During a visit to Waldsieversdorf in 1958, Dr. Eifler showed me a tetraploid specimen, found in natural conditions, displaying all the outward characteristics normally associated with the diploid species, B. pendula. Trees of this nature, if fertile and assuming a normal meiosis, could provide a sound basis for gene flow from the Silver to the Pubescent birch.

Although botanists have tended to use the word hybrid and hybridization with greater care since the discoveries of Johnsson and others, they have not hesitated, fortunately, to record intermediates where these have arisen, e.g. Laing and Carlisle (1955 and 1956), Steven and Carlisle (1959), and have not attempted to force such specimens into one species or the other. The fact that they have not done so is particularly valuable in view of the development and study of the concept of introgressive hybridization. Introgression, or introgressive hybridization, a term first used by Anderson (1949) is defined by King (1968) as;
'The incorporation of genes of one species into the gene pool of another. If the ranges of the two species overlap and fertile hybrids are produced, they tend to backcross with the more abundant species. This process results in a population of individuals most of whom resemble the more abundant parents but which possess also some characters of the other parent species.'

A fuller description of the various stages of the process is available in Stebbins (1950). He writes "Three phases are essential to this process; the initial formation of F_1 hybrids, their backcrossing to one or other of the parental species and natural selection of certain recombinant types. In this way, populations of genotypes which contain a few genes or chromosal segments derived from one species on the genetic background of the other can become established. Although this result of hybridization is to be expected whenever two populations with different adaptive norms hybridize, it is likely to be the commonest outcome if the parental species are separated by well-developed barriers of reproductive isolation, and are cross-fertilizing. Under such circumstances, the relatively uncommon F_1 hybrids are much more likely to mate with members of the parental species than with each other, and the back-cross individuals derived from such matings are likewise more apt to be viable and fertile than are progeny from $F_1 \times F_1$ matings".

Since the time of Anderson's work on Tradescantia spp., the number of study cases of introgressive hybridization in the literature has continued to increase. In the genus Betula, Froiland (1952) and Clausen (1962) have used the methods of hybrid indices to investigate the status of some American species. In Europe, Natho (1954, 1959) has already examined a number of European birches from this point of view. His review (Natho 1961) shows that he is concerned that hybridization of this nature takes place between several species including B. pendula and B. pubescens. In what he describes as the Central European birch families (populations?) he found a smooth transition from one species to another. By the use of a hybrid index of sixteen characters, he demonstrates the two-peaked frequency distribution that one expects to find in typical cases of introgressive hybridization. He considers that, although the fertility of first generation back crosses is low, this fertility increases in the products of back crossing and cites Clausen's studies in America as an example. In the western, southern and eastern limits of the Central European area, the two species remain distinct, as he met with populations of pure forms of both species on these boundaries. Like others before him, Natho stresses the conditions under which introgression is likely to take place, namely the close proximity of both parental species and the presence of a disturbed habitat which provides a suitable niche for the products of cross-fertilization. From what we already know about the ecological conditions and taxonomic status (Laing and Carlisle 1955 and 1956, Steven and Carlisle, 1959, Walters 1964 and 1968, Brown and Tuley 1971), there can be little remaining doubt that we can expect to meet with introgression in further taxonomic studies of British birch.

A suggested morphometric method for studying introgression

The writer believes that a morphometric method may have advantages over other methods more strictly numerical, which seek to combine a number of morphological features in the form of a hybrid index. An examination of the description of both species given in Clapham, Tutin and Warburg (1962) demonstrates that, in some of the gross morphological characters, there will be overlaps between the species. For example, specimens of the Silver birch may exhibit very little or no true rough bark in the basal portion of the stem - a phenomenon well known to birch breeders. Also, within the broad category of smooth bark, there are variations in colour, lustre, peeling-type and lentical-type as the studies of Lindquist (1946), Olofson (1953) and Delius (1935) have shown. Some of the forms produced by this variation are shared by both species. It seems likely that bark type is dependent, to some extent, on age and soil conditions. If rough and smooth bark are used as the extremities of a simple interspecific scoring system, the resulting classification, in the writer's opinion, will be confounded by this finer variation.

If we are to regard the Silver and Pubescent birches as separate species which occasionally hybridize, we ought to be looking for those features which will give us the best means for discrimination. From their botanical description, it would appear that the distinction between the two becomes sharper when we turn our attention to the leaves and the fruits. The characteristics of leaf shape are particularly amenable to morphometric methods of study. In this respect, the leaves of the vegetative dwarf shoots have been used extensively by Jentys-Szaferowa (1949-51). In the case of the Silver and Pubescent birches, she found distinctive patterns of departure in samples of one species when contrasted with the other. Leaf shape also influenced Natho (1959) in his choice of characters. Of the sixteen characters he selected, nine are associated with the leaves, of which four are measures of leaf shape. After he completed his study he wrote the following; 'Most impressive perhaps are the introgressions of leaf shape. In the tree birches, they exhibit a smooth continuation between the families. The leaf shape of _B. pubescens_ and _B. pendula_ is very distinctly different. Next to the extreme forms are those which show only a faint degree of introgression, until an intermediate shape in oval leaves is reached' (writer's translation). However, in an earlier paper he states that the 'line of shape' method of Jentys-Szaferowa failed completely to make distinction between individual trees.

In his opinion, the reason for this failure lay in the inflexibility of the method and not in the material examined. Because there are considerable overlaps between the two species in certain size characters, he felt that the 'line-of-shape' method as defined by Jentys-Szaferowa lacked the precision necessary for classifying individual trees. The problems associated with size and shape, particularly a method for determining the latter, are also discussed by Berrie (1953). The present writer noted that the distinction between the two species became sharper when the linear variables were transformed into ratios of a standard variable (Gardiner, 1972). Although a set of nine variables (five linear, four angular) was capable of distinguishing between sample means of both species following a principal component analysis, it produced, in the writer's opinion, a number of misclassifications during a study of the individual leaves. On the basis of these three studies, it appeared worthwhile to conduct experiments designed to measure only leaf shape where future investigations are designed to classify individual trees and where introgression is suspected.

The following method was developed and showed promise of success when tried out on two samples, one of each species, collected from a woodland near Borden, Hants. First, carbon leaf rubbings of the dwarf-shoot leaves were made, using the method described by Berrie (1953). The rubbings were then transferred to transparent sheets of film by the use of a photo-copier. Next, the images, of each leaf in turn, were projected on to a screen bearing a dotted line grid of an arbitrary scale (see Fig. 1). The projector, the overhead type, was moved backwards or forwards until the image of each leaf fitted the extremities of the grid base line. The number of dots on each arm of the grid contained by the periphery of the leaf were counted and recorded. The earlier analysis (Gardiner, 1972) revealed the importance of relative petiole length and this character was included in the exploratory method under discussion. The forty-five leaves were treated as single taxonomic units; these numbered 1-22 and 23-45 being regarded as putative specimens of the Pubescent and Silver birch respectively.

The resulting data sets contained eleven variables. However a principal components analysis showed that the variation could be expressed in four significant components. Component values were calculated for each leaf and subjected to a cluster analysis (minimum spanning tree). The nearest-neighbours and the distance between them in four dimensional space is shown in Fig. 2. These relationships produced thirteen primary clusters which are illustrated in Fig. 3. By re-running the two stages of the analysis on the component values of the primary groupings, two secondary clusters were obtained whose structure is shown in Fig. 4.

One of the major difficulties, in interpreting the results of a cluster analysis where four or more dimensions are involved, is to obtain a clear picture of the relationships between the suggested groupings. However in a recent paper, Andrews (1972) describes a method for plotting multi-dimensional data, which overcomes this problem by providing a visual representation.

An example of the use of this method together with a summary of the background is given by Jeffers (1972). This method calculates a series of functions for each point or taxon over the range $-\pi < t < \pi$ In the BASIC program used at Merlewood the limits of t are set at -3.0 and 3.0 with a step size of 0.5. This produces a series of thirteen functions over this range. The distribution of the mean component values of the primary clusters in terms of these functions is shown in Fig. 5. The capital letters A-M denote the same primary clusters as in Fig. 3 and Fig. 4. The continuous and dashed lines signify primary clusters which were grouped together in the two respective secondary clusters. An examination of Fig. 5 shows that those functions which produce discriminations approaching the suggested secondary groupings are found in the lower part of the display within the range t = 1.5 to 3.0. This part of the display can be isolated and magnified by modifying the range of t and decreasing the step size to 0.25. The results of the modification are shown in Fig. 6.

If we proceed to study Fig. 6, it can be seen that if we are to maintain the picture of two secondary clusters which corresponds with the hypothesis that there are two taxa in the sample, we have to remove the primary clusters F, I and J (see **Fig.** 7). Both F and J display affinities with both secondary clusters and change sides in a reciprocal manner. The primary cluster I has adopted an intermediate position although the direction of the connecting line indicates a behaviour similar to the secondary cluster containing the primary groups G, ML and K. This particular secondary group embraces the bulk of the leaves classified originally as B. pendula. The reason for this behaviour can be seen if the values over this range of functions are calculated for the individual leaves which make up the primary cluster I (see Fig. 8). In Fig. 8 the five leaves have been labelled A to E. Originally the leaves A to D were classified as Silver birch, whilst E was included in the Pubescent birch sample; the continuous and dashed lines indicate the original classification.

Of the five leaves shown, the leaf labelled C adopts the most intermediate position, but, if the leaf E was removed, it is probable that the primary cluster I would take up a position closer to the group G, M, L, K. The primary clusters F and J contain leaves which were included originally in the Pubescent and Silver birch samples respectively. However, it is obvious from their behaviour that they each contain some characteristics of the basic leaf geometry of the other species - the situation one would expect to meet if some introgression was taking place.

Throughout this analysis, we have been dealing with individual leaves which on the basis of their leaf shape have grouped themselves generally into the two species samples in which they were originally collected. There are, at the same time, some signs of intermediacy the effects of which can be examined by the use of the method put forward by Andrews. It is submitted therefore that if we can classify individual leaves, we should also be able to classify individual trees on the basis of their leaf shape. By applying the method to a woodland in which we suspect that introgression is taking place, we may be enabled to estimate both the frequency and form of the introgression.

the following stages are suggested for carrying out such an investigation.

1) Divide the wood into a convenient number of sample areas.

2) Lay out, from a random point on the periphery, a transect line across each sample area.

3) Collect 10 leaves from 10 vegetative short shoots (one leaf per shoot, collect the largest leaf in each case, if this is damaged or malformed, discard the shoot and choose another), from every tree within a specified distance from the transect line.

4) Measure the variables illustrated in Fig. 1 and calculate the means for each tree.

5) Subject the data to a principal components analysis and calculate the component values for each tree.

6) Carry out a cluster analysis to obtain an estimate of the primary groupings.

7) Employ Andrew's method to obtain a graphic representation of the relationship between the primary clusters.

8) Select for more detailed examination that part of the display which accords most closely with the suggested groupings.

9) Examine the behaviour of the trees forming the primary cluster nearest the centre of the display.

10) Compare the centre cluster with those immediately on either side of it.

11) Continue with the comparisons between pairs of clusters towards the limits of the display.

There are obviously limitations to the number of lines on the graphic display that can be studied conveniently at one time. If there are a large number of primary clusters it may be advantageous to form secondary groupings before proceeding to examine the primary clusters and individual trees in more detail.

References

Anderson, E. (1949) Introgressive hybridization. London and New York.

Andrews, D. F. (1972) Plots of high-dimensional data. Biometrics 28(1), 125-36.

Behrndt, A. (1951) Zur Birkenzuchtung. Z. Forstgenet. Forstpfl.-Zucht., 1(2), 33-5.

Berrie, A. M. (1953) A Study of the Scottish Birch with special reference to the genetics and ecology of the species. Thes. Univ. Glasgow, 1951-52.

Clausen, K. E. (1962) Introgressive hybridization between two Minnesota birches. Silvae Genet. 11(5/6), 125-176.

Clapham, A. R. Tutin, T. G. and Warburg, E. F. (1962) Flora of the British Isles, 2nd Editn. Cambridge.

Delius (1935) Untersuchungen uber Wertbirken in Forstamt Pfeil in Ostpreussen Z. Forst u. Jagdw. 67, 239-65.

Eifler, I. (1956) Artkreuzungen der Birke. Zuchter, 26, 342-346.

Eifler, I (1958) Kreuzungen zwischen _Betula verrucosa_ and _B. pubescens_. Zuchter, 28, 331-36.

Eifler, I (1961) Untersuchungen zur individuellen Bedingtheit des Kreuzungserfolges zwischen _B. pendula_ and B. pubescens Silvae Genet, 9(6), 149-76.

Froiland, S. G. (1952) The Biological status of _Betula Andrewsii_. Evolution, 6(3).

Gardiner, A. S. (1972) A biometric study of leaf variation in some British Birch populations. Forestry, 45(1), 37-47.

Hagman, M. (1963) Incompatibility in _B. verrucosa_ Ehrh. and _B. pubescens_ Ehrh. Proc. XI Int. Cong. Genetics, The Hague, Sec. 13, 8.

Helms, A. and Jorgensen, C. A. (1925) Birkene pa Moglemose. Bot. Tidskr. 39, 57-133.

Jeffers, J. N. R. (1972) Plotting of multidimensional data. Merlewood R & D paper No. 35.

Jentys-Szaferowa, J. (1949-51) Analysis of the collective species Betula alba L. on the basis of leaf measurements. Parts 1-3 Bull. Acad. Pol. Sci. et Lettres, Ser. B. Crawcow.

Johnsson, H. (1945) Interspecific hybridization with the genus Betula. Hereditas, 31, 163-76.

King, R. C. (1968) A dictionary of genetics. Oxford Univ. Press, New York, London, Toronto.

Laing, E. V. and Carlisle, A. (1955) Studies in morphological variation in conifers. Rep. For. Res. For. Comm.. London 79-80.

Laing, E. V. and Carlisle, A. (1956) Studies of the morphological variation of forest trees. Rep. For. Res. For. Comm., London, 95-96.

Lindquist, B. (1946) Studies uber die Stammrindentypen der Gattung Betula L. Acta Horti Bergiani, Bd. 14, no. 4.

Lindquist, B. (1947) On the variation of Skandinavian Betula verrucosa Ehrh. with some notes on the series Verrucosae Sukacz. Svensk. Bot. Tidskr. 41 (1) Uppsala.

Natho, G. (1954/55) Methoden zur Untersuchung von Hybridpopulationen. Wiss. Ztschr. Univ. Greifswald Jahrg. 4 Math.-nat. R.5.

Natho, G. (1959) Variationsbreite und Bastardbildung bei mitteleuropaschen Birkensippen. Feddes rep. nov. sp. regni veg. 61 (3), 211-273.

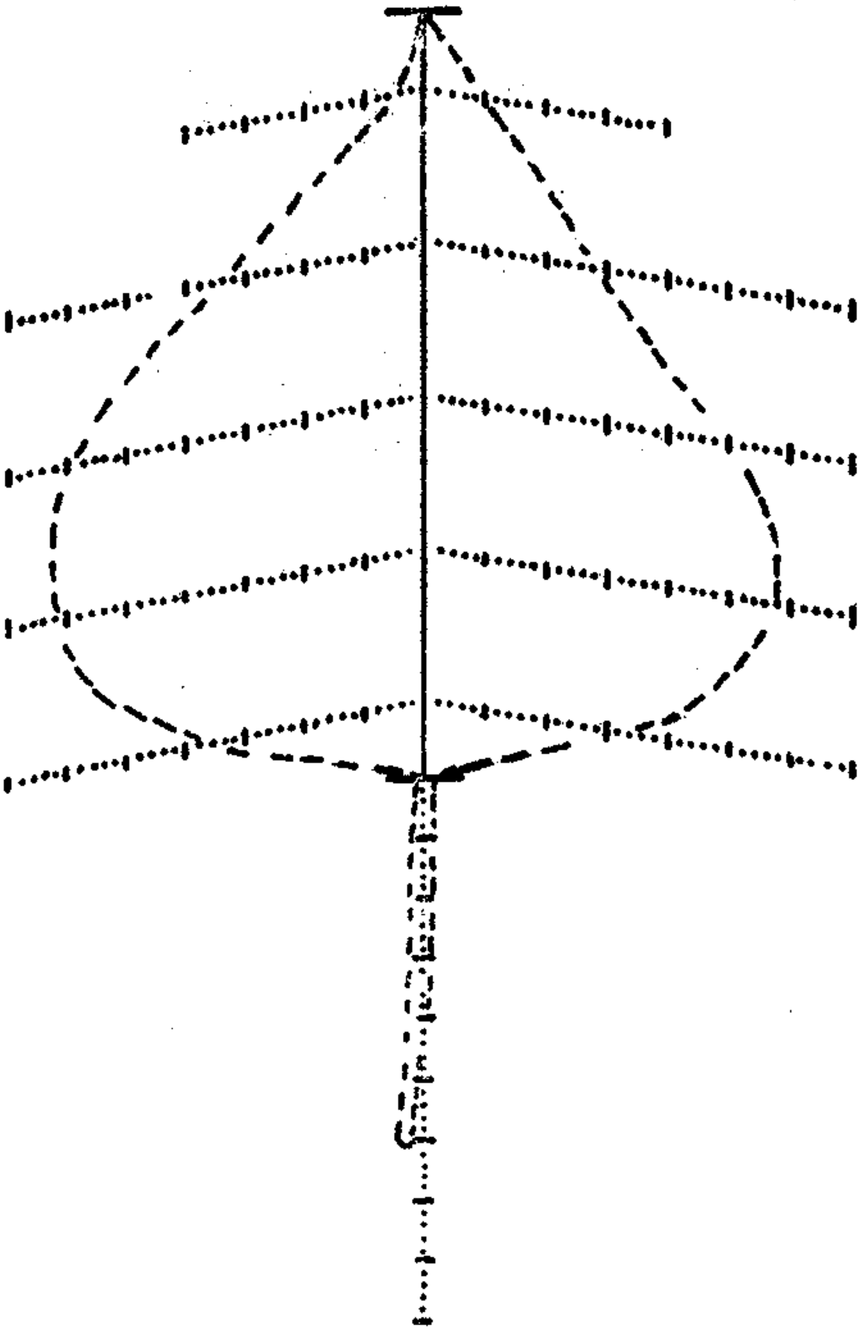
Natho, G. (1967) Zum Problem der Introgressiven Hybridization. Feddes Repert, 75(1-2) 5560.

Olofson, A. (1953) Om de Svenske bjorkartenas morfologiska variation ock skogliga varde. Svensk Papp-Tidn. 56(20/21), 785-98, 818-39.

Stebbins, G. L. (1959) The role of hybridization in evolution. Proc. Amer. Phil. Soc. 103(2), 240-8.

Steven, H. M. and Carlisle, A. (1959) The Native Pinewoods of Scotland. Edinburgh, Oliver and Boyd, p.69.

Fig. 1



INPUT:BNNAN

ENTER NOS. OF VARIABLES AND SETS Fig. 2

4 45

1	18	0.693126E+0
2	15	0.933359E+0
3	20	0.614357E+0
4	3	0.139346E+1
5	21	0.123721E+1
6	13	0.483850E+0
7	22	0.173107E+1
8	36	0.358376E+1
9	19	0.882641E+0
10	17	0.541875E+1
11	6	0.693503E+0
12	5	0.188466E+1
13	6	0.483850E+0
14	23	0.218119E+1
15	2	0.933359E+0
16	7	0.218388E+1
17	22	0.761713E+0
18	1	0.693126E+0
19	9	0.882641E+0
20	3	0.614357E+0
21	5	0.123721E+1
22	17	0.761713E+0
23	42	0.141221E+1
24	38	0.924376E+0
25	27	0.179579E+1
26	41	0.146282E+1
27	25	0.179579E+1
28	27	0.299304E+1
29	37	0.247278E+1
30	38	0.742309E+0
31	44	0.100708E+1
32	40	0.113730E+1
33	38	0.153241E+1
34	45	0.111920E+1
35	26	0.153354E+1
36	43	0.131484E+1
37	35	0.245437E+1
38	30	0.742309E+0
39	36	0.181067E+1
40	32	0.113730E+1
41	40	0.134841E+1
42	34	0.123773E+1
43	36	0.131485E+1
44	31	0.100708E+1
45	34	0.111920E+1

Fig. 3

Primary Clusters

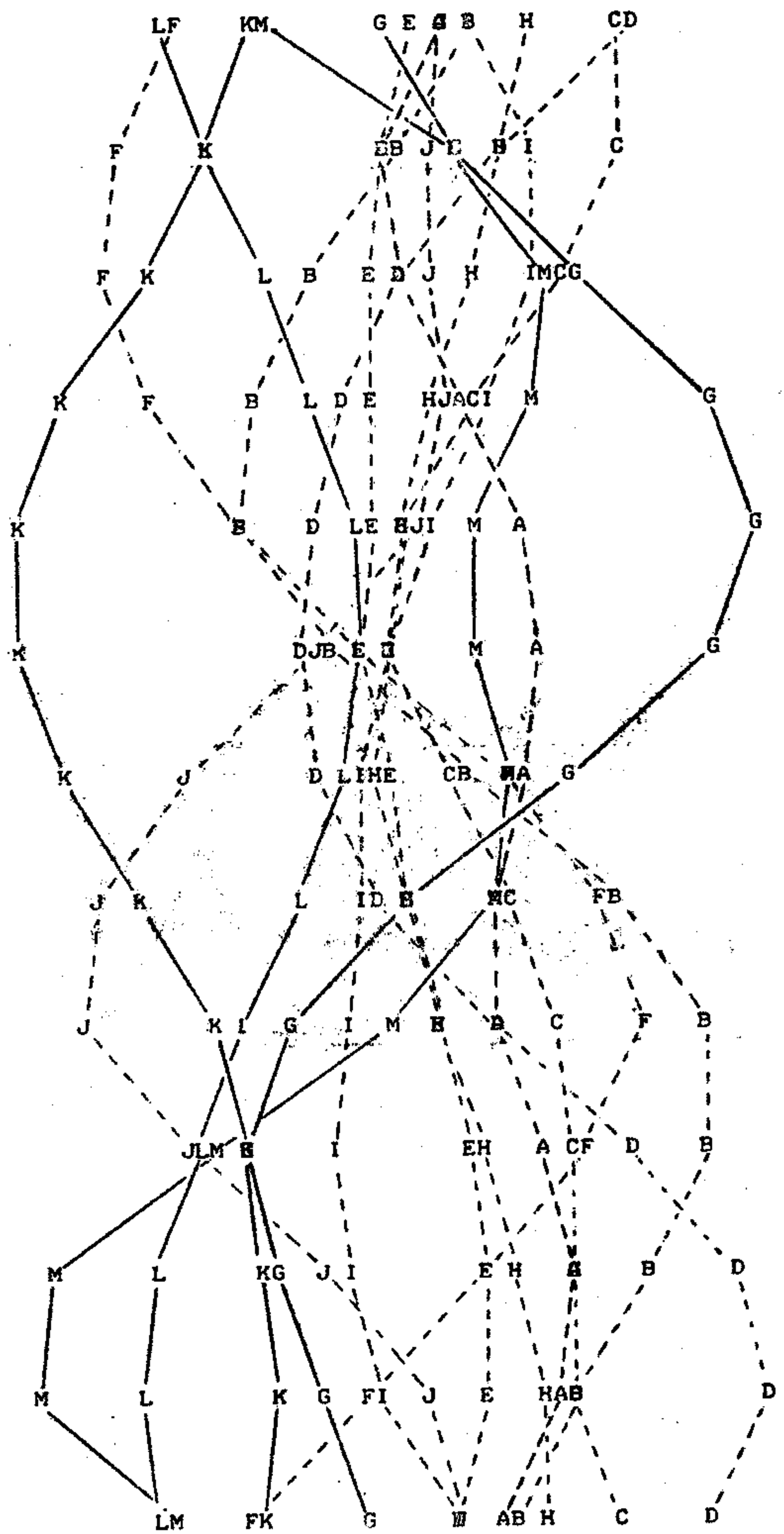
A	1	18					
B	2	15					
C	3	20	4				
D	5	21	12				
E	6	13	11				
F	7	22	17	16	10		
G	8	36	43	39			
H	9	9	19				
I	14	23	42	34	45		
J	24	38	30	33			
K	25	27	28				
L	26	41	40	32	35	37	29
M	31	44					

Fig. 4

Secondary Clusters

A	E	H	C	D	I	B	J	F
G	M	L	K					

Fig. 5



LF KM G E A B H CD
F K EB J C B I C
F K L B E D J H IMCG
K F B L D E HJACI M G
K B D LE HJI M A G
K DJB E M A G
K J D LIHE CB MA G
J K L ID E MC FB
J K I G I M H B C F B
JLM I EH A CF D B
M L KG J I E H A B D
M L K G FI J E HAB D
LM FK G AB H C D

READY

Fig. 6

RUN

NO OF VARIABLES AND SETS? 4,13
? A
? B
? C
? D
? E
? F
? G
? H
? I
? J
? K
? L
? M

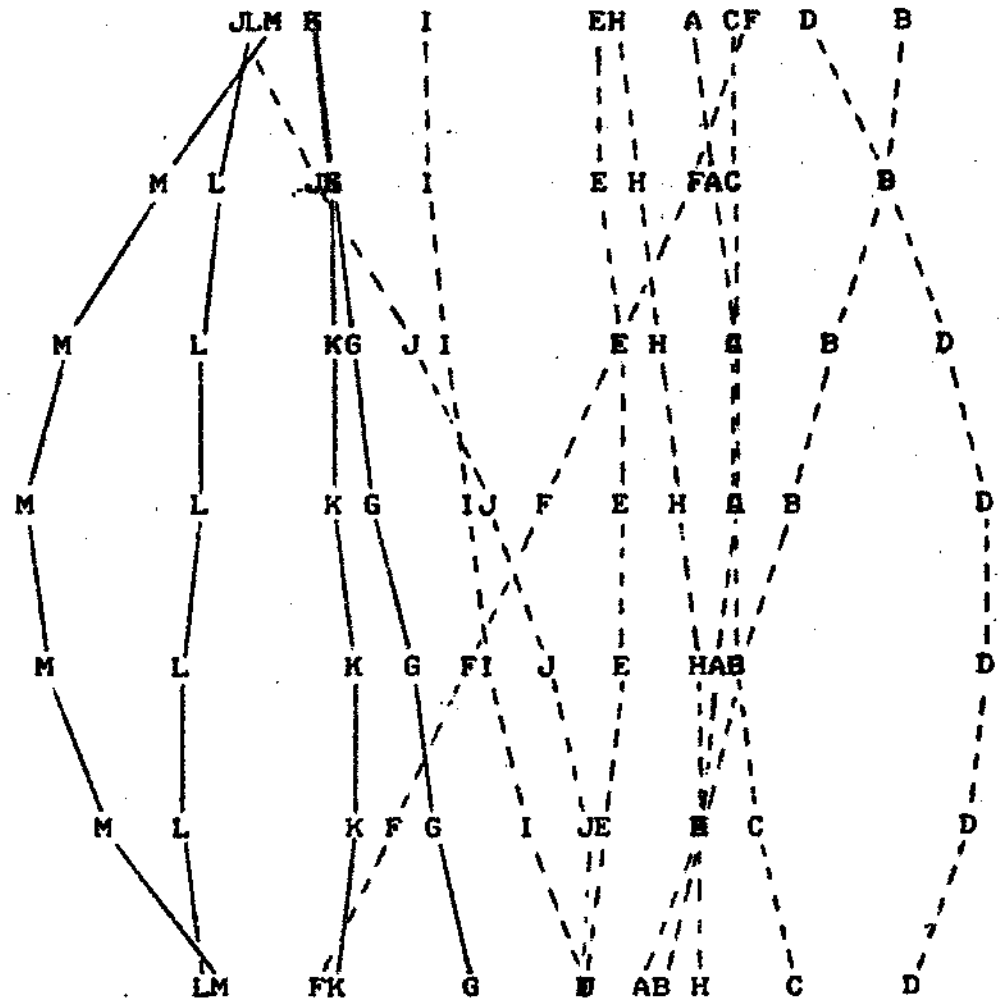
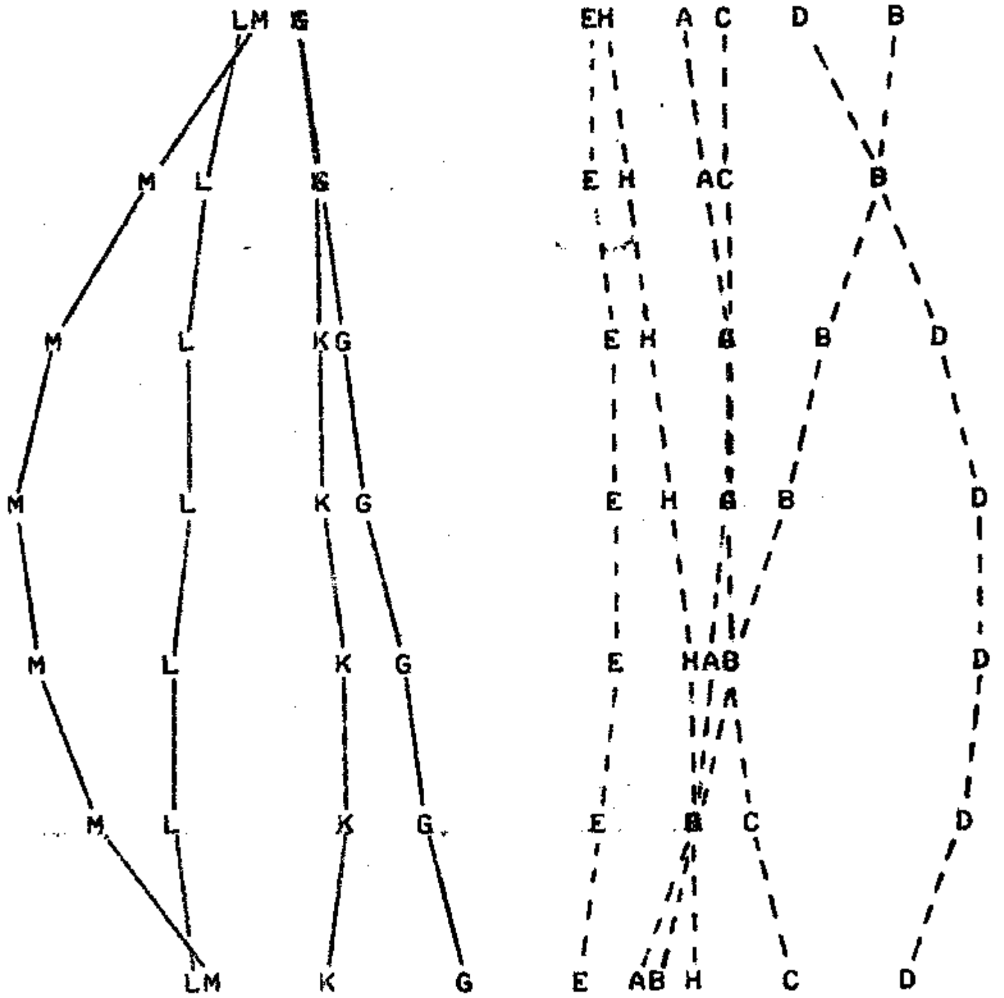


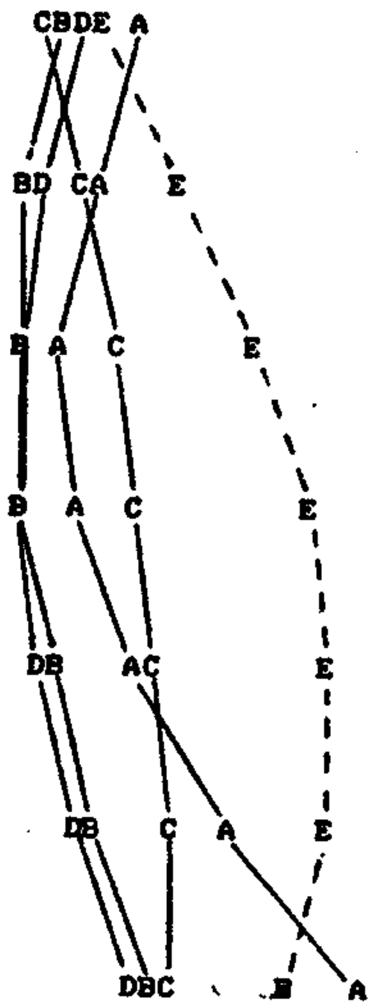
Fig. 7

```
READY

50 FOR J=1.5 TO 3.0 STEP 0.25
LIST 50
  50 FOR J=1.5 TO 3.0 STEP 0.25

READY

RUN

NO OF VARIABLES AND SETS? 4,13
? A
? B
? C
? D
? E
?
? G
? H
?
?
? K
? L
? M
```



```
READY

LIST 50
  50 FOR J=-3.0 TO 3.0 STEP 0.5

READY

50 FOR JZ
50 FOR J=1.5 TO 3.0 STEP 0.25
LIST 50
  50 FOR J=1.5 TO 3.0 STEP 0.25

READY

RUN

NO OF VARIABLES AND SETS? 4,5
? A
? B
? C
? D
? E
```

Fig. 8




```
READY
```